

Design, Synthesis and Structure-Activity-Relationships of Potent Mas-Related G Protein-Coupled Receptor X (MRGPRX) Modulators

KUMULATIVE DISSERTATION

Zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

Angefertigt am Pharmazeutischen Institut der Universität Bonn

Vorgelegt der Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

von

Daniel Marx

geboren in Siegburg

Bonn

2021

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn.

1. Gutachterin: Prof. Dr. Christa E. Müller
2. Gutachter: Prof. Dr. Andreas Gansäuer

Erscheinungsjahr: 2021

Tag der Promotion: 26.03.2021

Danksagungen

Hiermit möchte ich mich herzlich bei Frau Prof. Dr. Müller für das interessante Thema und die Ermöglichung dieser Arbeit bedanken. Vielen Dank für Ihre Betreuung und Unterstützung.

Ich bedanke mich bei Prof. Dr. Gansäuer für die Übernahme des Zweitgutachtens

I am deeply grateful to Dr. Vigneshwaran Namasivayam for the close cooperation and support regarding homology modeling and docking studies.

Bei Yvonne Riedel, Dr. Dominik Thimm, Ghazl Al Hamwi und Beatriz Büschbell bedanke ich mich für die Durchführung der biologischen Assays.

Mein Dank gilt auch Frau Sabine Terhart-Krabbe, Marion Schneider und Andreas Schneider für die Durchführung der NMR- und LC/MS-Analysen.

Des Weiteren bedanke ich bei meinen Labor- und Bürokollegen Dr. Ahmed Elgokha, Dr. Nader Boshta und Tim Klapschinski für die tolle Zeit während der Anfertigung dieser Doktorarbeit.

Auch bei Dr. Jörg Hockemeyer und Dr. Thanigaimalai Pillaiyar bedanke ich mich, die jederzeit für Fragen zur Verfügung standen.

Zuletzt bedanke ich mich noch bei dem kompletten Arbeitskreis von Frau Prof. Dr. Müller und den weiteren Kooperationspartner wie Prof. Herdewijn und Prof. Brüstle.

Table of Contents

1. Introduction	1
1.1. Drug discovery	1
1.2. Importance of the xanthine scaffold in medicinal chemistry	5
1.2.1. Classification of alkaloids.....	5
1.2.2. Structure, biosynthesis and catabolism of xanthine alkaloids.....	5
1.2.3. Pharmacology of methylxanthines.....	10
1.2.4. Approved xanthine drugs	12
1.2.4.1. Caffeine and theophylline	12
1.3. References	20
2. Article I: Fast, Efficient, and Versatile Synthesis of 6-amino-5-carboxamidouracils as Precursors for 8-substituted Xanthines	29
3. Article II: Structural and Conformational Studies on Carboxamides of 5,6-Diaminouracils—Precursors of Biologically Active Xanthine Derivatives.....	86
4. Medicinal Chemistry of Primate-Specific Mas-Related G Protein-Coupled Receptors (MRGPRX)	118
4.1. G protein-coupled receptors	118
4.2. Primate specific Mas-related G protein-coupled receptor	121
4.2.1. MRGPRX1	124
4.2.2. MRGPRX2 and its role in human mast cells	126
4.2.2.1. Peptide derived MRGPX2 agonists.....	127
4.2.2.2. Small-molecule MRGPRX2 modulators.....	129
4.2.3. MRGPRX3	130
4.2.4. Role of MRGPRX3 and MRGPRX4 in keratinocytes.....	130
4.2.5. MRGPRX4.....	132
4.3. References	135
5. Patent I: MRGPRX4 Agonists and Antagonists	143
6. Homology Modeling and Docking Studies of the MRGX4 receptor.....	218

6.1.	Homology model of the human MRGPRX4	218
6.2.	Docking studies of MRGPRX4 agonists	221
7.	Patent II: MRGX Receptor Antagonists – US 16/670,149	227
8.	Enhancement of the water solubility of MRGPRX2 antagonists.....	375
8.1.	Synthesis.....	375
8.2.	Experimental.....	381
8.2.1.	Crystal structure of 41	381
8.2.2.	Materials and Methods.....	381
8.3.	References	393
9.	Summary and Outlook	394
10.	Annex - Supporting Information to MRGPRX4 Agonists	402

ABSTRACT

The G protein-coupled receptor (GPCR) superfamily with more than 800 members is one of the most important target classes for drug development. After an agonist binds to the receptor, it induces a conformational change leading to the activation of a heterotrimeric guanine nucleotide-binding protein (G protein). G proteins play a key role in signal transduction from GPCRs activated by extracellular signals to intracellular second messenger systems. GPCRs are involved in almost all physiological and pathophysiological processes. About 100 of the more than 800 human GPCRs are so-called orphan receptors, whose endogenous ligands remain to be identified or confirmed.

In 2001, Dong *et al.* discovered a subfamily of orphan receptors comprising over 50 rodent and human GPCRs, which were termed Mas-related gene receptors (MRGs). Later, in 2002, Lembo *et al.* discovered the same GPCR family to be expressed in rat primary cultures of dorsal root ganglia (DRG) and designated them sensory neuron-specific receptors (SNSRs). The current nomenclature declared by the International Union of Pharmacology (IUPHAR) of these orphan receptors is Mas-related G protein-coupled receptors (MRGPR).

This receptor family can be divided into nine distinct subfamilies (MRGPRA-H and -X). They belong to the δ -branch of class A, rhodopsin-like GPCR subgroup. Among the nine subfamilies of MRGPRs, four members (MRGPRX1-4) are primate-specific. Due to their expression profile, they are supposed to be involved, e.g., in pain transmission, immune responses, itching and wound healing.

The present study was aimed at developing potent, selective ligands as pharmacological tool compounds for investigating the poorly studied subtypes MRGPRX2 and -4 based on previously discovered hit compounds.

To achieve this, we initially developed an optimized synthesis of carboxamides of 5,6-diaminouracils, which are crucial intermediates for the synthesis of xanthines, a novel scaffold for MRGPRX4 agonists. Subsequently, their physicochemical properties were examined in detail. These studies led to two publications (Marx, D., Wingen, L.M., Schnakenburg, G., Müller, C.E., Scholz, M.S. Synthesis of 6-amino-5-carboxamidouracils as precursors for 8-substituted xanthines. *Front. Chem.*, **2019**, 7, 56, 1–15 and Marx, D., Schnakenburg, G., Grimme, S., Müller, C.E. Structural and conformational studies on carboxamides of 5,6-diaminouracils—precursors of biologically active xanthine derivatives. *Molecules* **2019**, 24, 2168).

The optimization of the synthesis of key precursors for MRGPRX4 ligands provided the basis for the development of a large number of diverse MRGPRX4 agonists, and part of this study was submitted as a patent application (Marx, D.; Müller, C.; Alnouri, W.; Riedel, Y.; Namasivayam, V.; Pillaiyar, T.; Thimm, D.; Hockemeyer, J. MRGPRX4 agonists and antagonists. *UBN0005(EP)* **2020**). Since no synthetic procedure for the synthesis of these selective and highly potent MRGPRX4 agonists was known in the literature, a broad range of chemical methods had to be investigated to finally obtain the desired target compounds in 6 to 9 synthesis steps. This reaction usually starts with 6-aminouracils, which can be selectively alkylated at the N3 position, followed by nitrosylation and subsequent reduction to obtain 5,6-diaminouracil derivatives. A further regioselective amide coupling reaction yields the 6-amino-5-carboxamidouracil derivatives described above. With diethyl(4-iodobutyl)phosphonate as alkylating agent, followed by a ring closure reaction using 2N NaOH, the desired xanthine derivatives bearing a alkyle phosphonate group in the N3 position can be obtained after cleavage of the protective group of the phosphonic acid ester.

The compounds were designed supported by molecular modeling, synthesized and structure-activity relationship (SARs) were analyzed. Homology models were established, and molecular docking studies were performed to support the drug development efforts. In cooperation with pharmacologists, the potencies of the newly synthesized compounds were determined in calcium mobilization and β -arrestin assays.

A further patent application describes the design, synthesis and structure-activity relationships of a series of novel tricyclic benzimidazole derivatives as antagonists for MRGPRX2 (Müller, C.; Alnouri, W.; Riedel, Y.; Thimm, D.; Marx, D.; Namasivayam, V.; Gattner, S.; Herdewyn, P.; De Jonghe, S.; Leonczak, P.; Verdonck, S. MRGX receptor antagonists. *US16/670,149* **2019**). The new MRGPRX2 antagonists were shown to inhibit mast cell degranulation and thus constitute potential novel therapeutics for asthma, allergic rhinitis, anaphylaxis, chronic urticaria, atopic dermatitis and other inflammatory diseases.

The final part of the present cumulative thesis describes the development of MRGPRX2 antagonists that show significantly improved water solubility compared to the compounds disclosed in the above-mentioned patent.

The present thesis constitutes a significant advance in the field of MRGPRX receptors and their modulators, which have great potential as future drugs.

1. Introduction

1.1. Drug discovery

The European Medicines Agency defined a drug as “a substance or combination of substances that is intended to treat, prevent or diagnose a disease, or to restore, correct or modify physiological functions by exerting a pharmacological, immunological or metabolic action.” These substances may be of natural, synthetic or semi-synthetic origin. Very early in history, people were aware that different plants could cause different biological effects in humans.¹ For a medicinal product to be effective, it must interact at a molecular level with a component of the body or an infectious microorganism. Many plants contain a large number of chemical molecules, some of which can exert disease-modifying properties on humans. But they also contain numerous substances that have toxic and undesirable properties on the human organism. The goal in early drug development at the beginning of the 19th century was the identification and isolation of individual substances from plants, microbes or animals that were able to alleviate or prevent diseases and cause as few or no side effects as possible. After several successful and still used drugs such as morphine from the plant *Papaver somniferum L.* (opium poppy) could be isolated, progress in organic synthesis led to the semi-synthetic and synthetic production of biologically active substances. The first synthetic drug was the hypnotic chloral hydrate (**1**, Figure 1), which was discovered in 1869 and is still marketed today under the trade name Noctec[®].¹⁻²

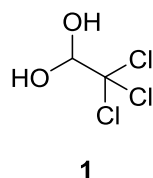


Figure 1: Structure of the hypnotic chloral hydrate, the first synthetic drug.

Modern drug discovery often follows the following procedure. First a target is identified and selected for further discovery. This is often designated as target validation procedure.³ Currently, the most important classes of drug targets are G protein-coupled receptors (GPCRs, 33%), ion channels (18%), nuclear receptors (16%) and kinases (3%). Together they account for 70% of all drug targets.⁴

OVERVIEW OF DRUG TARGETS

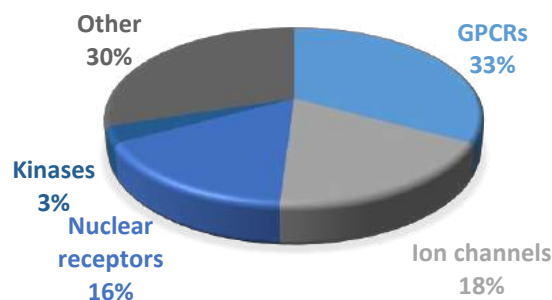


Figure 2: Overview of the most important drug targets.⁴

Target validation is an extremely versatile process, ranging from *in vitro* techniques and animal models to clinical testing in suitable patients. Target validation can include among others, transgenic animal models for the observation of phenotypic endpoints, monoclonal antibodies or chemical genomics which is defined as the study of genomic responses to chemical compounds.³ The next step is the identification of a hit molecule. A breakthrough in drug development was the establishment of high throughput screening (HTS) which had its origins in the 1980s and 1990s.¹ This method has now been further developed to ultra-high throughput screening (uHTS), which can screen more than one million compounds for biological activity in less than two days.⁵ Since this method can usually be used to find weakly effective hit molecules, these must be optimized with regard to their biological activity, selectivity, physicochemical and pharmacokinetic properties. Methods such as homology modeling, molecular docking and quantitative structure-activity relationship (QSAR) support the process of hit to lead compound development.⁶

In the field of small molecules, researchers often follow the Lipinski rule of five, which states that:

- a drug should typically have a molar mass of less than 500 Dalton,

- a drug should have less than 5 hydrogen bridge donors,
- a drug should feature less than 10 hydrogen bridge acceptors,
- a drug should have a logP of no more than 5.

Further *in vitro* assays are used to obtain important information about absorption, distribution, metabolism and excretion properties (ADME). Different assays for the determination of water solubility, LogD value, the determination of peroral bioavailability, possible blood-brain barrier permeability, microsomal stability, CYP450 inhibition, and various toxicological screenings are required.³ Once a substance has been found that meets most of the above requirements, this compound can further be tested in *in vivo* models. The pharmacodynamic and pharmacokinetic properties of the drug candidates will then be determined in a suitable animal model.

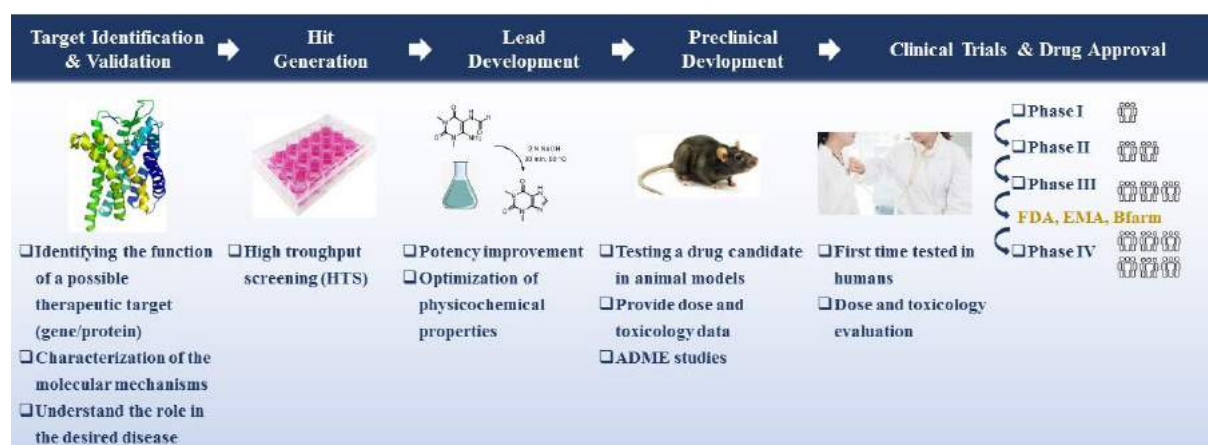


Figure 3: Overview of the development of drugs, from target validation up to their approval by a drug approval authority such as the U.S. Food & Drug Administration (FDA) or the European Medicines Agency (EMA) for the EU market.³

Once the preclinical studies have been successfully completed, the drug candidate can be tested in clinical trials on humans. These are divided into four different phases. In phase one, the drug is tested for safety in a small group of healthy volunteers. After successful completion of phase one, the drug candidate can then be tested for the first time in patients (phase II). Different doses are evaluated to determine active and at the same time safe doses, and the efficacy of the active substance is usually tested against placebo and/or the current gold standard. If this second phase is also successful, the active substance is tested on a very large number (often more than 1000 patients, depending on the indication) for efficacy and rare side effects to evaluate the risk-benefit ratio. If the first three phases have been successfully completed, the drug candidate can be approved for marketing by the relevant regulatory authorities. In a fourth phase, the long-term effects of the substance as well as side-effects in large groups of heterogenous patients

under less controlled conditions can then be examined.⁷ The costs for the development of drugs are mostly in the hundreds of millions or even billions of Euros, and it can take more than 10 years from the discovery of the hit molecule to approval.⁸

Since 1982, after the approval of the first biological drug insulin, biopharmaceuticals have played an increasingly important role in drug development. In 2018, 59 new drugs were approved by the FDA, of which 42 were new chemical entities (NCE) and 17 were biologicals, meaning that almost one third of the approved drugs are now biological molecules such as antibodies or enzymes.⁹ However, the development of biologicals is often more costly and risky compared to NCEs, and peroral application is not feasible at present.¹⁰ Therefore, small molecules are still the most important substance class in drug development, and between 2007 and 2016, 212 (77%) of the 275 new FDA-approved drugs were small molecules.¹¹ Many of these synthetic compounds have a natural origin in plants or animals.

1.2. Importance of the xanthine scaffold in medicinal chemistry

Plants are at the beginning of the food chain, as they belong to the autotrophic organisms that are able to produce their food autonomously via photosynthesis. Plants, algae and phytoplankton are therefore primary producers and usually consumed by heterotrophs. To protect plants from herbivores and microbes, they have developed many defense mechanisms over the course of evolution. Plants are using mechanical defense systems such as spines, thorns, hooks, trichomes, glandular and stinging hairs, or chemical defense systems known as allelochemicals or secondary metabolites (SM).¹² They have no immune system to protect themselves against microbes and herbivores and are therefore dependent on SMs. Maplestone *et al.* defined SMs as “naturally produced substances which do not play an explicit role in the internal economy of the organism that produces it”.¹³ SMs can be distinguished into nitrogen-containing SMs and SMs without nitrogen (terpenoids, phenolics, fatty acids, steroids or waxes). The largest group of nitrogen-containing SMs are alkaloids which are biosynthesized from amino acids.¹⁴

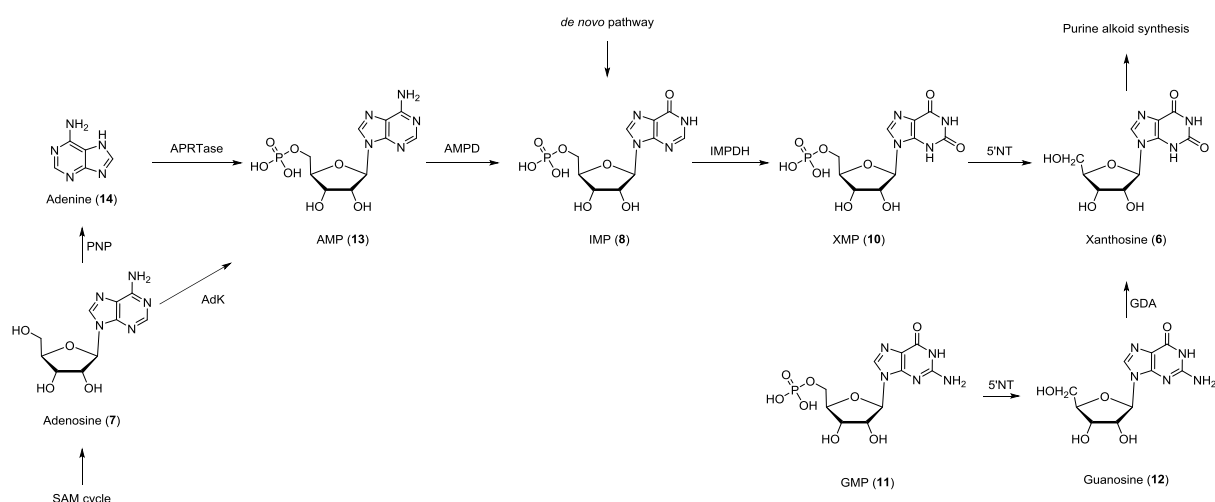
1.2.1. Classification of alkaloids

At the beginning of the 19th century, in 1804, the first bioactive plant alkaloid morphine (**1**) was extracted by the young German pharmacist Friedrich Sertürner. The term “alkaloid” was introduced in 1819 by the pharmacist Carl Friedrich Wilhelm Meißner.¹⁵ An exact definition for alkaloids is sometimes debated today, but in general they are known as natural nitrogen-containing cyclic molecules of limited distribution.¹⁶

1.2.2. Structure, biosynthesis and catabolism of xanthine alkaloids

An important subgroup of alkaloids are xanthines. The main methylxanthines (Table 1) are caffeine (1,3,7-trimethylxanthine, **2**), theobromine (3,7-dimethylxanthine, **3**), theophylline (1,3-dimethylxanthine, **4**) and methyluric acid (**5**). The methylxanthines are frequently consumed central stimulants present, e.g., in tea (*Camellia sinensis* L.), coffee (*Coffea* sp.) and cacao (*Theobroma cacao* L.).¹⁷ Methylxanthines were isolated and characterized at the beginning of the 19th century and could also be produced synthetically by Emil Fischer at the end of the 19th century, for which he was awarded with the Nobel Prize in Chemistry in 1902. In plants, the methylxanthines are biosynthetically formed from purine nucleotides. The first initial precursor, xanthosine (**6**), can be produced by *de novo* purine synthesis (*de novo* route),

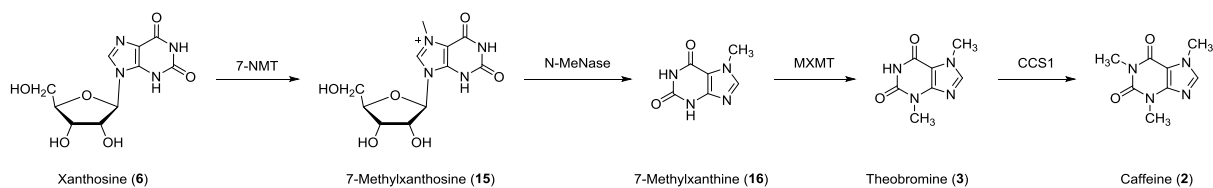
from the cellular adenine nucleotide pool (AMP route), from adenosine (7) released from the *S*-adenosyl-L-methionine cycle (SAM cycle route) or from the guanine nucleotide pool (GMP route). The *de novo* pathway represents the synthesis of inosine-5'-monophosphate (IMP, 8) from 5-phosphoribosylamine (PRA). PRA can be synthesized from 5-phosphoribosyl-1-pyrophosphate (PRPP), which is obtained by ribose-5-phosphate, an intermediate of the pentose phosphate pathway. For the synthesis of xanthosine, IMP (8) is dehydrogenated by the NAD⁺-dependent IMP dehydrogenase to the nucleotide xanthosine monophosphate (XMP, 10). Finally, XMP (10) is converted by a 5'-nucleotidase to the nucleoside xanthosine (6). In the GMP route, guanine monophosphate (GMP, 11) is first dephosphorylated to guanosine (12) and afterwards converted to xanthosine (6) by guanosine deaminase. In the SAM cycle *S*-adenosyl-L-homocysteine (SAH), obtained by the methyl group donor *S*-adenosylmethionine (SAM), is hydrolyzed to homocysteine and adenosine (7). Adenosine monophosphate (AMP, 13) is obtained by transformation of adenine (14) via adenine phosphoribosyl transferase and can be further converted to IMP (8, Scheme 1).¹⁸⁻¹⁹



Scheme 1: Biosynthesis of xanthosine (7), the initial intermediate in the purine alkaloid synthesis.¹⁸⁻¹⁹ The important enzymes involved are as follows: AdK (adenosine kinase, EC 2.7.1.20), PNP (purine nucleoside phosphorylase, EC 2.4.2.1), APRTase (adenine phosphoribosyltransferase, EC 2.4.2.7), AMPD (adenosine 5'-monophosphate deaminase, EC 3.5.4.6), IMPDH (inosine-5'-monophosphate dehydrogenase, EC 1.1.1.205), 5'NT (5'-nucleotidase EC 3.1.3.5), GDA (guanine deaminase, EC 3.5.4.3).

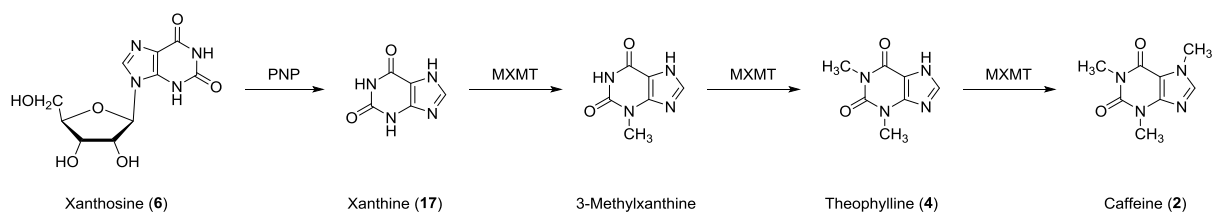
Most of the methylxanthine derivatives are then formed via a four step synthesis.¹⁷ The first step is the conversion of xanthosine (6) to 7-methylxanthosine (15) catalyzed by 7-methylxanthosine synthase. The next step is the hydrolysis of 15 to 7-methylxanthine (16) followed by a methylation to theobromine (3), which is catalyzed by the *S*-adenosyl-L-

methionine (SAM)-dependent *N*-methyltransferase(s). Further methylation yields the caffeine (2) and is catalyzed by the dual-functional caffeine synthase (Scheme 2 and 3).¹⁸



Scheme 2: Proposed biosynthesis of the purine alkaloids caffeine (5) and theobromine (7) in plants.¹⁸⁻¹⁹ The important enzymes involved are as follows: 7-NMT (N-7-methyltransferase, EC 2.1.1.158), N-MeNase (N-methyl nucleosidase, EC 3.2.2.25), MXMT (theobromine synthase, EC 2.1.1.159), CCS1 (caffeine synthase, EC 2.1.1.160).

Another proposed biosynthetic approach is the synthesis of xanthine (17) from xanthosine (6) which can be further methylated to theophylline (4) and caffeine (2).



Scheme 3: Further approach for the biosynthesis of the purine alkaloids caffeine (2) and theophylline (4) in plants.¹⁸⁻¹⁹ The important enzymes involved are as follows: PNP (purine nucleoside phosphorylase, EC 2.4.2.1) and MXMT (monomethylxanthine methyltransferase, EC 2.1.1.159).

There are two theories about the physiological role of purine alkaloids. One assumes that purine alkaloids serve as chemical defenders of the plant to protect itself from herbivores and pathogens. Hollingsworth *et al.*²⁰ verified in tobacco plants that caffeine acts as a neurotoxin against snails and kills or expels them. The second theory assigns purine alkaloids an important role in allelopathic or autotoxic function. It is assumed that caffeine is released in the seed coat and the falling leaves in the soil and prevents the seeds from germinating around the parent plants.²¹⁻²³ Methylxanthines differ in their molecular structure by the number and position of *N*-methyl groups (see Table 1).

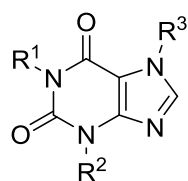
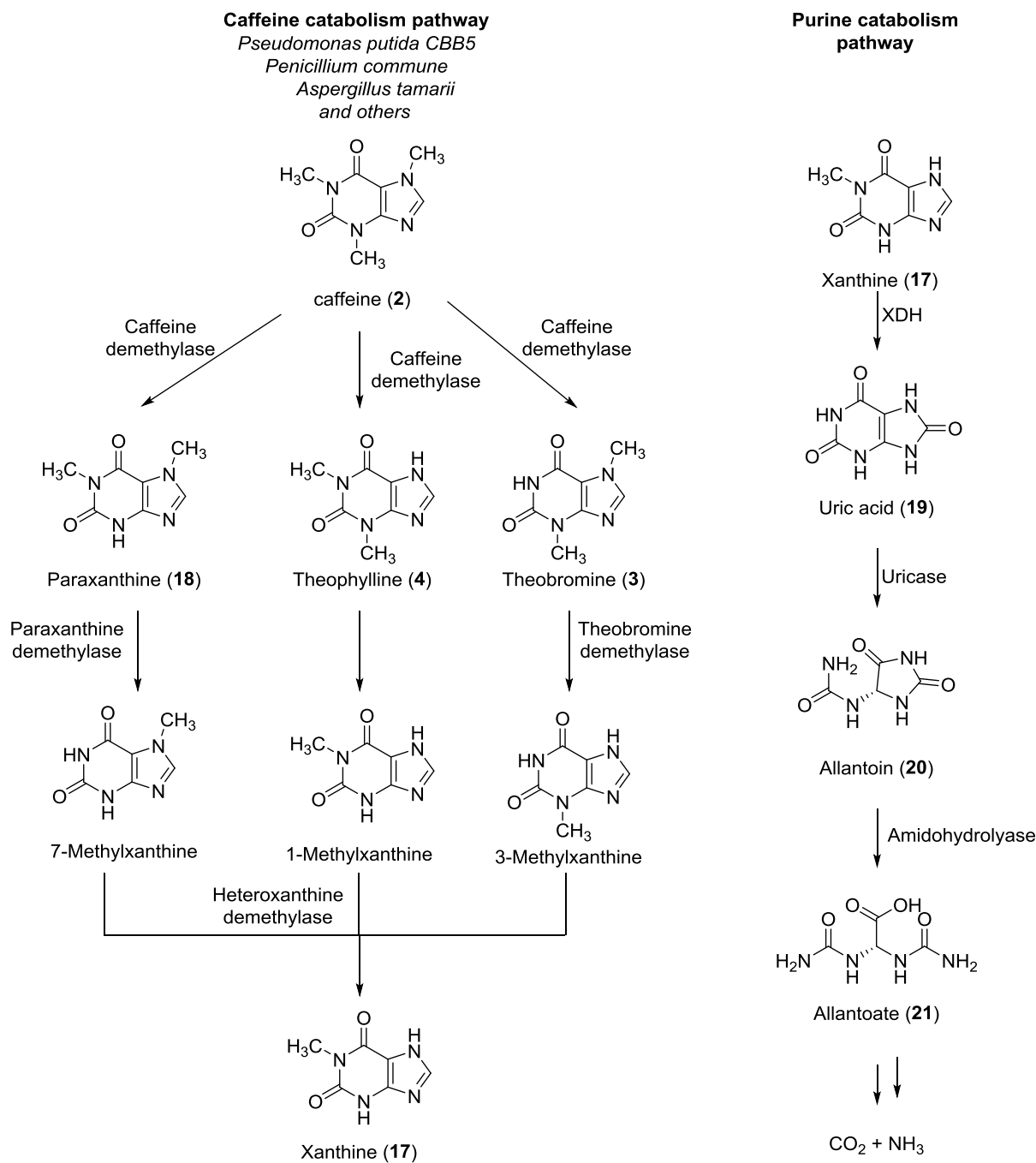


Table 1: Structure and occurrence of most important methylxanthines.

<i>Name</i>	<i>R¹</i>	<i>R²</i>	<i>R³</i>	<i>Occurrence</i>
<i>Xanthine</i> (17)	H	H	H	Product of the purine degradation pathway. Oxidation product of hypoxanthine by xanthine oxidase. ²⁴
<i>Paraxanthine</i> (18)	CH ₃	H	CH ₃	Paraxanthine is the main product of caffeine metabolism catalyzed by cytochrome P450 (subtypes 1A2 and 2E1). ²⁵
<i>Theobromine</i> (3)	H	CH ₃	CH ₃	Theobromine occurs mainly in cocoa products and in smaller amounts in tea. ²⁶⁻²⁸
<i>Theophylline</i> (4)	CH ₃	CH ₃	H	Theophylline is found in green coffee beans ²⁹ , in smaller amounts in black tea (<i>Camellia sinensis</i>) ²⁸ and in traces in cacao cotyledon and dried mate. ³⁰
<i>Caffeine</i> (2)	CH ₃	CH ₃	CH ₃	Caffeine can be found in more than 60 plant species including coffee, tea, cacao beans, guarana, mate, and kola nuts. ³¹

In the catabolism of caffeine (2) and other methylxanthines in plants the compounds are first degraded by demethylases to xanthine (17). Xanthine (17) is afterwards oxidized to uric acid (19) via xanthine oxidase or NAD⁺-dependent xanthine dehydrogenase. The uric acid (19) is then converted to allantoin (20) by an uricase via several intermediate steps. In the next step allantoin (20) is degraded into allantoate (21) catalyzed by an amidohydrolyase. After 21 has been converted to ureidoglycolate, it can be excreted as ammonia and CO₂ (Scheme 4).¹⁸⁻¹⁹



Scheme 4: Catabolism of caffeine (2) and other methylxanthines in bacteria and fungi. Caffeine (2) is first demethylated to xanthine (17) in the caffeine metabolism pathway and afterwards degraded during the purine catabolism pathway over several steps catalyzed by numerous enzymes and finally excreted as CO₂ and NH₃. The most important enzymes are as follows: demethylase enzymes (EC 1.13.12.-); XDH (xanthine dehydrogenase or xanthine oxidase, EC 1.17.3.2); uricase or uricate oxidase (EC 1.7.3.3); amidohydrolyase and ureases (EC 3.5.1.5).¹⁸⁻¹⁹

1.2.3. Pharmacology of methylxanthines

Caffeine (2) and theophylline (4) are known for their CNS stimulation and their diuretic and antiasthmatic effects. Caffeine (2) is a component of numerous beverages, such as coffee, tea or energy drinks, and is therefore considered the most consumed psychoactive substance in the world.³² The psychoactive reactions that can be triggered by cocoa, coffee and tea are mediated by the blockade of adenosine receptors (ARs) caused by these methylxanthines (Table 2).³³ ARs are activated by the important signaling molecules, the nucleoside adenosine (7). The AR family comprises four subtypes A₁-, A_{2A}-, A_{2B}- and A₃ARs, which belong to the large family of GPCRs.³⁴⁻³⁵ GPCRs are characterized by their 7 transmembrane α -helices and will be discussed in more detail in chapter 4.

The A₁AR is highly expressed in the brain³⁶ but also present in heart³⁷, kidney³⁸, adipose tissue³⁹ and pancreas. Adenosine (7) and most naturally occurring methylxanthines show high affinity for this receptor subtype (Table 2). The A₁AR is a G_{i/o} coupled receptor, and upon activation the A₁AR leads to an inhibition of adenylate cyclase (AC) activity resulting in decreased cAMP production. This leads to reduced protein kinase A (PKA) activity.

The A_{2A}AR is coupled to G_s and G_{olf} (olfactory neuron specific G protein). In peripheral tissues or in the brain, activation of A_{2A}AR increases AC activity and the increased cAMP concentration stimulates the cyclic AMP-protein kinase A (PKA) pathway. This leads to the phosphorylation of various proteins, ion channels and phosphodiesterases.^{34, 36, 40-41} Identified A₁AR-A_{2A}AR heterodimers might be involved in the regulation of neurotransmitter release.⁴² A deficiency of the neurotransmitter dopamine plays a decisive role in Parkinson's disease. Parkinson's disease (PD) is the second most common neurodegenerative disease after the Alzheimer's disease (AD) and is characterized by symptoms such as bradykinesia, akinesia, rigor and tremor. It is caused by a lack of dopamine in the brain due to a progressive degeneration of dopaminergic neurons in the *pars compacta* of the substantia nigra, possibly caused by an accumulation of misfolded α synuclein. Dopamine receptor agonists such as ropinirol are used to increase the dopamine level. But also the non-proteinogenic α -amino acid levodopa (L-DOPA), which is a precursor in the biosynthesis of messenger substances such as adrenalin and dopamine, plays an important role in the therapy of PD. Due to the colocalization of A_{2A} and D₂ receptors in the striatum and the adenosine-dopamine antagonism, A_{2A}AR antagonists are potential novel therapeutics for the treatment of PD, and the first drug with this mechanism of action, istradefylline, has been approved in Japan and the USA.⁴³

The A_{2B}AR shows a lower affinity for adenosine compared to the AR subtypes A₁ and A_{2A}. The receptor is ubiquitously expressed, but at low levels under physiological conditions. However, it can be significantly upregulated upon hypoxia, inflammation, and cancer. Blockade of the A_{2B}AR has been proposed for the treatment of asthma, cardiopulmonary disease and cancer.⁴⁰ The A_{2B}AR is a G_s and G_{q/11} coupled receptor and activation of the A_{2B}AR can increase AC and phospholipase C (PLC) activity.⁴¹

The A₃AR can be found in lung, kidneys, placenta, eye, microglia and astrocytes and inhibits AC activity via a G_iG_{q/11} coupling. The receptor is proposed to have cardioprotective and analgesic effects and might have an important antitumoral role.⁴⁴

Table 2: Adenosine receptor affinities of natural occurring xanthines.

<i>Natural xanthine derivatives</i>	<i>A₁</i> [nM]	<i>A_{2A}</i> [nM]	<i>A_{2B}</i> [nM]	<i>A₃</i> [nM]
<i>Caffeine (2)</i>	10,700 (h) ⁴⁵	23,400 (h) ⁴⁶	33,800 (h) ⁴⁷	13,300 (h) ⁴⁵
	44,900 (h) ⁴⁶	9,560 (h) ⁴⁵	10,400 (h) ⁴⁸	>100,000 (r) ⁴⁹
	41,000 (r) ⁵⁰	45,000 (r) ⁵¹	20,500 (h) ⁵²	
	44,000 (r) ⁵¹	32,500 (r) ⁵³	30,000 (r) ⁵⁴	
		48,000 (r) ⁴⁵	13,000 (m) ⁵⁴	
<i>Theophylline (4)</i>	6,770 (h) ⁵⁵	1,710 (h) ⁵⁵	9,070 (h) ⁴⁸	22,300 (h) ⁴⁵
	14,000 (r) ⁵⁶	6,700 (h) ⁴⁵	74,000 (h) ⁵²	86,400 (h) ⁵⁵
	8,740 (r) ⁴⁵	22,000 (r) ⁵⁶	15,100 (r) ⁴⁸	>100,000 (r) ⁴⁹
		25,300 (r) ⁴⁵	5,630 (m) ⁵⁷	85,000 (r) ⁵⁸
<i>Theobromine (3)</i>	105,000 (r) ⁵⁶	>250,000 (r) ⁵⁶	130,000 (h) ⁵⁹	>100,000 (r) ⁴⁹
	83,400 (r) ⁵⁹	187,000 (r) ⁵⁹		
<i>Paraxanthine (18)</i>	21,000 (r) ⁵⁶	32,000 (r) ⁵⁶	4,500 (h) ⁶⁰	>100,000 (r) ⁴⁹

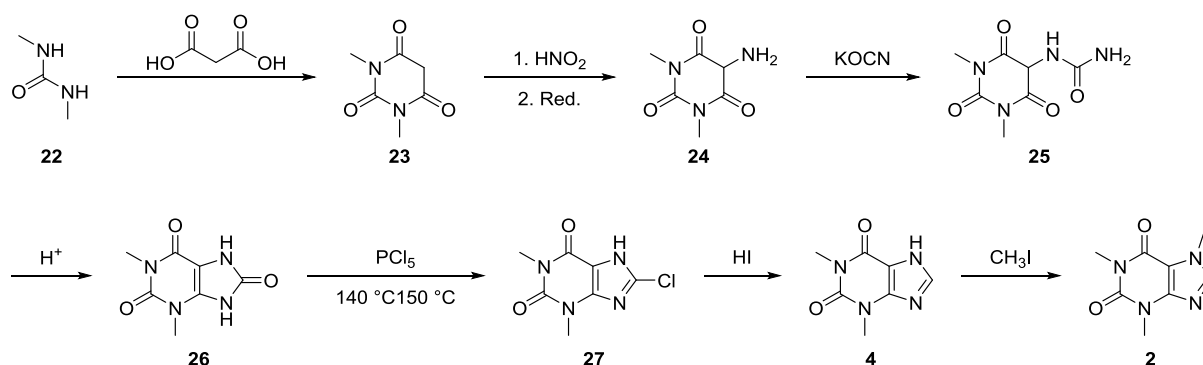
h: human, r: rat, m: mouse

Methylxanthines mainly act as antagonists for ARs but further mechanisms like phosphodiesterase inhibition, direct effects on ryanodine receptors inducing intracellular calcium release, modulation of GABA_A receptor activity, or histone deacetylase activation were described for caffeine and also suggested for other methylxanthines.^{33, 61}

1.2.4. Approved xanthine drugs

1.2.4.1. Caffeine and theophylline

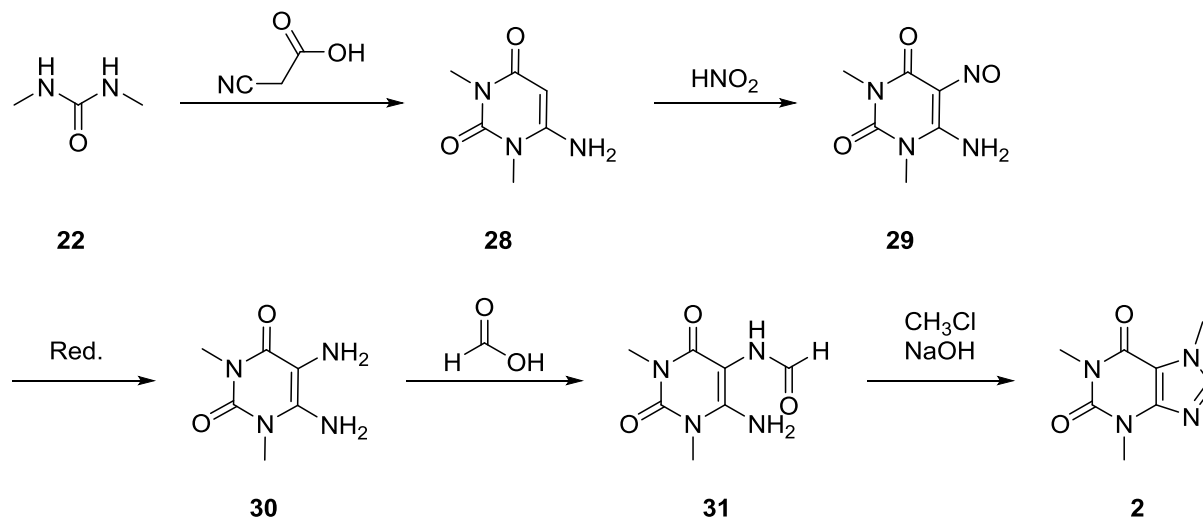
The first described synthesis of xanthine derivatives was that of caffeine (**2**) and theophylline (**4**) by Emil Fischer and Lorenz Ach. Emil Fischer was awarded with the Nobel Prize for Chemistry in 1902, based on his research on the synthesis of sugars and purines.⁶²⁻⁶⁴ The synthesis starts with an amide coupling reaction of 1,3-dimethylurea (**22**) with malonic acid yielding 1,3-dimethylbarbituric acid (**23**) followed by nitrosation and reduction to the 5-amino-1,3-dimethylbarbituric acid (**24**). Potassium cyanate is used to convert **24** to the urea derivative **25**. Cyclization reaction yields 1,3-dimethyluric acid (**26**). After chlorination (**27**) using PCl_5 and dehalogenation with HI the methylxanthine theophylline (**4**) can be obtained. Methylation of the *N7* position yielded the widely consumed stimulant caffeine (**2**, Scheme 5).



Scheme 5: Total synthesis of theophylline (**4**) and caffeine (**2**) by Emil Fischer.⁶²

Caffeine (**2**) is produced on a large scale both synthetically and by extraction. Decaffeination produces caffeine (**2**) as a by-product, which can then be resold. The industrial synthesis of caffeine (**2**) and theophylline (**4**) is based on the Traube purine synthesis (Scheme 5). The largest producers of caffeine (**2**) are companies such as CSPC and Shandong Xinhua from China, Kudos Chemie Limited from India and BASF for the European market. In 2018, the global caffeine market amounted to around US\$340 million and is expected to grow further in the coming years.⁶⁵ The synthesis also starts with ring closure reaction using 1,3-dimethylurea (**22**) and 2-cyanoacetic acid to obtain 6-amino-1,3-dimethyluracil (**28**). The next steps are quite similar as described for the first total synthesis of caffeine by Emil Fischer, including nitrosation to **29** followed by reduction to diaminouracil **30** and subsequent amide coupling to the 6-amino-

5-amido-uracil derivative **31**, which is further cyclized to caffeine (**2**) under basic condition (Scheme 6).



Scheme 6: Modification of the Traube purine synthesis for the large-scale production of caffeine (**2**).⁶⁶

Due to their biological activity, xanthine derivatives have gained increasing attention with regard to the development of new drugs. After Emil Fischer showed that the compound class could also be produced synthetically, numerous modifications were made to the basic structure of xanthine, on the one hand to increase biological activity, and on the other hand to increase the selectivity for specific targets. In order to highlight the medical significance of this class of compounds, a systematic analysis of the approved drugs and those in clinical trials was carried out. According to the DrugBank database, 12 xanthine derivatives are approved as drugs (Table 3) and another 13 are currently in clinical trials (data from February 2020). Further xanthine derivatives are approved in individual countries in the Far East or have a high significance in research and development. The FDA has assigned the suffix “fylline” to the theophylline and caffeine derivatives and analogs.

Henry Hyde Salter reported for the first time in 1859 about the effects of methylxanthines for the treatment of bronchoconstriction. Salter was an asthmatic and studied the effects of strong coffee on his respiratory symptoms.⁶⁷ After theophylline (**4**) was first used as a diuretic, various clinical studies were carried out by the US American David I. Macht in 1921⁶⁸ and by Samson Hirsch from Germany in 1922,⁶⁹ which showed that theophylline (**4**) is a much more effective agent for the relaxation of smooth muscles than caffeine (**2**).⁶⁷ Theophylline was clinically approved as ethylenediamine salt known as aminophylline (**32**) or in its pure form for asthma

treatment in 1922.⁷⁰ This makes theophylline (**4**) one of the oldest and for a long time most prescribed drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD) worldwide.⁷¹ However, due to its narrow safety window, it is nowadays not used as a first-line treatment anymore. Derivatives of theophylline (**4**) have also been approved over time, such as 8-bromotheophylline (**33**), the active ingredient in pamabrom[®], which is metabolized to theophylline (**4**) and then develops its weak diuretic action and can be used to treat primary dysmenorrhea and premenstrual syndrome.⁷² 8-Bromotheophylline (**33**) can be synthesized from theophylline (**4**) by the addition of bromine under acidic conditions.⁷³ Oxtriphylline (**34**) the choline salt of theophylline (**4**) is also used to relax the smooth muscles of bronchial and pulmonary vessels and to induce antiasthmatic effects.⁷⁴ Theophylline (**4**) and the other methylxanthines have a moderate water solubility and sometimes incompatibilities and unpleasant effects when administered.

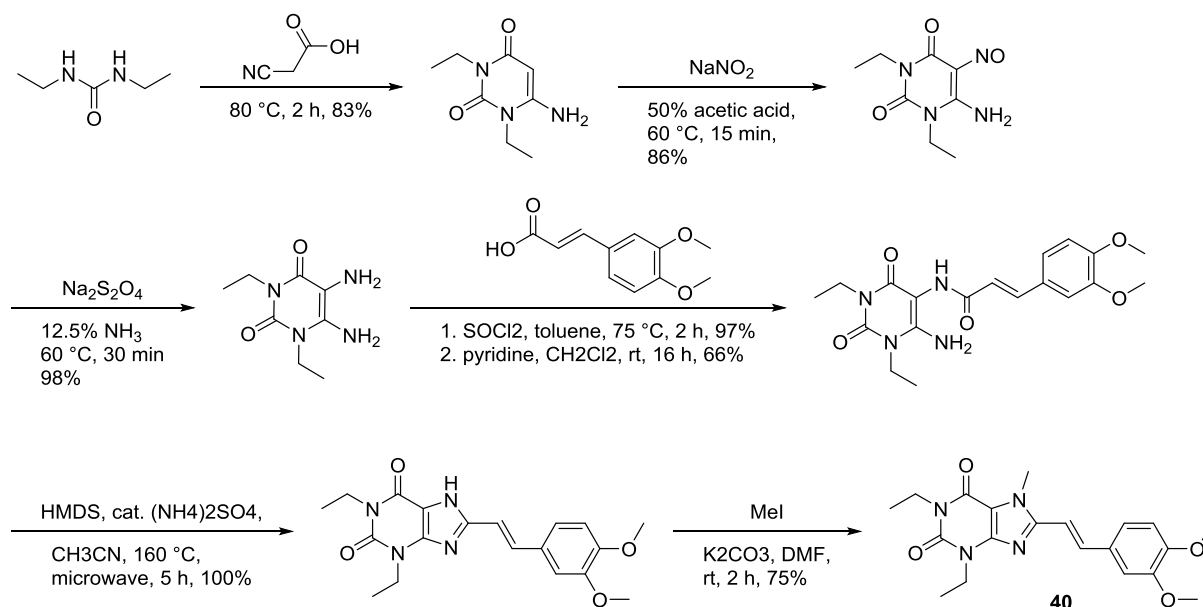
In 1946, the group around Maney⁷⁵ synthesized the *N*7-dihydroxypropyl-substituted theophylline derivative dyphylline (**35**) with good tolerability and less nausea compared to aminophylline (**32**). Xanthine derivative **35**, also known as diprophylline, is marketed under the trade name Dilor[®] (U.S.) for the treatment of respiratory diseases such as asthma, cardiac dyspnoea and bronchitis due to its bronchodilating and vasodilating effects.⁷⁶⁻⁷⁷

A further disadvantage of **4** is the possibility of severe CNS toxicity at high plasma concentrations.⁷⁸ In addition, the plasma half-life of **4** can vary considerably due to unpredictable metabolic rates.⁷⁹ In order to avoid the disadvantages of **4** mentioned above, further substitutions were made to the theophylline scaffold by Persson's group. Persson *et al.* developed the 3-propylxanthine (enprofylline, **36**) in 1981 and identified it as a clinical candidate due to its bronchodilator effects.⁸⁰

The replacement of the *N*1-methyl group by a 5-oxohexyl group resulted in the drug pentoxifylline (**37**), which was approved by the FDA in 1984 for the treatment of intermittent claudication, a symptom of peripheral arterial occlusive disease (PAD).⁸¹ Pentoxifylline acts primarily by inhibiting PDE, which leads to increased cAMP levels. This results in an increased synthesis of thromboxane and prostacyclin (prostaglandin I₂) leading to a reduction in blood viscosity and a decrease in the potential for platelet aggregation and thrombus formation.⁸²

By further functionalization of the *N*7-position of theophylline (**4**), other important drugs such as the vasodilator xanthinol (**38**) and the antihypertensive drug Akrinor[®] (**39**) a mixture of cafedrine and theodrenaline (20:1) were obtained.⁸³⁻⁸⁴

By derivatizing the C8-position of the xanthine scaffold it was possible to produce selective AR antagonists, such as the drug istradefylline (**40**) developed by Kyowa Kirin (Japan). After the FDA rejected approval in the USA in 2008, the drug was approved in Japan in 2013 as the first A_{2A}AR antagonist for the treatment of Parkinson's disease. Six years later, on 28 August 2019, istradefylline was finally approved by the FDA in the USA as NOURIANZ[®] on the basis of new clinical studies.

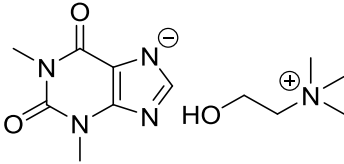
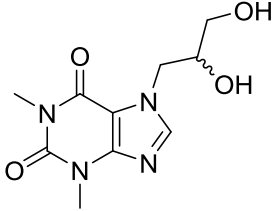
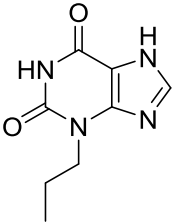
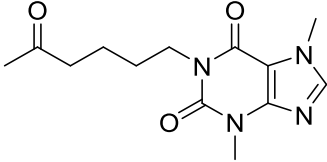
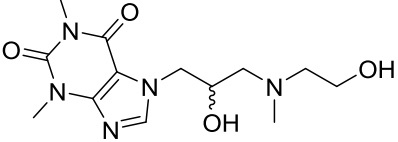


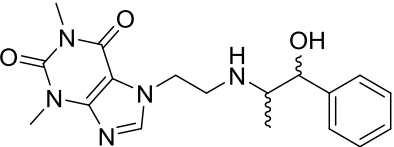
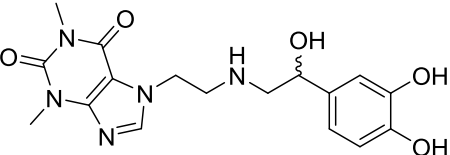
Scheme 7: Istradefylline (**40**) synthesis.⁸⁵⁻⁸⁶

Due to the variety of possibilities for derivatizing the xanthine scaffold, it has been possible to target other enzymes than PDEs and other receptors than ARs. In 2007, Boehringer Ingelheim developed linagliptin (**41**), a selective dipeptidyl peptidase 4 (DPP-4) inhibitor, which was approved in 2011 in Europe and the U.S for the treatment of type 2 diabetes.⁸⁷⁻⁸⁸ The drug was also approved in Germany but the Institute for Quality and Efficiency in Health Care (IQWiG) was unable to demonstrate any additional benefit compared to the alternative treatment options with metformin and sulfonylurea. The synthesis of Linagliptin (**41**) is shown in Scheme 8.⁸⁹

Table 3: Xanthine derivatives approved as drugs*

Name (Trade name)	Structure	Target	Disease
Caffeine (2) (Cafcit [®] , NoDoz [®] , Vivarin [®] , Revive [®] , Stay Awake [®] , Enerjets [®] , Lucidex [®])		Nonselective AR antagonist, PDE inhibitor and HDAC activator. ^{33, 61}	Caffeine citrate (Cafcit) has been shown to reduce the incidence of premature apnea. ⁹⁰⁻⁹¹ Furthermore, caffeine can be taken as a stimulant in capsule form (NoDoz, Vivarin,...). ⁹²
Theophylline (4) (Uniphyll [®] , Theochron [®])		Nonselective AR antagonist, PDE inhibitor and HDAC activator.	Theophylline is indicated for the treatment of chronic asthma and other chronic lung diseases such as emphysema and chronic bronchitis and remains the most widely prescribed anti-asthma drug worldwide. ⁹³⁻⁹⁴
17 Aminophylline (32) (Norphyl [®])		Nonselective AR antagonist ⁹⁵ , PDE Inhibitor (isoenzyme type III and IV) ⁹⁶ and HDAC activator ⁹⁷ .	Approved for asthma or other chronic lung diseases such as chronic bronchitis and emphysema to relieve the symptoms of reversible airway obstruction. Also approved for the prevention of apnea in premature infants. ⁹⁸
8-Bromotheophyllin (33) (Pamabrom [®])		Nonselective AR antagonist, PDE inhibitor and HDAC activator. ^{61, 99}	The weak diuretic pamabrom is effective in the treatment of primary dysmenorrhea and premenstrual syndrome. The active xanthine derivative of pamabromo is theophylline. It has been shown to relieve adenosine-induced angina-like chest pain, postdural puncture headache and pain during experimental ischaemia in humans. ^{72, 100-101}

Oxtriphylline (34) (Choledyl SA [®])		Nonselective AR antagonist PDE inhibitor and HDAC activator.	Oxtriphylline is the choline salt of theophylline and is converted into it after ingestion. Theophylline then relaxes the smooth muscles of the bronchial and pulmonary vessels and reduces the reaction of the airways to histamine, methacholine, adenosine and allergen. ¹⁰²⁻¹⁰³
Diphylline or Diprophylline (35) (Dilor [®] , Lufyllin [®])		Nonselective A ₁ -, A _{2A} AR antagonist and PDE inhibitor.	Used in the treatment of respiratory diseases such as asthma, heart dyspnoea and bronchitis due to its bronchodilator and vasodilator effects. ⁷⁶⁻⁷⁷
Enprofylline (36) (Nilyph [®])		Nonselective AR antagonist and PDE inhibitor.	Due to its bronchodilator effects it is used in the treatment of asthma. ¹⁰⁴⁻¹⁰⁵
Pentoxifylline (37) (Trental [®] , Pentoxil [®])		Nonselective AR antagonist PDE inhibitor and HDAC activator.	Pentoxifyllin increases the walking distance of patients with intermittent claudication compared to placebo or vasodilators. ¹⁰⁶
Xanthinol (38) (Complamina [®])		Increased synthesis and secretion of prostaglandin D ₂	Used for cerebrovascular disorders, peripheral vascular disease and other conditions. Xanthinol nicotinate is a peripheral vasodilator and, when injected intralesionally in OSMF patients, leads to increased mouth opening, tongue protrusion, cheek flexibility and relief of burning sensation. ⁸³

Cafedrine/Theodrenaline 20:1 (39)	<p data-bbox="622 196 734 225">Cafedrine</p> 	<p data-bbox="907 196 1187 225">Proposed interaction with</p> <p data-bbox="907 244 1187 363">β1-adrenoceptor, as PDE inhibitor and with α1-adrenoceptor¹⁰⁷</p>	Approved as an anti-hypotensive agent. ⁸⁴
	<p data-bbox="678 399 701 414">+</p> <p data-bbox="604 435 766 464">Theodrenaline</p> 		

*Unless otherwise stated, the data were taken from the DrugBank database.

1.3. References

- (1) Dias, D. A.; Urban, S.; Roessner, U. A historical overview of natural products in drug discovery. *Metabolites* **2012**, *2* (2), 303–336.
- (2) Jones, A. W. Early drug discovery and the rise of pharmaceutical chemistry. *Drug Test. Anal.* **2011**, *3* (6), 337–344.
- (3) Hughes, J. P.; Rees, S.; Kalindjian, S. B.; Philpott, K. L. Principles of early drug discovery. *Br. J. Pharmacol.* **2011**, *162* (6), 1239–1249.
- (4) Santos, R.; Ursu, O.; Gaulton, A.; Bento, A. P.; Donadi, R. S.; Bologa, C. G.; Karlsson, A.; Al-Lazikani, B.; Hersey, A.; Oprea, T. I. A comprehensive map of molecular drug targets. *Nat. Rev. Drug Discov.* **2017**, *16* (1), 19–34.
- (5) Clare, R. H.; Bardelle, C.; Harper, P.; Hong, W. D.; Börjesson, U.; Johnston, K. L.; Collier, M.; Myhill, L.; Cassidy, A.; Plant, D. Industrial scale high-throughput screening delivers multiple fast acting macrofilaricides. *Nat. Commun.* **2019**, *10* (1), 1–8.
- (6) Leelananda, S. P.; Lindert, S. Computational methods in drug discovery. *Beilstein J. Org. Chem.* **2016**, *12* (1), 2694–2718.
- (7) Umscheid, C. A.; Margolis, D. J.; Grossman, C. E. Key concepts of clinical trials: a narrative review. *Postgrad. Med.* **2011**, *123* (5), 194–204.
- (8) Prasad, V.; Mailankody, S. Research and development spending to bring a single cancer drug to market and revenues after approval. *JAMA Intern. Med.* **2017**, *177* (11), 1569–1575.
- (9) G de la Torre, B.; Albericio, F. The pharmaceutical industry in 2018. An analysis of FDA drug approvals from the perspective of molecules. *Molecules* **2019**, *24* (4), 809.
- (10) Thomas Morrow; Felcone, L. H. Defining the difference: what makes biologics unique. *Biotechnol. Healthc.* **2004**, *1* (4), 24–26, 28–29.
- (11) Beall, R. F.; Hwang, T. J.; Kesselheim, A. S. Pre-market development times for biologic versus small-molecule drugs. *Nat. Biotechnol.* **2019**, *37* (7), 708–711.
- (12) Wink, M. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theor. Appl. Genet.* **1988**, *75* (2), 225–233.

- (13) Maplestone, R. A.; Stone, M. J.; Williams, D. H. The evolutionary role of secondary metabolites — a review. *Gene* **1992**, *115* (1), 151–157.
- (14) Schäfer, H.; Wink, M. Medicinally important secondary metabolites in recombinant microorganisms or plants: Progress in alkaloid biosynthesis. *Biotechnol. J.* **2009**, *4* (12), 1684–1703.
- (15) Friedrich, C.; Von Domarus, C. Carl Friedrich Wilhelm Meissner (1792-1853)--pharmacist and alkaloid researcher. *Pharmazie* **1998**, *53* (1), 67–73.
- (16) Alves de Almeida, A. C.; de-Faria, F. M.; Dunder, R. J.; Manzo, L. P. B.; Souza-Brito, A. R. M.; Luiz-Ferreira, A. Recent trends in pharmacological activity of alkaloids in animal colitis: potential use for inflammatory bowel disease. *Evid. Based Complement Alternat. Med.* **2017**, *2017*, 8528210.
- (17) Monteiro, J. P.; Alves, M. G.; Oliveira, P. F.; Silva, B. M. Structure-bioactivity relationships of methylxanthines: trying to make sense of all the promises and the drawbacks. *Molecules* **2016**, *21* (8), 974.
- (18) Ashihara, H.; Kato, M.; Crozier, A. Distribution, biosynthesis and catabolism of methylxanthines in plants. *Handb. Exp. Pharmacol.* **2011**, (200), 11–31.
- (19) Ashihara, H.; Crozier, A. Biosynthesis and metabolism of caffeine and related purine alkaloids in plants. *Adv. Bot. Res.* **1999**, *30*, 117–205.
- (20) Hollingsworth, R. G.; Armstrong, J. W.; Campbell, E. Pest control: caffeine as a repellent for slugs and snails. *Nature* **2002**, *417* (6892), 915–916.
- (21) Anaya, A. L.; Cruz-Ortega, R.; Waller, G. R. Metabolism and ecology of purine alkaloids. *Front. Biosci.* **2006**, *11*, 2354–70.
- (22) Kim, Y.-S.; Uefuji, H.; Ogita, S.; Sano, H. Transgenic tobacco plants producing caffeine: a potential new strategy for insect pest control. *Transgenic Res.* **2006**, *15* (6), 667–672.
- (23) Uefuji, H.; Tatsumi, Y.; Morimoto, M.; Kaothien-Nakayama, P.; Ogita, S.; Sano, H. Caffeine production in tobacco plants by simultaneous expression of three coffee *N*-methyltransferases and its potential as a pest repellent. *Plant Mol. Biol.* **2005**, *59* (2), 221–227.
- (24) Brychkova, G.; Fluhr, R.; Sagi, M. Formation of xanthine and the use of purine metabolites as a nitrogen source in Arabidopsis plants. *Plant Signal. Behav.* **2008**, *3* (11), 999–1001.

- (25) Guerreiro, S.; Toulorge, D.; Hirsch, E.; Marien, M.; Sokoloff, P.; Michel, P. P. Paraxanthine, the primary metabolite of caffeine, provides protection against dopaminergic cell death via stimulation of ryanodine receptor channels. *Mol. Pharmacol.* **2008**, *74* (4), 980–989.
- (26) Stavric, B. Methylxanthines: toxicity to humans. 3. Theobromine, paraxanthine and the combined effects of methylxanthines. *Food Chem. Toxicol.* **1988**, *26* (8), 725–733.
- (27) Balentine, D. A.; Harbowy, M. E.; Graham, H. N. Tea: the plant and its manufacture; chemistry and consumption of the beverage. *Prog. Clin. Biol. Res.* **1998**, *158*, 29–74.
- (28) Graham, H. Tea: the plant and its manufacture; chemistry and consumption of the beverage. *Prog. Clin. Biol. Res.* **1984**, *158*, 29–74.
- (29) Spiller, M. A. The chemical components of coffee. *Prog. Clin. Biol. Res.* **1984**, *158*, 97–147.
- (30) Shively, C.; Tarka, J. S. Methylxanthine composition and consumption patterns of cocoa and chocolate products. *Prog. Clin. Biol. Res.* **1984**, *158*, 149–178.
- (31) Willson, C. The clinical toxicology of caffeine: a review and case study. *Toxicol. Rep.* **2018**, *5*, 1140–1152.
- (32) Lisko, J. G.; Lee, G. E.; Kimbrell, J. B.; Rybak, M. E.; Valentin-Blasini, L.; Watson, C. H. Caffeine concentrations in coffee, tea, chocolate, and energy drink flavored e-liquids. *Nicotine Tob. Res.* **2016**, *19* (4), 484–492.
- (33) Franco, R.; Oñatibia-Astibia, A.; Martínez-Pinilla, E. Health benefits of methylxanthines in cacao and chocolate. *Nutrients* **2013**, *5* (10), 4159–4173.
- (34) Baraldi, P. G.; Tabrizi, M. A.; Gessi, S.; Borea, P. A. Adenosine receptor antagonists: translating medicinal chemistry and pharmacology into clinical utility. *Chem. Rev.* **2008**, *108* (1), 238–263.
- (35) Müller, C.; Jacobson, K. A. Xanthines as adenosine receptor antagonists. *Handb. Exp. Pharmacol.* **2011**, (200), 151–99.
- (36) Chen, J.-F.; Eltzhig, H. K.; Fredholm, B. B. Adenosine receptors as drug targets—what are the challenges? *Nat. Rev. Drug Discov.* **2013**, *12* (4), 265–286.
- (37) Li, N.; Csepe, T. A.; Hansen, B. J.; Sul, L. V.; Kalyanasundaram, A.; Zakharkin, S. O.; Zhao, J.; Guha, A.; Van Wagoner, D. R.; Kilic, A. Adenosine-induced atrial fibrillation: localized reentrant drivers in lateral right atria due to heterogeneous expression of adenosine A₁ receptors and GIRK4 subunits in the human heart. *Circulation* **2016**, *134* (6), 486–498.

- (38) Kim, M.; Chen, S. W.; Park, S. W.; Kim, M.; D'Agati, V. D.; Yang, J.; Lee, H. T. Kidney-specific reconstitution of the A₁ adenosine receptor in A₁ adenosine receptor knockout mice reduces renal ischemia–reperfusion injury. *Kidney Int.* **2009**, *75* (8), 809–823.
- (39) Barakat, H.; Davis, J.; Lang, D.; Mustafa, S. J.; McConnaughey, M. M. Differences in the expression of the adenosine A₁ receptor in adipose tissue of obese black and white women. *J. Clin. Endocrinol. Metab.* **2006**, *91* (5), 1882–1886.
- (40) Baraldi, P. G.; Tabrizi, M. A.; Gessi, S.; Borea, P. A. Adenosine receptor antagonists: translating medicinal chemistry and pharmacology into clinical utility. *Chemical Reviews* **2008**, *108* (1), 238–263.
- (41) Borea, P. A.; Gessi, S.; Merighi, S.; Vincenzi, F.; Varani, K. Pharmacology of adenosine receptors: the state of the art. *Physiol. Rev.* **2018**, *98* (3), 1591–1625.
- (42) Ciruela, F.; Ferré, S.; Casado, V.; Cortés, A.; Cunha, R.; Lluís, C.; Franco, R. Heterodimeric adenosine receptors: a device to regulate neurotransmitter release. *Cell. Mol. Life Sci.* **2006**, *63* (21), 2427–2431.
- (43) Cieślak, M.; Komoszyński, M.; Wojtczak, A. Adenosine A_{2A} receptors in Parkinson's disease treatment. *Purinergic Signal.* **2008**, *4* (4), 305–312.
- (44) Borea, P. A.; Varani, K.; Vincenzi, F.; Baraldi, P. G.; Tabrizi, M. A.; Merighi, S.; Gessi, S. The A₃ adenosine receptor: history and perspectives. *Pharmacol. Rev.* **2015**, *67* (1), 74–102.
- (45) Jacobson, K. A.; Ijzerman, A. P.; Linden, J. 1, 3-Dialkylxanthine derivatives having high potency as antagonists at human A_{2B} adenosine receptors. *Drug Dev. Res.* **1999**, *47* (1), 45–53.
- (46) Abo-Salem, O. M.; Hayallah, A. M.; Bilkei-Gorzo, A.; Filipek, B.; Zimmer, A.; Müller, C. E. Antinociceptive effects of novel A_{2B} adenosine receptor antagonists. *J. Pharmacol. Exp. Ther.* **2004**, *308* (1), 358–366.
- (47) Borrmann, T.; Hinz, S.; Bertarelli, D. C.; Li, W.; Florin, N. C.; Scheiff, A. B.; Müller, C. E. 1-Alkyl-8-(piperazine-1-sulfonyl) phenylxanthines: development and characterization of adenosine A_{2B} receptor antagonists and a new radioligand with subnanomolar affinity and subtype specificity. *J. Med. Chem* **2009**, *52* (13), 3994–4006.
- (48) Kim, S.-A.; Marshall, M. A.; Melman, N.; Kim, H. S.; Müller, C. E.; Linden, J.; Jacobson, K. A. Structure–activity relationships at human and rat A_{2B} adenosine receptors of xanthine derivatives substituted at the 1-, 3-, 7-, and 8-positions. *J. Med. Chem* **2002**, *45* (11), 2131–2138.

(49) Van Galen, P. J.; VAN BERGEN, A. H.; Gallo-Rodriguez, C.; Melman, N.; Olah, M. E.; IJzerman, A. P.; Stiles, G. L.; Jacobson, K. A. A binding site model and structure-activity relationships for the rat A₃ adenosine receptor. *Mol. Pharmacol.* **1994**, *45* (6), 1101–1111.

(50) Grahner, B.; Winiwarter, S.; Lanzner, W.; Mueller, C. E. Synthesis and structure-activity relationships of deazaxanthines: analogs of potent A₁- and A₂-adenosine receptor antagonists. *J. Med. Chem.* **1994**, *37* (10), 1526–1534.

(51) Daly, J. W.; Hide, I.; Müller, C. E.; Shamim, M. Caffeine analogs: structure-activity relationships at adenosine receptors. *Pharmacology* **1991**, *42* (6), 309–321.

(52) Bertarelli, D. C.; Diekmann, M.; Hayallah, A. M.; Rüsing, D.; Iqbal, J.; Preiss, B.; Verspohl, E. J.; Müller, C. E. Characterization of human and rodent native and recombinant adenosine A_{2B} receptors by radioligand binding studies. *Purinergic Signal.* **2006**, *2* (3), 559–571.

(53) Müller, C. E.; Maurinsh, J.; Sauer, R. Binding of [³H] MSX-2 (3-(3-hydroxypropyl)-7-methyl-8-(m-methoxystyryl)-1-propargylxanthine) to rat striatal membranes—a new, selective antagonist radioligand for A_{2A} adenosine receptors. *Eur. J. Pharm. Sci.* **2000**, *10* (4), 259–265.

(54) Brackett, L. E.; Daly, J. W. Functional characterization of the A_{2B} adenosine receptor in NIH 3T3 fibroblasts. *Biochem. Pharmacol.* **1994**, *47* (5), 801–814.

(55) Klotz, K.-N.; Hessling, J.; Hegler, J.; Owman, C.; Kull, B.; Fredholm, B.; Lohse, M. Comparative pharmacology of human adenosine receptor subtypes—characterization of stably transfected receptors in CHO cells. *Naunyn Schmiedebergs Arch. Pharmacol.* **1997**, *357* (1), 1–9.

(56) Mueller, C. E.; Shi, D.; Manning Jr, M.; Daly, J. W. Synthesis of paraxanthine analogs (1, 7-disubstituted xanthines) and other xanthines unsubstituted at the 3-position: structure-activity relationships at adenosine receptors. *J. Med. Chem.* **1993**, *36* (22), 3341–3349.

(57) Auchampach, J. A.; Kreckler, L. M.; Wan, T. C.; Maas, J. E.; van der Hoeven, D.; Gizewski, E.; Narayanan, J.; Maas, G. E. Characterization of the A_{2B} adenosine receptor from mouse, rabbit, and dog. *J. Pharmacol. Exp. Ther.* **2009**, *329* (1), 2–13.

(58) Jacobson, K. A.; Fischer, B.; Ji, X.-d. "Cleavable Trifunctional" approach to receptor affinity labeling: regeneration of binding to A₁-adenosine receptors. *Bioconjug. Chem.* **1995**, *6* (3), 255–263.

(59) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A₂ adenosine receptor labeled by [³H] NECA in rat striatal membranes. *Mol. Pharmacol.* **1986**, *29* (4), 331–346.

(60) Bruns, R. F. Adenosine antagonism by purines, pteridines and benzopteridines in human fibroblasts. *Biochem. Pharmacol.* **1981**, *30* (4), 325–333.

(61) Singh, N.; Shreshtha, A. K.; Thakur, M.; Patra, S. Xanthine scaffold: scope and potential in drug development. *Heliyon* **2018**, *4* (10), e00829.

(62) Kunz, H. Emil Fischer—unequalled classicist, master of organic chemistry research, and inspired trailblazer of biological chemistry. *Angew. Chem. Int. Ed.* **2002**, *41* (23), 4439–4451.

(63) Fischer, E. Ueber die Verbindungen der Zucker mit den Alkoholen und Ketonen. *Ber. Dtsch. Chem. Ges* **1895**, *28*, 1145–1167.

(64) Fischer, E. Einfluss der Salzbildung auf die Verseifung von Amidn und Estern durch Alkalien. *Ber. Dtsch. Chem. Ges* **1907**, 479–491.

(65) Research Reports World. Caffeine market 2019 industry size, share, demands, sales, trends, global growth, insights and forecast research report 2025. *Research Reports World* **2019**, <https://www.marketwatch.com/press-release/caffeine-market-2019-industry-size-share-demands-sales-trends-global-growth-insights-and-forecast-research-report-2025-2019-02-19>.

(66) Campbell, K. N.; Campbell, B. K. Method of preparing xanthine and methylated xanthines. *US2523496A*, **1950**.

(67) Persson, C. On the medical history of xanthines and other remedies for asthma: a tribute to HH Salter. *Thorax* **1985**, *40* (12), 881–886.

(68) Macht, D. I.; Ting, G.-C. A study of antispasmodic drugs on the bronchus. *J. Pharmacol. Exp. Ther.* **1921**, *18*, 373–398.

(69) Hirsch, S. Klinischer und experimenteller Beitrag zur krampflösenden Wirkung der Purinderivate. *Klin. Wschr.* **1922**, *1* (13), 615–618.

(70) Barnes, P. J. Theophylline. *Am. J. Respir. Crit. Care Med.* **2013**, *188* (8), 901–906.

(71) Battaglia, J. Pharmacological management of acute agitation. *Drugs* **2005**, *65* (9), 1207–1222.

(72) Ortiz, M. I.; Murguía-Cánovas, G.; Vargas-López, L. C.; Silva, R.; González-de la Parra, M. Naproxen, paracetamol and pamabrom versus paracetamol, pyrilamine and pamabrom in primary dysmenorrhea: a randomized, double-blind clinical trial. *Medwave* **2016**, *16* (9), e6587.

(73) Favier, C.; Pinhas, H.; Beranger, S.; Pascal, J.-C. Piperazine derivatives of theophylline. *EP0033674A3*, **1983**.

(74) Fox, R. W.; Samaan, S. S.; Lockey, R. F.; Bukantz, S. C. Study of oxtriphylline SA in 50 asthmatics. *J. Asthma* **1983**, *20* (3), 177–181.

(75) Maney, P. V.; Jones, J. W.; Gross, E.; Korn, H. Dihydroxypropyl theophylline: Its preparation and pharmacological and clinical study. *J. Am. Pharm. Assoc. Am. Pharm. Assoc.* **1946**, *35* (9), 266–272.

(76) Schwabe, U.; Ukena, D.; Lohse, M. J. Xanthine derivatives as antagonists at A₁ and A₂ adenosine receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1985**, *330* (3), 212–221.

(77) Iancu, L.; Shneur, A.; Cohen, H. Trials with xanthine derivatives in systemic treatment of psoriasis. *Dermatologica* **1979**, *159* (1), 55–61.

(78) Persson, C. G.; Erjefält, I. Convulsive effect of theophylline in conscious and anaesthetized guinea-pigs. *Acta Pharmacol. Toxicol.* **1981**, *48* (1), 32–38.

(79) Jenne, J. W.; Nagasawa, H. T.; Thompson, R. D. Relationship of urinary metabolites of theophylline to serum theophylline levels. *Clin. Pharmacol. Ther.* **1976**, *19* (3), 375–381.

(80) Persson, C.; Kjellin, G. Enprofylline, a principally new antiasthmatic xanthine. *Acta Pharmacol. Toxicol.* **1981**, *49* (4), 313–316.

(81) Gillings, D. B. Pentoxifylline and intermittent claudication: review of clinical trials and cost-effectiveness analyses. *J. Cardiovasc. Pharmacol.* **1995**, *25*, S44–50.

(82) Ward, A.; Clissold, S. P. Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* **1987**, *34* (1), 50–97.

(83) Singh, U. Efficacy and safety of intralesional xantinol nicotinate in the treatment of various stages of oral submucous fibrosis. *J. Clin. Diagn. Res.* **2016**, *10* (10), ZC34–ZC37.

(84) Heller, A. R.; Heger, J.; de Abreu, M. G.; Müller, M. Cafedrine/theodrenaline in anaesthesia. *Anaesthesist* **2015**, *64* (3), 190–196.

(85) 王雪根何凌云史全全金皓洁汪洋夏珺妤 Istradefylline synthesis process. *CN104974157A*, **2014**.

(86) Jörg, M.; Shonberg, J.; Mak, F. S.; Miller, N. D.; Yuriev, E.; Scammells, P. J.; Capuano, B. Novel adenosine A_{2A} receptor ligands: A synthetic, functional and computational

investigation of selected literature adenosine A_{2A} receptor antagonists for extending into extracellular space. *Bioorg. Med. Chem. Lett.* **2013**, 23 (11), 3427-3433.

(87) Eckhardt, M.; Langkopf, E.; Mark, M.; Tadayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P. 8-(3-(*R*)-Aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3, 7-dihydropurine-2, 6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2007**, 50 (26), 6450–6453.

(88) McGill, J. B. Linagliptin for type 2 diabetes mellitus: a review of the pivotal clinical trials. *Ther. Adv. Endocrinol. Metab.* **2012**, 3 (4), 113–124.

(89) Dong, W.; Huang, Y.; Su, S.; Sun, Y.; Zhang, P. Process for the preparation of a xanthine-based compound. *WO2016207364A1*, **2016**.

(90) Erenberg, A.; Leff, R. D.; Haack, D. G.; Mosdell, K. W.; Hicks, G. M.; Wynne, B. A.; Group, C. C. S. Caffeine citrate for the treatment of apnea of prematurity: a double-blind, placebo-controlled study. *Pharmacotherapy* **2000**, 20 (6), 644–652.

(91) Comer, A. M.; Perry, C. M.; Figgitt, D. P. Caffeine citrate. *Paediatr. Drugs* **2001**, 3 (1), 61–79.

(92) Cappelletti, S.; Daria, P.; Sani, G.; Aromatario, M. Caffeine: cognitive and physical performance enhancer or psychoactive drug? *Curr. Neuropharmacol.* **2015**, 13 (1), 71–88.

(93) Barnes, P.; Pauwels, R. Theophylline in the management of asthma: time for reappraisal? *Eur. Respir. J.* **1994**, 7 (3), 579–591.

(94) Mahemuti, G.; Zhang, H.; Li, J.; Tielwaerdi, N.; Ren, L. Efficacy and side effects of intravenous theophylline in acute asthma: a systematic review and meta-analysis. *Drug Des. Devel. Ther.* **2018**, 12, 99–120.

(95) Cvietusa, P.; Mascali, J. J.; Negri, J.; Borish, L. Anti-inflammatory effects of theophylline: modulation of cytokine production. *Ann. Allergy Asthma Immunol.* **1996**, 77 (1), 34–38.

(96) Rabe, K.; Magnussen, H.; Dent, G. Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants. *Eur. Respir. J.* **1995**, 8 (4), 637–642.

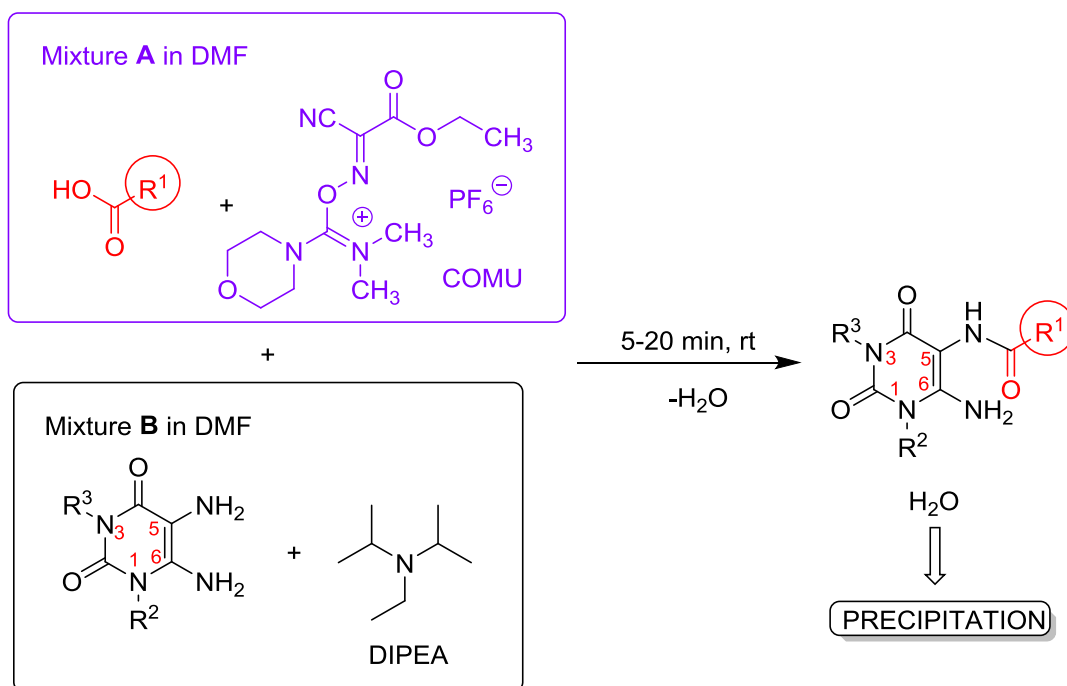
(97) Ranjani, R.; Vinotha, A. S. A prospective randomized controlled study: theophylline on oxidative stress and steroid sensitivity in chronic obstructive pulmonary disease patients. *Int. J. Pharm. Investig.* **2017**, 7 (3), 119–124.

- (98) Armanian, A. M.; Badiie, Z.; Afghari, R.; Salehimehr, N.; Hassanzade, A.; Sheikhzadeh, S.; Tehrani, M. S.; Rezvan, G. Prophylactic aminophylline for prevention of apnea at higher-risk preterm neonates. *Iran. Red. Crescent Med. J.* **2014**, *16* (8), e12559.
- (99) Cosio, B. G.; Tsaprouni, L.; Ito, K.; Jazrawi, E.; Adcock, I. M.; Barnes, P. J. Theophylline restores histone deacetylase activity and steroid responses in COPD macrophages. *J. Exp. Med.* **2004**, *200* (5), 689–695.
- (100) Rao, S. S.; Mudipalli, R. S.; Remes-Troche, J. M.; Utech, C. L.; Zimmerman, B. Theophylline improves esophageal chest pain—a randomized, placebo-controlled study. *Am. J. Gastroenterol.* **2007**, *102* (5), 930–938.
- (101) Mahoori, A.; Hassani, E.; Noroozinia, H.; Javaheri, N.; Hatami, S. Theophylline versus acetaminophen in the treatment of post-dural puncture headache (PDPH). *Middle East J. Anaesthesiol.* **2013**, *22* (3), 289–292.
- (102) Duesel, B.; Fand, T. Choline theophyllinate, a new stable theophylline derivative of enhanced solubility. *Int. Rec. Med. Gen. Pract. Clin.* **1954**, *167* (5), 245–247.
- (103) Brown, E.; Clancy, R. The use of choline theophyllinate in asthmatic patients. *Ann. Allergy.* **1955**, *13* (5), 543–550.
- (104) Laursen, L. C. Anti asthmatic effects and pharmacokinetics of enprofylline—a new xanthine derivate. *Dan. Med. Bull.* **1987**, *34* (6), 289–297.
- (105) Lunell, E.; Svedmyr, N.; Andersson, K. E.; Persson, C. G. Effects of enprofylline, a xanthine lacking adenosine receptor antagonism, in patients with chronic obstructive lung disease. *Eur. J. Clin. Pharmacol.* **1982**, *22* (5), 395–402.
- (106) Aviado, D. M.; Porter, J. M. Pentoxifylline: a new drug for the treatment of intermittent claudication; mechanism of action, pharmacokinetics, clinical efficacy and adverse effects. *Pharmacotherapy* **1984**, *4* (6), 297–306.
- (107) Bein, B.; Christ, T.; Eberhart, L. H. Cafedrine/Theodrenaline (20: 1) is an established alternative for the management of arterial hypotension in Germany—a review based on a systematic literature search. *Front. Pharmacol.* **2017**, *8*, 68.

1. Article I: Fast, Efficient, and Versatile Synthesis of 6-Amino-5-carboxamidouracils as Precursors for 8-Substituted Xanthines

Daniel Marx, Lukas M. Wingen, Gregor Schnakenburg, Christa E. Müller* and Matthias S. Scholz

The characterization and synthesis of methylxanthines originated at the end of the 19th century through work by Emil Fischer and Lorenz Ach. Today the synthesis of xanthine derivatives is mostly performed by nitrosylation of the 5-position of 6-aminouracils with subsequent reduction to the corresponding 5,6-diaminouracil derivatives. These intermediates can be selectively coupled with carboxylic acids to the corresponding 5-amidouracils thereby introducing different substitution patterns at the 8-position of the final xanthine scaffold. This coupling reaction is often performed by using hazardous chlorinating reagents to convert the carboxylic acids into the activated carboxylic acid chlorides or using the irritant and moisture-sensitive 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl). These reactions show several drawbacks, because, on the one hand, long reaction times are required, and, on the other hand, the yields are only moderate. In the following article a novel, fast and versatile synthesis without the necessity of hazardous coupling or chlorinating reagents to obtain 5-amido-6-aminouracils is described.



The coupling reagent COMU proved to be a safe and convenient alternative to the classical coupling reagents. COMU contains no explosive benzotriazole moiety and is characterized by high solubility and stability in various solvents. It can be easily removed from the system, since only water-soluble by-products are generated. With the described method, significantly accelerated reaction times of only five to ten minutes with quantitative yields and 99% purity were achieved without the requirement of complex column chromatographic purification procedures.



Fast, Efficient, and Versatile Synthesis of 6-amino-5-carboxamidouracils as Precursors for 8-Substituted Xanthines

Daniel Marx^{1†}, Lukas M. Wingen^{1†}, Gregor Schnakenburg², Christa E. Müller^{1*} and Matthias S. Scholz¹

¹ Pharmaceutical Chemistry 1, Pharmaceutical Institute, University of Bonn, Bonn, Germany, ² Department of Chemistry, Institute of Inorganic Chemistry, University of Bonn, Bonn, Germany

OPEN ACCESS

Edited by:

Naohiko Yoshikai,
Nanyang Technological University,
Singapore

Reviewed by:

Mariafrancesca Fochi,
University of Bologna, Italy
Indubhusan Deb,
Indian Institute of Chemical Biology
(CSIR), India

*Correspondence:

Christa E. Müller
christa.mueller@uni-bonn.de

[†]These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Organic Chemistry,
a section of the journal
Frontiers in Chemistry

Received: 15 November 2018

Accepted: 21 January 2019

Published: 18 February 2019

Citation:

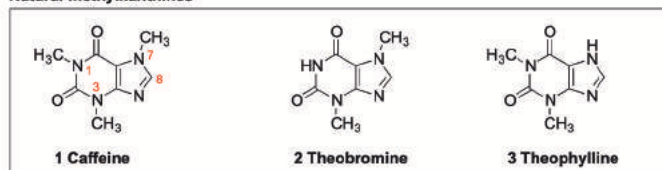
Marx D, Wingen LM,
Schnakenburg G, Müller CE and
Scholz MS (2019) Fast, Efficient, and
Versatile Synthesis of 6-amino-5-
carboxamidouracils as Precursors for
8-Substituted Xanthines.
Front. Chem. 7:56.
doi: 10.3389/fchem.2019.00056

Substituted xanthine derivatives are important bioactive molecules. Herein we report on a new, practical synthesis of 6-amino-5-carboxamidouracils, the main building blocks for the preparation of 8-substituted xanthines, by condensation of 5,6-diaminouracil derivatives and various carboxylic acids using the recently developed non-hazardous coupling reagent COMU (1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholinomethylene)]methanaminium hexafluoro phosphate). Optimized reaction conditions led to the precipitation of pure products after only 5 to 10 min of reaction time. The method tolerates a variety of substituted 5,6-diaminouracil and carboxylic acid derivatives as starting compounds resulting in most cases in more than 80% isolated yield. Regioselectivity of the reaction yielding only the 5-carboxamido-, but not the 6-carboxamidouracil derivatives, was unambiguously confirmed by single X-ray crystallography and multidimensional NMR experiments. The described method represents a convenient, fast access to direct precursors of 8-substituted xanthines under mild conditions without the necessity of hazardous coupling or chlorinating reagents.

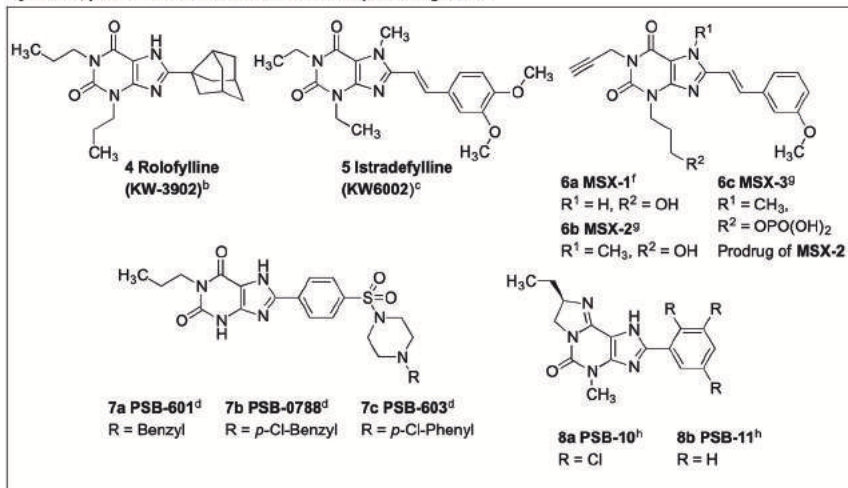
Keywords: amide, COMU, purine, uracil, xanthine, X-ray crystal structure

INTRODUCTION

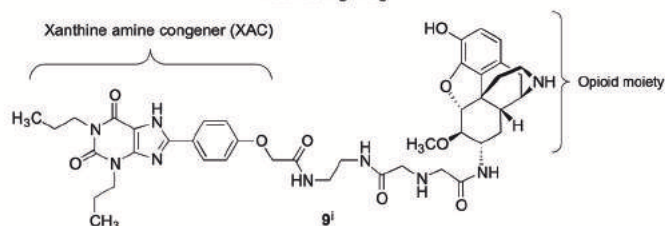
Xanthines are privileged structures in medicinal chemistry (Jacobson et al., 1993; Scammells et al., 1994; Kim et al., 2000; Baraldi et al., 2004; Müller and Jacobson, 2011). The methylxanthines caffeine (compound **1**, Figure 1), theobromine (**2**) and theophylline (**3**) are frequently consumed and therapeutically applied natural products (Franco et al., 2013). The biological activities of **1** and **2**, including central nervous system stimulatory, diuretic and antiasthmatic effects, are due to their blockade of adenosine receptors (ARs). The ARs, which belong to the family of G protein-coupled receptors (GPCRs), are (potential) drug targets for several diseases, in particular for heart and brain diseases (Baraldi et al., 2008; Müller and Jacobson, 2011; Chen et al., 2013). Recent findings point toward a great potential of A_{2A} and A_{2B} AR antagonists in immuno-oncology (Leone et al., 2015; Müller et al., 2018).

Natural methylxanthines^a

Synthetic, potent and selective adenosine receptor antagonists



Dual-acting drug



Xanthines for various targets

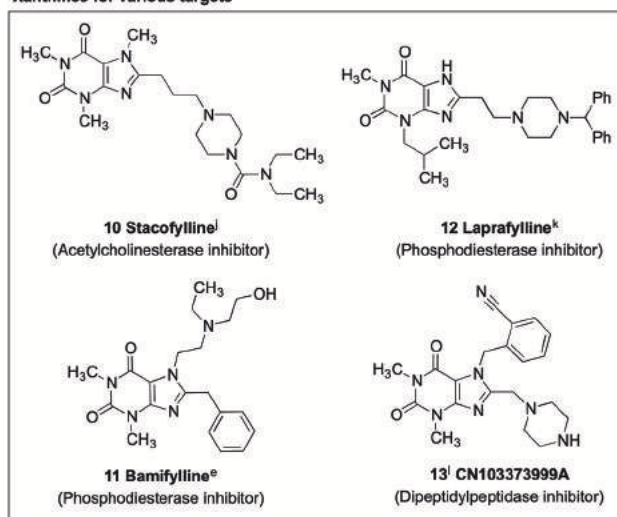


FIGURE 1 | Xanthine-based drugs. ^a(Franco et al., 2013); ^b(Slawsky and Givertz, 2009); ^c(Park and Stacy, 2012); ^d(Borrmann et al., 2009); ^e(Alciato et al., 1990); ^f(Sauer et al., 2000); ^g(Hockemeyer et al., 2004); ^h(Ozola et al., 2003); ⁱ(Jacobson, 2009); ^j(Gallagher, 2004); ^k(Baraldi et al., 2007); ^l(Costante et al., 2015).

Caffeine and theophylline are weak, non-selective AR antagonists (Nieber, 2017; Oñatibia-Astibia et al., 2017). Replacing the hydrogen atom at C8 by a larger residue, in combination with suitable substituents at the xanthine nitrogen atoms, may result in highly potent and subtype-selective AR antagonists (Baraldi et al., 2007; Müller and Jacobson, 2011). Inspired by the natural methylxanthines, several drugs have been developed, which were designated by the suffix “fylline” (**Figure 1**) (Alciato et al., 1990; LeWitt et al., 2008). An example is rolofylline (KW-3902, **4**, **Figure 1**), which carries a bulky noradamantanyl residue at the 8-position and acts as a selective A₁ AR receptor antagonist (LeWitt et al., 2008). Istradefylline (KW-6002, **5**, **Figure 1**), a potent, selective A_{2A} AR antagonist was approved for the treatment of Parkinson’s disease (PD) in Japan (LeWitt et al., 2008; Dungo and Deeks, 2013; Kondo and Mizuno, 2015). It features a styryl residue at the xanthine 8-position and ethyl groups at the xanthine N1 and N3 nitrogen atoms. An N1-propargyl residue in combination with a C8-styryl substitution yielded the potent and selective A_{2A} AR antagonist MSX-2 (**6b**) and its prodrug MSX-3 (**6c**) prepared from the precursor MSX-1 (**6a**) (Sauer et al., 2000; Hockemeyer et al., 2004). PSB-601 (**7a**), PSB-0788 (**7b**) and PSB-603 (**7c**) are potent, selective A_{2B} AR antagonists. These xanthines carry a *para*-sulfonamido-substituted phenyl ring at the 8-position and are potential therapeutics for the treatment of asthma, pain and cancer (Feoktistov et al., 1998; Yan et al., 2006; Singh and Yadav, 2016; Hinz et al., 2018; Müller et al., 2018). The tricyclic purine derivatives PSB-10 (**8a**) and PSB-11 (**8b**) are selective A₃ AR antagonists (Müller et al., 2002; Ozola et al., 2003).

Crystal structures of the AR subtypes A₁ (Cheng et al., 2017; Glukhova et al., 2017) and A_{2A} (Doré et al., 2011; Liu et al., 2012; Sun et al., 2017) showed that large 8-substituents of xanthine derivatives point out of the receptor binding pocket toward the extracellular space. This makes C8 a privileged position for the attachment of fluorophores (Köse et al., 2018), solubilizing moieties (Daly et al., 1985), spin labels for electron paramagnetic resonance (EPR) studies (Ilaš et al., 2005) or linkers for dual-acting compounds (Jacobson, 2009). An example of a dual ligand is compound **9** (Jacobson, 2009).

Receptors other than ARs, and enzymes can also be addressed by selecting appropriate substituents at the xanthine scaffold. Stacofylline (**10**) inhibits the enzyme acetylcholinesterase; it contains a diethylaminocarbonylpiperazinyl residue connected via a propyl spacer to the 8-position of caffeine (Gallagher, 2004). Bamifylline (**11**), a phosphodiesterase inhibitor, carries a benzyl-substituent at C8 and is used as an analgesic, bronchodilatory and vasodilatory drug (Alciato et al., 1990). The phosphodiesterase inhibitor laprafylline (**12**) features, similar to stacofylline (**10**), a piperazinyl residue attached by an ethyl linker to the 8-position of 1-methyl-3-isobutylxanthine. Recently, dipeptidylpeptidase 4 (DPP-4) inhibitors have gained attention for the treatment of type 2 diabetes (Crepaldi et al., 2007; Costante et al., 2015). Xanthine-derived compounds, such as CN103373999A (**13**), bearing a piperazinylmethyl residue at the xanthine 8-position have been identified as potent DPP-4 inhibitors (Costante et al., 2015).

8-Substituted xanthines can be synthesized by reacting 5,6-diaminouracil derivatives with carboxylic acids or aldehydes (**Scheme 1**).

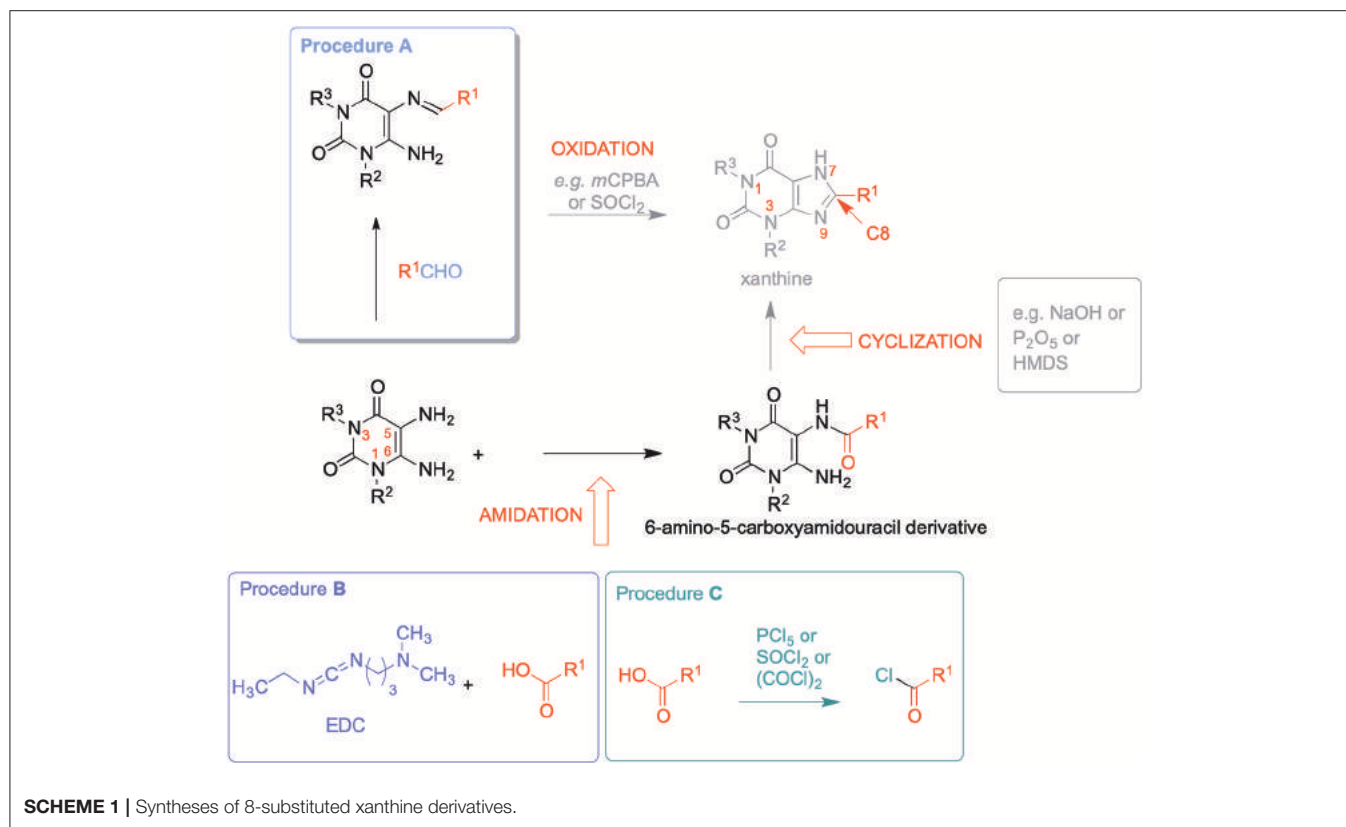
Different routes have been employed to obtain the required xanthine precursors. Condensation of 5,6-diaminouracils with aldehydes forming the corresponding imines [5-(arylidene- or alkylidene-amino)-6-aminouracils] as precursors, followed by oxidative cyclization is a commonly used route for the synthesis of 8-substituted xanthine derivatives (Hayallah et al., 2002; El-Sabbagh et al., 2007). However, aldehydes are less stable than the corresponding carboxylic acids, and commercial availability is often limited (Procedure A, **Scheme 1**) (Daly et al., 1985; Hayallah et al., 2002). Alternatively, 6-amino-5-carboxamidouracils can be prepared, which are the most frequently utilized xanthine precursors, that can be cyclized using a variety of methods, e.g., by sodium hydroxide or methylate, trimethylsilyl polyphosphate (PPSE), hexamethyldisilazane (HMDS) (Hayallah et al., 2002), or phosphorus pentoxide (Müller et al., 2008), depending on their reactivity and stability.

An established method for their preparation is the coupling of 5,6-diaminouracil derivatives with carboxylic acids in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) as a coupling reagent (Procedure B, **Scheme 1**) (Sauer et al., 2000; Hayallah et al., 2002; Hockemeyer et al., 2004; Basu et al., 2017). Another method requires the activation of the carboxylic acid by formation of the carboxylic acid chloride (Procedure C, **Scheme 1**) (Jacobson et al., 1989; Hockemeyer et al., 2004). Procedure C had been used to establish a multigram-scale synthesis of istradefylline (**5**). Drawbacks of this reaction are long reaction times (16 h) for the formation of the amide, only moderate yields (65%), and importantly, an additional step due to conversion of the acid into the corresponding acid chloride using hazardous chlorinating reagents. Furthermore, carboxylic acid chlorides are less stable than the corresponding carboxylic acids rendering storage and handling more demanding (Hockemeyer et al., 2004). Coupling reactions with the irritant and moisture-sensitive EDC-HCl also suffer from rather long reaction times, and typically provide moderate yields requiring tedious purification (Sauer et al., 2000; Hockemeyer et al., 2004).

All of these disadvantages motivated us to search for an alternative amide coupling procedure for the preparation of 6-amino-5-carboxamidouracil derivatives being the most stable and easily storable xanthine precursors. Our aim was to develop a fast and effective coupling method applicable to a variety of diaminouracils and carboxylic acids that would allow simple work-up and straightforward isolation of the desired product (**Scheme 3**).

EXPERIMENTAL

Chemicals were purchased from Merck (Darmstadt, Germany), ABCR (Karlsruhe, Germany) or TCI (Eschborn, Germany). Analytical thin layer chromatography (TLC) was performed on TLC plates F₂₅₄ (Merck) and analyzed using UV light.



High resolution mass spectra (HR-MS) were recorded on a micrOTOF-Q mass spectrometer (Bruker), low resolution mass spectra (LR-MS) on an API 2000 (Applied Biosystems) mass spectrometer. ¹H NMR and ¹³C NMR spectra were recorded in CDCl₃ or (CD₃)₂SO on a Bruker Ascend 600 MHz NMR-spectrometer operating at 600.18 MHz (¹H), and 150.93 MHz (¹³C). Chemical shifts (δ) are reported in ppm and are referenced to the chemical shifts of the residual solvent proton(s) present in chloroform δ [(CHCl₃) = 7.26 ppm for the ¹H NMR spectra and δ (CDCl₃) = 77.16 ppm for the ¹³C NMR spectra] and in dimethylsulfoxide δ ((CH₃)₂SO) = 2.50 ppm for the ¹H NMR spectra and δ ((CD₃)₂SO) = 39.52 ppm for the ¹³C NMR spectra. Multiplicity: s, singlet; d, doublet; q, quartet; m, multiplet. Coupling constants (*J*) are shown in Hertz (Hz). The infrared spectra were recorded as solid samples on an ALPHA-T (Bruker) with a Platinum ATR Module using the Opus software. The IR spectra were measured in the attenuated total reflection (ATR) mode in the region of 4,000–385 cm⁻¹ (s, strong; m, medium; w, weak) and are reported in cm⁻¹.

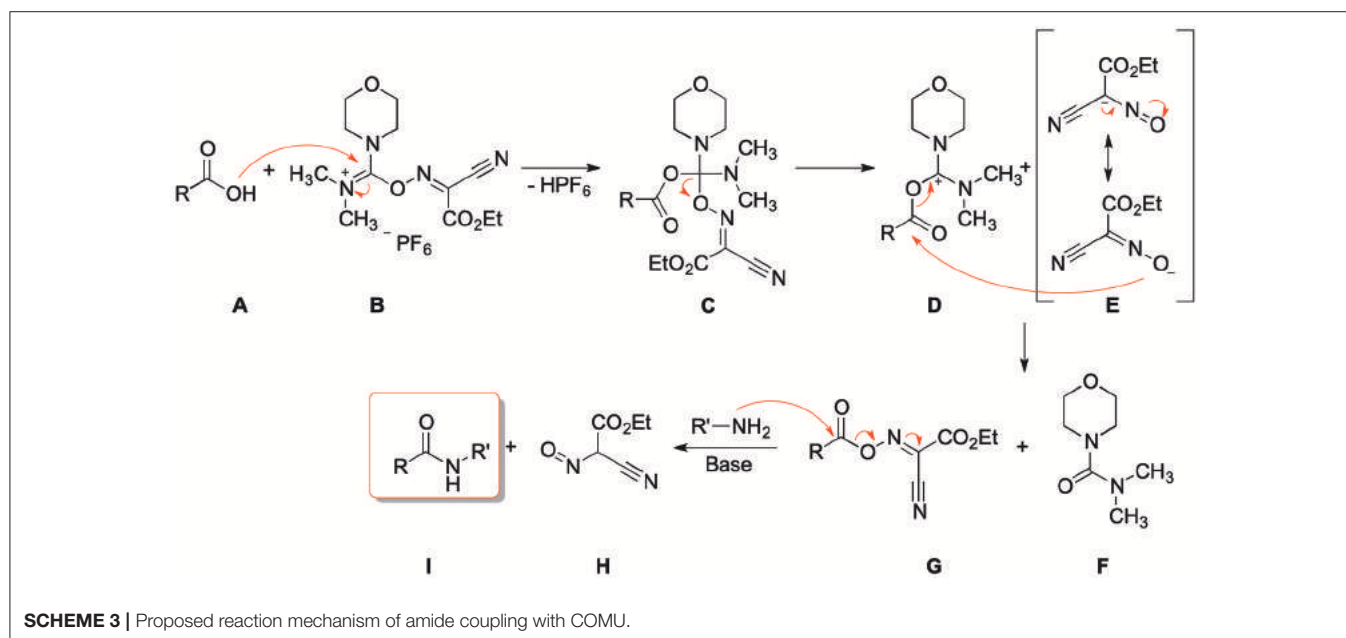
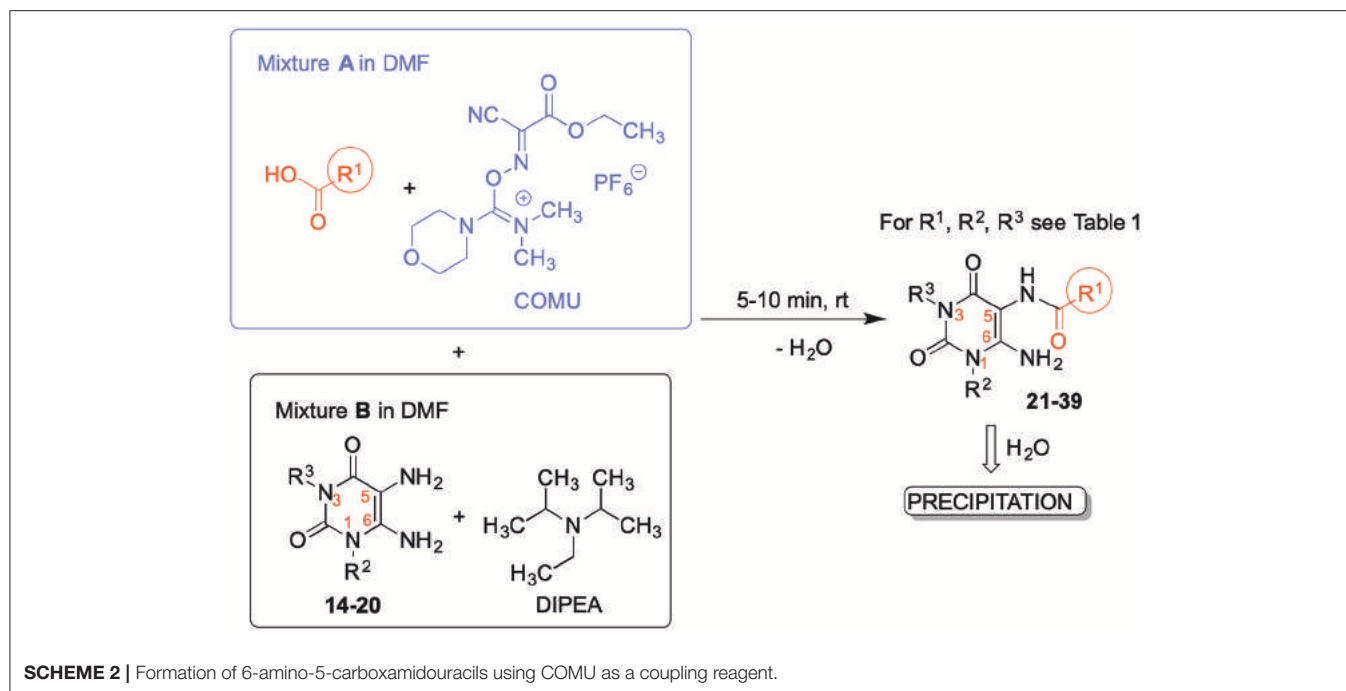
General Amide Formation Procedure

To a solution of the respective carboxylic acid (1.0 equiv.) and COMU (1.1 equiv.) dissolved in a minimum of dimethylformamide (DMF), a mixture of diaminouracil (1.1 equiv.) and *N,N*-diisopropylethylamine (DIPEA) (1.1 equiv.) dissolved in a minimum DMF was added dropwise. The reaction was stirred for 5–10 min at room

temperature, and water was added. The resulting precipitate was filtered off, washed with water and dried under reduced pressure. Most of the reactions were performed using 300 mg of the respective diaminouracil and 4 ml of DMF. The product was precipitated using 20 ml of water and washed with small portions of water (10 ml). The reaction generally performed well from 60 mg up to 1.5 g of diaminouracil as a precursor. For the 1.5 g scale 8 ml of DMF were used for dissolution, and 40 ml of water for precipitation, and 20 ml for the subsequent washing step. All other conditions were identical, and virtually the same percentage of yield as obtained independent of the scale of the reaction.

(9*H*-fluoren-9-yl)methyl 4-(2-((6-amino-3-methyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl)amino)-2-oxoethyl)piperazine-1-carboxylate (21)

Yield: 62% (white solid); mp 181–184°C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.48 (s, 1H, N1-H), 8.21 (s, 1H, CONH), 7.90 (d, *J* = 7.5 Hz, 2H, H_{arom}), 7.63 (d, *J* = 7.5 Hz, 2H, H_{arom}), 7.42 (t, *J* = 7.4 Hz, 2H, H_{arom}), 7.35 (td, *J* = 7.4, 1.1 Hz, 2H, H_{arom}), 6.01 (s, 2H, NH₂), 4.40 (d, *J* = 6.5 Hz, 2H, CH₂), 4.28 (t, *J* = 6.4 Hz, 1H, CH), 3.40–3.32 (m, 4H, 2 × CH₂), 3.06 (s, 3H, CH₃), 3.05–3.01 (m, 2H, CH₂), 2.48–2.37 (m, 4H, 2 × CH₂). ¹³C NMR (126 MHz, DMSO) δ 169.5 (CON), 160.7 (C6), 154.3 (OCON), 149.9 (CO), 149.7 (CO), 143.8 (2C, C_{arom}), 140.8 (2C,



$\nu_{\text{C=O}}$ (C_{arom}), 127.6 (2C, C_{arom}), 127.1 (2C, C_{arom}), 124.9 (2C, C_{arom}), 120.1 (2C, C_{arom}), 86.7 (C5), 66.4 (CH₂), 61.0 (CH₂), 52.2 (2C, 2 × CH₂), 46.8 (2C, 2 × CH₂), 43.3 (CH), 26.4 (CH₃). IR (cm⁻¹): $\tilde{\nu} = 3,556$ (w), 33,481 (w), 3,327 (w), 3,208 (w), 3,010 (w), 2,949 (w), 2,895 (w), 2,811 (w), 2,757 (w), 1,730 (m), 1,688 (s), 1,655 (m), 1,556 (s), 1,505 (s), 1,458 (s), 1,442 (s), 1,289 (w), 1,237 (s), 1,203 (w), 1,124 (s), 1,082 (m), 1,006 (m), 966 (m), 755 (s), 737 (s), 641 (w), 621 (w), 576 (m), 499 (s), 412 (s). HRMS (ESI-QTOF) calculated for C₂₆H₂₈N₆O₅ [M+H]⁺: 505.2194; found: 505.2190.

N-(6-amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)benzamide (22)

Yield: 78% (white solid); mp > 320°C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.59 (s, 1H, N3-H), 8.83 (s, 1H, CONH), 7.99 (d, *J* = 7.8 Hz, 2H, H_{arom}), 7.54 (t, *J* = 6.8 Hz, 1H, H_{arom}), 7.48 (q, *J* = 7.9, 7.3 Hz, 2H, H_{arom}), 6.65 (s, 2H, NH₂), 3.27 (s, 3H, CH₃). ¹³C NMR (DMSO, 126 MHz) δ 166.6 (CON), 160.0 (C6), 153.7 (CO), 150.4 (CO), 134.7 (C_{arom}), 131.2 (C_{arom}), 128.1 (2C, C_{arom}), 128.1 (2C, C_{arom}), 87.8 (C5), 29.2 (CH₃). IR (cm⁻¹): $\tilde{\nu}$

= 3,342 (w), 3,201 (w), 3,063 (w), 1,778 (w), 1,713 (s), 1,638 (s), 1,584 (s), 1,506 (s), 1,484 (s), 1,390 (w), 1,294 (m), 1,263 (w), 1,220 (w), 1,176 (w), 1,072 (w), 1,012 (w), 891 (w), 782 (m), 744 (w), 715 (s), 686 (w), 584 (m), 545 (s), 477 (s), 428 (w). HRMS (ESI-QTOF) calculated for $C_{12}H_{12}N_4O_3$ $[M+H]^+$: 261.0982; found: 261.0981.

***N*-(6-amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)-4-methoxybenzamide (23)**

Yield: 87% (off-white solid); mp 109–112°C; 1H NMR (600 MHz, DMSO- d_6) δ 8.73 (s, 1H, CONH), 7.95 (d, J = 8.9 Hz, 2H, H_{arom}), 7.00 (d, J = 8.9 Hz, 2H, H_{arom}), 6.65 (s, 2H, NH_2), 3.86–3.82 (m, 2H, N1- CH_2 or N3- CH_2), 3.82 (s, 3H, OCH_3), 3.75–3.68 (m, 2H, N1- CH_2 or N3- CH_2), 1.57 (dt, J = 15.1, 7.5 Hz, 2H, CH_2), 1.51 (dt, J = 14.8, 7.6 Hz, 2H, CH_2), 0.89 (t, J = 7.4 Hz, 3H, CH_3), 0.83 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 151 MHz) δ 166.1 (CON), 161.6 (C_{arom}), 159.2 (C6), 151.7 (CO), 150.4 (CO), 129.8 (C_{arom}), 126.8 (C_{arom}), 113.1 (C_{arom}), 87.6 (C5), 55.3 (OCH_3), 43.7 (N1- CH_2 or N3- CH_2), 41.8 (N1- CH_2 or N3- CH_2), 20.8 (2C, CH_2), 11.2 (CH_3), 10.7 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,416 (w), 3,348 (w), 3,219 (w), 2,963 (w), 2,939 (w), 2,877 (w), 2,841 (w), 1,695 (m), 1,636 (m), 1,605 (s), 1,488 (s), 1,415 (m), 1,381 (w), 1,259 (s), 1,191 (m), 1,114 (w), 1,080 (w), 1,029 (m), 901 (w), 852 (m), 762 (m), 551 (s), 513 (s). HRMS (ESI-QTOF) calculated for $C_{18}H_{24}N_4O_4$ $[M+H]^+$: 361.1870; found: 361.1885.

4-Nitrophenyl 4-((6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-carbamoyl) benzenesulfonate (24)

Product was purified by column chromatography ($CH_2Cl_2/MeOH$, 95:5). Yield: 78% (yellowish solid); mp 203–206°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.49 (s, 1H, N1-H), 9.21 (s, 1H, CONH), 8.30–8.26 (m, 2H, H_{arom}), 8.21–8.17 (m, 2H, H_{arom}), 8.06–8.02 (m, 2H, H_{arom}), 7.42–7.36 (m, 2H, H_{arom}), 6.22 (s, 2H, NH_2), 3.75 (q, J = 7.0 Hz, 2H, N3- CH_2), 1.06 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 164.8 (CON), 160.3 (C6), 153.0 (C_{arom}), 150.5 (CO), 149.7 (CO), 146.2 (C_{arom}), 140.6 (C_{arom}), 135.6 (C_{arom}), 129.4 (2C, C_{arom}), 128.2 (2C, C_{arom}), 125.9 (2C, C_{arom}), 123.3 (2C, C_{arom}), 86.4 (C5), 34.4 (N3- CH_2), 13.3 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,304 (w), 3,185 (w), 3,078 (w), 2,971 (w), 2,917 (w), 2,851 (w), 1,734 (m), 1,627 (m), 1,507 (s), 1,480 (s), 1,374 (s), 1,349 (s), 1,314 (m), 1,293 (m), 1,203 (s), 1,153 (s), 1,091 (m), 1,012 (w), 866 (s), 757 (s), 733 (w), 692 (m), 630 (w), 606 (s), 564 (s), 500 (s), 445 (m). HRMS (ESI-QTOF) calculated for $C_{19}H_{17}N_5O_8S$ $[M+H]^+$: 476.0871; found: 476.0860.

(2*R*,3*as*,5*S*,6*as*)-*N*-(6-amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)octahydro-2,5-methanopentalene-3*a*-carboxamide (25)

Most of the compound precipitated overnight. To increase the yield, the filtrate was extracted with diethyl ether, dried over $MgSO_4$, and after filtration the solvent was removed *in vacuo*. Yield: 99% (slightly brown solid); mp 153–157°C; 1H NMR (600

MHz, DMSO- d_6) δ 7.74 (s, 1H, CONH), 6.33 (s, 2H, NH_2), 3.88–3.78 (m, 2H, NCH_2), 3.74–3.62 (m, 2H, NCH_2), 2.74–2.69 (m, 1H, CH), 2.24 (s, 2H, $H_{adamantyl}$), 2.05 (d, J = 9.8 Hz, 2H, $H_{adamantyl}$), 1.83–1.76 (m, 4H, $H_{adamantyl}$), 1.55 (p, J = 7.2 Hz, 6H, $H_{adamantyl}$ and CH_2CH_3), 1.49 (q, J = 7.4 Hz, 2H, CH_2CH_3), 0.88 (t, J = 7.4 Hz, 3H, CH_3), 0.82 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 151 MHz) δ 177.1 (CON), 158.9 (C6), 151.4 (CO), 150.3 (CO), 88.2 (C5), 54.7 ($C_{adamantyl}$), 46.8 (NCH_2), 43.6 (NCH_2), 43.2 ($C_{adamantyl}$), 42.3 ($C_{adamantyl}$), 41.8 ($C_{adamantyl}$), 37.0 ($C_{adamantyl}$), 34.5 ($C_{adamantyl}$), 20.8 ($C_{adamantyl}$), 11.2 ($C_{adamantyl}$), 10.7 ($C_{adamantyl}$). IR (cm^{-1}): $\tilde{\nu}$ = 3,425 (w), 3,331 (w), 2,925 (w), 2,871 (w), 1,694 (s), 1,627 (m), 1,556 (s), 1,492 (s), 1,374 (w), 1,338 (w), 1,272 (m), 1,226 (m), 1,204 (m), 1,111 (w), 1,085 (w), 899 (w), 843 (w), 763 (w), 716 (w), 549 (m), 475 (w), 429 (w). HRMS (ESI-QTOF) calculated for $C_{20}H_{30}N_4O_3$ $[M+H]^+$: 375.2391; found: 375.2389.

***N*-(6-amino-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)cyclopentane-carboxamide (26)**

Yield: 70% (white solid); mp > 320°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.38 (s, 1H, N1-H), 8.21 (s, 1H, CONH), 5.77 (s, 2H, NH_2), 3.04 (s, 3H, NCH_3), 2.74 (p, J = 8.0 Hz, 1H, CH), 1.84–1.75 (m, 2H, $H_{cyclopentyl}$), 1.74–1.66 (m, 2H, $H_{cyclopentyl}$), 1.61 (qt, J = 10.3, 4.3 Hz, 2H, $H_{cyclopentyl}$), 1.50 (dt, J = 9.2, 5.6, 2.9 Hz, 2H, $H_{cyclopentyl}$). ^{13}C NMR (DMSO, 126 MHz) δ 176.0 (CON), 160.9 (C6), 150.1 (CO), 150.0 (CO), 87.7 (C5), 44.1 ($C_{cyclopentyl}$), 30.1 (2C, $C_{cyclopentyl}$), 26.6 (CH_3), 25.9 (2C, $C_{cyclopentyl}$). IR (cm^{-1}): $\tilde{\nu}$ = 3,328 (w), 3,173 (w), 2,967 (w), 2,951 (w), 2,872 (w), 1,720 (s), 1,651 (s), 1,633 (s), 1,552 (s), 1,497 (s), 1,456 (s), 1,380 (w), 1,302 (w), 1,211 (m), 1,170 (w), 1,120 (w), 1,024 (w), 996 (w), 961 (w), 945 (w), 755 (s), 711 (m), 662 (m), 592 (s), 549 (m), 512 (s), 471 (m), 417 (s). HRMS (ESI-QTOF) calculated for $C_{11}H_{16}N_4O_3$ $[M+H]^+$: 253.1295; found: 253.1294.

(*E*)-*N*-(6-amino-1,3-diethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3,4-dimethoxyphenyl)acrylamide (27)

Yield: 70% (white solid); mp 108–112°C; 1H NMR (500 MHz, DMSO- d_6) δ 8.49 (s, 1H, CONH), 7.39 (d, J = 15.8 Hz, 1H, CH), 7.18 (d, J = 1.9 Hz, 1H, H_{arom}), 7.15 (dd, J = 8.3, 1.9 Hz, 1H, H_{arom}), 7.01 (d, J = 8.4 Hz, 1H, H_{arom}), 6.71 (d, J = 15.8 Hz, 1H, CH), 6.62 (s, 2H, NH_2), 3.93 (q, J = 6.9 Hz, 2H, N1- CH_2 or N3- CH_2), 3.80 (d, J = 6.3 Hz, 8H, N1- CH_2 or N3- CH_2 and 2 \times OCH_3), 1.14 (t, J = 7.0 Hz, 3H, CH_3), 1.07 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 165.6 (CON), 158.8 (C6), 151.0 (C_{arom}), 150.1 (CO), 149.8 (CO), 148.9 (C_{arom}), 138.8 (CH), 127.8 (C_{arom}), 121.1 (C_{arom}), 120.4 (C_{arom}), 111.9 (C_{arom}), 110.2 (CH), 87.8 (C5), 55.5 (OCH_3), 55.4 (OCH_3), 37.6 (N1- CH_2), 35.4 (N3- CH_2), 13.2 (2C, CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,370 (w), 3,197 (w), 2,987 (w), 2,939 (w), 2,840 (w), 1,705 (s), 1,661 (m), 1,644 (m), 1,581 (s), 1,509 (s), 1,464 (s), 1,419 (m), 1,374 (w), 1,325 (w), 1,267 (s), 1,238 (s), 1,185 (s), 1,161 (s), 1,139 (s), 1,024 (m), 974 (m), 848 (w), 794 (m), 760 (m), 671 (m), 554 (s), 529 (s), 448 (s). HRMS (ESI-QTOF) calculated for $C_{19}H_{24}N_4O_5$ $[M+H]^+$: 389.1819; found: 389.1812.

(E)-N-(6-amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-methoxyphenyl)acrylamide (28)

Yield: 83% (white solid); mp 295–298°C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.59 (s, 1H, N1-H), 8.67 (s, 1H, CONH), 7.44 (d, J = 15.8 Hz, 1H, CH), 7.35 (t, J = 7.9 Hz, 1H, H_{arom}), 7.17 (d, J = 7.7 Hz, 1H, H_{arom}), 7.15–7.12 (m, 1H, H_{arom}), 6.98–6.95 (m, 1H, H_{arom}), 6.82 (d, J = 15.8 Hz, 1H, CH), 6.13 (s, 2H, NH_2), 4.44 (d, J = 2.4 Hz, 2H, N3- CH_2), 3.80 (s, 3H, OCH_3), 3.03 (t, J = 2.4 Hz, 1H, $\text{H}_{\text{propargyl}}$). ^{13}C NMR (DMSO, 126 MHz) δ 165.0 (CON), 159.6 (C6), 150.3 (CO), 149.1 (CO), 138.8 (C_{arom} or CH), 136.4 (C_{arom} or CH), 130.0 (C_{arom}), 122.7 (C_{arom}), 119.7 (C_{arom}), 115.2 (C_{arom}), 112.7 (CH), 86.9 (C5), 79.9 ($\text{C}_{\text{propargyl}}$), 72.4 ($\text{C}_{\text{propargyl}}$), 55.1 (OCH_3), 28.9 (N3- CH_2). IR (cm^{-1}): $\tilde{\nu}$ = 3,393 (w), 3,290 (w), 3,252 (w), 3,120 (w), 1,727 (s), 1,707 (m), 1,650 (s), 1,625 (m), 1,598 (s), 1,550 (s), 1,508 (s), 1,492 (s), 1,447 (s), 1,410 (w), 1,388 (w), 1,340 (m), 1,313 (m), 1,295 (m), 1,250 (s), 1,187 (m), 1,159 (m), 1,038 (w), 1,016 (w), 976 (s), 944 (w), 930 (w), 903 (w), 885 (w), 836 (w), 778 (m), 759 (s), 698 (s), 643 (s), 564 (s), 456 (s). HRMS (ESI-QTOF) calculated for $\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}_4$ [$\text{M}+\text{H}$] $^+$: 341.1244; found: 341.1241.

N-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-phenylacetamide (29)

Yield: 85% (white solid); mp 258–261°C; ^1H NMR (500 MHz, DMSO- d_6) δ 8.58 (s, 1H, CONH), 7.38–7.33 (m, 2H, H_{arom}), 7.32–7.27 (m, 2H, H_{arom}), 7.21 (tt, J = 6.4, 1.1 Hz, 1H, H_{arom}), 6.54 (s, 2H, NH_2), 3.59 (s, 2H, CH_2), 3.31 (s, 3H, CH_3), 3.11 (s, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 170.7 (CON), 159.3 (C6), 152.0 (CO), 150.5 (CO), 136.5 (C_{arom}), 129.2 (C_{arom}), 128.0 (C_{arom}), 126.1 (C_{arom}), 87.5 (C5), 42.0 (CH_2), 30.0 (CH_3), 27.5 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,322 (w), 3,190 (w), 1,699 (m), 1,667 (s), 1,643 (m), 1,583 (s), 1,496 (s), 1,421 (w), 1,381 (w), 1,344 (w), 1,322 (w), 1,225 (m), 1,164 (w), 1,153 (m), 1,057 (w), 1,028 (w), 979 (w), 954 (w), 935 (w), 903 (w), 837 (w), 756 (m), 728 (s), 693 (m), 557 (s), 535 (m), 487 (s), 438 (m). HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_3$ [$\text{M}+\text{H}$] $^+$: 289.1295; found: 289.1296.

N-(6-amino-2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropyrimidin-5-yl)benzamide (30)

Yield: 85% (off-white solid); mp. 121–124°C; ^1H NMR (600 MHz, chloroform- d_1) δ 8.19 (s, 1H, CONH), 7.93 (d, J = 7.4 Hz, 2H, H_{arom}), 7.52 (t, J = 7.1 Hz, 1H, H_{arom}), 7.43 (t, J = 7.5 Hz, 2H, H_{arom}), 5.71 (s, 2H, NH_2), 3.83 (dt, J = 14.3, 7.8 Hz, 4H, 2 \times N CH_2), 1.72 (q, J = 7.4 Hz, 2H, CH_2), 1.61 (q, J = 7.4 Hz, 2H, CH_2), 0.99 (t, J = 7.3 Hz, 3H, CH_3), 0.91 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (CDCl_3 , 151 MHz) δ 166.9 (CON), 160.2 (C6), 150.2 (CO), 148.0 (CO), 133.4 (C_{arom}), 132.3 (C_{arom}), 128.8 (C_{arom}), 127.6 (C_{arom}), 92.3 (C5), 44.9 (N CH_2), 43.6 (N CH_2), 21.6 (CH_2), 21.3 (CH_2), 11.4 (CH_3), 11.3 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,364 (w), 3,216 (w), 2,963 (w), 2,931 (w), 2,874 (w), 1,696 (m), 1,664 (m), 1,578 (s), 1,508 (s), 1,463 (s), 1,414 (m), 1,278 (m), 1,160 (w), 1,073 (w), 1,000 (w), 900 (w), 842 (w), 764 (m), 689 (m), 543 (s), 456 (m). HRMS (ESI-QTOF) calculated for $\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_3$ [$\text{M}+\text{H}$] $^+$: 331.1765; found: 331.1767.

N-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)cinnamamide (31)

Yield: 80% (off-white solid); mp > 320°C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.43 (s, 1H, N1-H), 8.68 (s, 1H, CONH), 7.58 (d, J = 7.4 Hz, 2H, H_{arom}), 7.50–7.37 (m, 4H, H_{arom} + H_{vinyl}), 6.83 (d, J = 15.9 Hz, 1H, H_{vinyl}), 5.99 (s, 2H, NH_2), 3.74 (q, J = 6.5 Hz, 2H, CH_2), 1.06 (t, J = 6.7 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 164.9 (CON), 160.3 (C6), 149.7 (CO), 149.5 (CO), 138.8 (C_{vinyl} or C_{arom}), 135.0 (C_{vinyl} or C_{arom}), 129.4 (C_{vinyl} or C_{arom}), 129.0 (2C, C_{arom}), 127.4 (2C, C_{arom}), 122.4 (C_{vinyl} or C_{arom}), 87.4 (C5), 34.4 (N3- CH_2), 13.2 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,315 (w), 3,166 (w), 3,065 (w), 3,026 (w), 2,976 (w), 2,940 (w), 2,913 (w), 1,723 (s), 1,646 (s), 1,617 (s), 1,557 (s), 1,490 (s), 1,427 (m), 1,381 (w), 133 (m), 1,291 (w), 1,192 (m), 1,161 (w), 1,047 (w), 999 (m), 741 (s), 713 (m), 586 (s), 543 (s), 505 (s), 487 (s), 450 (w), 433 (w). HRMS (ESI-QTOF) calculated for $\text{C}_{15}\text{H}_{16}\text{N}_4\text{O}_3$ [$\text{M}+\text{H}$] $^+$: 301.1295; found: 301.1294.

N-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-phenylpropanamide (32)

Yield: 90% (white solid); mp > 320°C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.38 (s, 1H, N1-H), 8.39 (s, 1H, CONH), 7.28 (t, J = 7.4 Hz, 2H, H_{arom}), 7.24 (d, J = 6.9 Hz, 2H, H_{arom}), 7.18 (t, J = 7.1 Hz, 1H, H_{arom}), 5.82 (s, 2H, NH_2), 3.73 (q, J = 6.9 Hz, 2H, N3- CH_2), 2.91–2.80 (m, 2H, CH_2), 2.53 (dd, J = 9.2, 7.0 Hz, 2H, CH_2), 1.04 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 171.7 (CON), 160.4 (C6), 149.9 (CO), 149.6 (CO), 141.5 (C_{arom}), 128.3 (2C, C_{arom}), 128.1 (2C, C_{arom}), 125.8 (C_{arom}), 87.2 (C5), 36.8 (CH_2), 34.3 (N3- CH_2), 30.9 (CH_2), 13.2 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,341 (w), 3,290 (w), 3,180 (w), 3,066 (w), 3,029 (w), 2,913 (w), 1,725 (m), 1,637 (s), 1,552 (s), 1,486 (s), 1,382 (m), 1,333 (m), 1,301 (m), 1,192 (w), 1,157 (m), 1,124 (w), 1,044 (w), 970 (w), 921 (w), 799 (w), 78 (w), 760 (s), 730 (m), 695 (m), 662 (m), 571 (s), 501 (s), 481 (s). HRMS (ESI-QTOF) calculated for $\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_3$ [$\text{M}+\text{H}$] $^+$: 303.1452; found: 303.1454.

N-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-phenylcyclopropanecarboxamide (33)

Yield: 89% (white solid); mp 302–305°C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.35 (s, 1H, N1-H), 8.68 (s, 1H, CONH), 7.29 (t, J = 7.5 Hz, 2H, H_{arom}), 7.19 (d, J = 7.7 Hz, 1H, H_{arom}), 7.14 (d, J = 7.9 Hz, 2H, H_{arom}), 5.90 (s, 2H, NH_2), 3.72 (q, J = 6.9 Hz, 2H, N3- CH_2), 2.28 (dt, J = 9.5, 6.0 Hz, 1H, CH), 2.09 (dt, J = 8.8, 4.7 Hz, 1H, CH), 1.37 (dt, J = 9.0, 4.5 Hz, 1H, CH), 1.26–1.20 (m, 1H, CH), 1.04 (t, J = 6.9 Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 171.5 (CON), 160.4 (C6), 149.9 (CO), 149.5 (CO), 141.2 (C_{arom}), 128.2 (2C, C_{arom}), 125.9 (3C, C_{arom}), 87.4 (C5), 34.4 (N3- CH_2), 25.6 ($\text{C}_{\text{cyclopropyle}}$), 24.3 ($\text{C}_{\text{cyclopropyle}}$), 16.1 ($\text{C}_{\text{cyclopropyle}}$), 13.2 (CH_3). IR (cm^{-1}): $\tilde{\nu}$ = 3,355 (w), 3,312 (w), 3,186 (w), 3,082 (w), 3,032 (w), 3,011 (w), 2,978 (w), 2,941 (w), 1,726 (s), 1,650 (s), 1,628 (s), 1,555 (s), 1,497 (s), 1,454 (s), 1,427 (m), 1,382 (w), 1,334 (m), 1,300 (m), 1,199 (m), 1,160 (w), 1,080 (w), 1,026 (w), 957 (w), 760 (s), 693 (m), 662 (m), 592 (m), 543 (m), 518 (s), 499

(m). HRMS (ESI-QTOF) calculated for $C_{16}H_{18}N_4O_3$ $[M+H]^+$: 315.1452; found: 315.1460.

***N*-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-phenoxyacetamide (34)**

Yield: 88% (off-white solid); mp 289–293°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.45 (s, 1H, N1-H), 8.53 (s, 1H, CONH), 7.31 (td, $J = 7.4, 2.0$ Hz, 2H, H_{arom}), 7.05–6.99 (m, 2H, H_{arom}), 6.97 (t, $J = 7.3$ Hz, 1H, H_{arom}), 6.07 (s, 2H, NH_2), 4.57 (s, 2H, $COCH_2$), 3.73 (q, $J = 7.0$ Hz, 2H, $N3-CH_2$), 1.05 (t, $J = 7.0$ Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 167.9 (CON), 160.3 (C6), 157.9 (C_{arom}), 150.2 (CO), 149.6 (CO), 129.4 (2C, C_{arom}), 121.0 (C_{arom}), 114.7 (2C, C_{arom}), 85.9 (C5), 66.9 ($COCH_2$), 34.4 ($N3-CH_2$), 13.2 (CH_3). IR (cm^{-1}): $\tilde{\nu} = 3,364$ (w), 3,321 (w), 3,273 (w), 3,170 (w), 1,716 (m), 1,689 (m), 1,643 (m), 1,574 (s), 1,487 (s), 1,458 (m), 1,379 (w), 1,339 (w), 1,279 (w), 1,249 (w), 1,221 (s), 1,167 (w), 1,111 (w), 1,084 (w), 1,065 (w), 924 (w), 830 (w), 791 (w), 753 (s), 6,966 (w), 635 (m), 578 (w), 534 (s), 508 (m), 440 (w). HRMS (ESI-QTOF) calculated for $C_{14}H_{16}N_4O_4$ $[M+H]^+$: 305.1244; found: 305.1253.

***N*-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-3-phenylpropanamide (35)**

Yield: quantitative (white solid); mp 265–267°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.37 (s, 1H, N1-H), 8.42 (s, 1H, CONH), 7.28 (t, $J = 7.4$ Hz, 2H, H_{arom}), 7.25–7.21 (m, 2H, H_{arom}), 7.18 (t, $J = 7.2$ Hz, 1H, H_{arom}), 5.51 (s, 2H, $N3-NH_2$), 3.72 (q, $J = 6.9$ Hz, 2H, CH_2), 2.98 (dd, $J = 13.4, 6.2$ Hz, 1H, CH), 2.78–2.68 (m, 1H, CH), 2.57–2.51 (m, 1H, CH), 1.04 (t, $J = 7.0$ Hz, 3H, CH_3), 1.00 (d, $J = 6.8$ Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 175.4 (CON), 160.3 (C6), 149.6 (CO), 149.5 (CO), 140.1 (C_{arom}), 128.9 (2C, C_{arom}), 128.1 (2C, C_{arom}), 125.9 (C_{arom}), 87.5 (C5), 41.0 (CCH_3), 34.4 ($N3-CH_2$), 16.8 (CH_3), 13.2 (CH_3). IR (cm^{-1}): $\tilde{\nu} = 3,354$ (w), 3,318 (w), 3,178 (w), 3,082 (w), 3,022 (w), 3,002 (w), 2,975 (w), 2,938 (w), 2,875 (w), 1,723 (s), 1,632 (s), 1,552 (s), 1,492 (s), 1,457 (s), 1,426 (s), 1,378 (m), 1,331 (w), 1,299 (m), 1,226 (w), 1,181 (w), 1,160 (w), 1,116 (w), 1,044 (w), 948 (w), 759 (s), 745 (m), 698 (s), 659 (m), 543 (s), 505 (s). HRMS (ESI-QTOF) calculated for $C_{16}H_{20}N_4O_3$ $[M+H]^+$: 317.1608; found: 317.1617.

***N*-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)benzamide (36)**

Yield: 87% (off-white solid); mp > 320°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.38 (s, 1H, N1-H), 8.86 (s, 1H, CONH), 7.99–7.91 (m, 2H, H_{arom}), 7.56–7.51 (m, 1H, H_{arom}), 7.47 (t, $J = 7.5$ Hz, 2H, H_{arom}), 6.06 (s, 2H, NH_2), 3.75 (q, $J = 7.0$ Hz, 2H, $N3-CH_2$), 1.06 (t, $J = 7.0$ Hz, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO) δ 166.4 (CON), 160.5 (C6), 150.4 (CO), 149.7 (CO), 134.5 (C_{arom}), 131.1 (C_{arom}), 128.0 (C_{arom}), 127.8 (C_{arom}), 87.1 (C5), 34.4 ($N3-CH_2$), 13.3 (CH_3). IR (cm^{-1}): $\tilde{\nu} = 3,302$ (w), 3,166 (w), 3,061 (w), 2,976 (w), 1,718 (m), 1,627 (m), 1,552 (s), 1,504 (s), 1,481 (s), 1,456 (s), 1,426 (s), 1,381 (m), 1,334 (w), 1,299 (m), 1,165 (w), 1,047 (w), 926 (w), 883 (w), 797 (m), 760 (m), 692 (m), 657 (m), 544

(s), 503 (m), 473 (m), 445 (w). HRMS (ESI-QTOF) calculated for $C_{13}H_{14}N_4O_3$ $[M+H]^+$: 275.1139; found: 275.1142.

***N*-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-phenylacetamide (37)**

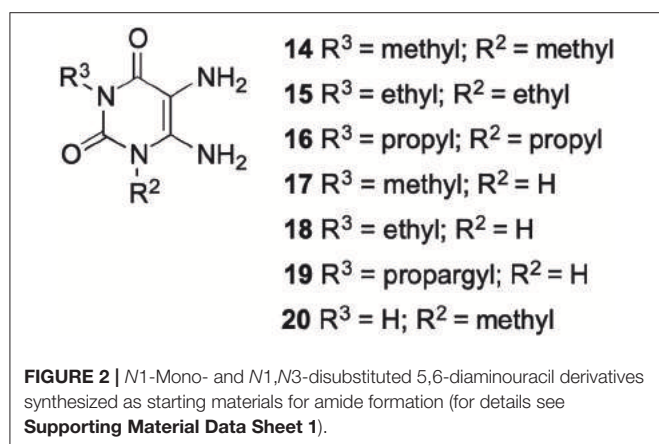
Yield: 80% (white solid); mp > 320°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.39 (s, 1H, N1-H), 8.58 (s, 1H, CONH), 7.35–7.31 (m, 2H, H_{arom}), 7.28 (m, 2H, H_{arom}), 7.23–7.19 (m, 1H, H_{arom}), 5.90 (s, 2H, NH_2), 3.71 (q, $J = 7.0$ Hz, 2H, $N3-CH_2$), 3.56 (s, 2H, CH_2), 1.03 (t, $J = 7.0$ Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 170.6 (CON), 160.5 (C6), 150.1 (CO), 149.7 (CO), 136.6 (C_{arom}), 129.4 (C_{arom}), 128.2 (C_{arom}), 126.3 (C_{arom}), 87.5 (C5), 42.1 ($COCH_2$), 34.5 ($N3-CH_2$), 13.4 (CH_3). IR (cm^{-1}): $\tilde{\nu} = 3,349$ (w), 3,297 (w), 3,184 (w), 3,065 (w), 2,980 (w), 2,909 (w), 2,885 (w), 1,729 (m), 1,638 (s), 1,547 (s), 1,483 (s), 1,421 (s), 1,331 (m), 1,294 (m), 1,216 (w), 1,180 (m), 1,155 (m), 1,031 (w), 963 (w), 926 (w), 793 (w), 758 (s), 694 (s), 661 (m), 599 (s), 488 (s). HRMS (ESI-QTOF) calculated for $C_{14}H_{16}N_4O_3$ $[M+H]^+$: 289.1295; found: 289.1304.

***N*-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-6-methylheptanamide (38)**

Yield: 81% (white solid); mp 278–281°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.34 (s, 1H, N1-H), 8.24 (s, 1H, CONH), 5.82 (s, 2H, NH_2), 3.70 (q, $J = 7.0$ Hz, 2H, $N3-CH_2$), 2.24–2.12 (m, 2H, $COCH_2$), 1.55–1.45 (m, 3H, CH_2 and CH), 1.34–1.23 (m, 2H, CH_2), 1.19–1.11 (m, 2H, CH_2), 1.03 (t, $J = 7.0$ Hz, 3H, CH_3), 0.85 (d, $J = 6.6$ Hz, 6H, $2 \times CH_3$). ^{13}C NMR (DMSO, 126 MHz) δ 172.7 (CON), 160.6 (C6), 150.0 (CO), 149.7 (CO), 87.6 (C5), 38.5 (CH_2), 35.4 (CH_2), 34.5 ($N3-CH_2$), 27.4 (CH_2), 26.7 (CH), 25.4 (CH_2), 22.7 ($2 \times CH_3$), 13.4 (CH_3). IR (cm^{-1}): $\tilde{\nu} = 3,341$ (w), 3,302 (w), 3,186 (w), 3,075 (w), 2,957 (w), 2,915 (w), 2,875 (w), 2,851 (w), 1,728 (m), 1,637 (s), 1,551 (s), 1,488 (s), 1,424 (s), 1,379 (m), 1,333 (m), 1,294 (m), 1,200 (w), 1,159 (m), 1,111 (w), 1,048 (w), 967 (w), 925 (w), 760 (s), 729 (w), 664 (m), 580 (s), 500 (s), 444 (m). HRMS (ESI-QTOF) calculated for $C_{14}H_{24}N_4O_3$ $[M+H]^+$: 297.1921; found: 297.1924.

***(3a*S*,4*S*,5*S*,7a*R*)-N*-(6-amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-*o*-octahydro-1*H*-2,5-methanoindene-4-carboxamide (39)**

Filtrate was extracted with Et_2O , dried over $MgSO_4$ and the solvent removed in vacuo. Yield: 75% (off-white solid); mp > 320°C; 1H NMR (500 MHz, DMSO- d_6) δ 10.33 (s, 1H, N1-H), 7.77 (s, 1H, CONH), 5.64 (s, 2H, NH_2), 3.71 (q, $J = 7.0$ Hz, 2H, $N3-CH_2$), 1.97 (s, 3H, $H_{noradamantane}$), 1.87 (d, $J = 2.7$ Hz, 6H, $H_{noradamantane}$), 1.69–1.63 (m, 6H, $H_{noradamantane}$), 1.04 (t, $J = 7.0$ Hz, 3H, CH_3). ^{13}C NMR (DMSO, 126 MHz) δ 177.3 (CON), 160.2 (C6), 149.7 (CO), 149.6 (CO), 87.6 (C5), 38.6 ($CH_2_{noradamantane}$), 36.2 ($CH_2_{noradamantane}$), 34.3 ($N3-CH_2$), 27.7 (4C, $CH_{noradamantane}$), 13.2 (CH_3). IR (cm^{-1}): $\tilde{\nu} = 3,478$ (w), 3,428 (w), 3,289 (w), 3,165 (w), 3,067 (w), 2,984 (w), 2,909 (w), 2,853 (w), 1,718 (m), 1,622 (s), 1,545 (s), 1,507 (s), 1,486 (s),



1,446 (s), 1,372 (w), 1,330 (w), 1,291 (m), 1,244 (w), 1,184 (w), 1,161 (w), 1,110 (w), 1,042 (w), 989 (w), 927 (w), 760 (s), 701 (w), 653 (m), 542 (s), 499 (m). HRMS (ESI-QTOF) calculated for C₁₇H₂₄N₄O₃ [M+H]⁺: 333.1921; found: 333.1922.

RESULTS AND DISCUSSION

Disadvantages of irritant and hazardous coupling procedures, long reaction times and moderate yields encouraged us to search for a new method to yield the desired 6-amino-5-carboxamidouracil derivatives. After initial experiments with various procedures, the coupling reagent COMU showed the most promising results. COMU, which was developed in 2009, does not contain a potentially explosive benzotriazole moiety, and is therefore safer than classical coupling reagents such as, for example, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU). COMU shows high solubility, is stable in typically used solvents, can be easily removed due to the water-solubility of its products, and may be used for a broad range of carboxylic acids and amines yielding the corresponding amides (El-Faham et al., 2009; El-Faham and Albericio, 2010, 2011; Hjørringgaard et al., 2012).

The synthetic procedure which led to differently substituted 6-amino-5-carboxamidouracils is shown in **Scheme 2**. Diaminouracil derivatives and carboxylic acids were used as starting materials and subjected to amide coupling using COMU. *N*1-mono- and *N*1,*N*3-disubstituted 5,6-diaminouracil derivatives (**14–20**, **Figure 2**) were individually prepared (for details see **Supporting Material Data Sheet 1**) according to previously described procedures and (Maxwell and Salivar, 1952; Müller et al., 1993; Hockemeyer et al., 2004), while the employed carboxylic acid derivatives were in most cases commercially available.

Amide Coupling Reaction

Amide formation with the coupling reagent required the adjustment of different parameters, including solvent, reaction time, temperature and base. With DMF, DIPEA and COMU the optimal conditions were found (**Scheme 2**). The reaction may

also be performed in other solvents, such as CH₂Cl₂, ethyl acetate or tetrahydrofuran (MacMillan et al., 2013), however, DMF is preferred resulting in short reaction times, and, importantly, the product can easily be precipitated in high purity by the addition of water. This renders a tedious isolation and purification procedure dispensable.

Scheme 3 depicts the proposed reaction mechanism, which is based on the mechanism proposed for the synthesis of esters using COMU (Twibanire and Grindley, 2011). The first step is the nucleophilic attack of the carboxylic acid (**A**) at the uronium moiety of COMU (**B**) resulting in intermediate **C**. Decomposition of **C**, followed by addition of the resulting anion **E** to the carbonyl group of **D** and subsequent elimination of the urea derivative **F** leads to the activated carboxylic acid **G**. Finally, the corresponding amide derivative is formed by nucleophilic attack of an amine and elimination of the water-soluble side product **H**.

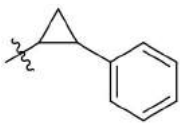
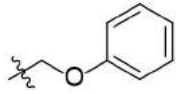
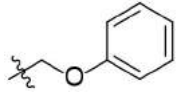
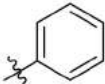
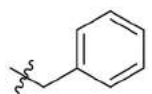
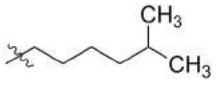
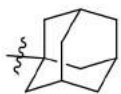
According to the proposed reaction mechanism, the carboxylic acid was converted to its active ester after dissolving it (1.0 equiv) together with COMU (1.1 equiv) in a minimum of DMF (mixture A, **Scheme 2**). Then, a solution of the 5,6-diaminouracil derivative (1.2 equiv) and diisopropylethylamine (DIPEA, 1.1 equiv) as a base dissolved in a minimum of DMF (mixture B) was added, followed by 5–10 min of stirring at room temperature (**Scheme 2**). Upon addition of cold water, the product precipitated. It was filtered off, washed with cold water, and dried under reduced pressure yielding the target compounds **21–39** (**Table 1**) in high purity and with yields ranging from 62 to 99%. Due to our interest in AR antagonists, we prepared various precursors for 8-substituted xanthines, which we could obtain in high yields and isolate by simple precipitation as shown for various examples (**22–29**). The 1,3-dipropyl derivatives **23** and **30** were formed in 87 and 85% yield, with 98 and 99% purity, respectively. Compound **23** is a precursor of the dual-acting A₁ AR-opioid receptor ligands, such as **9**. Compound **22** was obtained in 78% yield and provides access to the A₃ AR antagonists PSB-11 (**8b**). Compound **24**, the key compound for the synthesis of highly potent and selective A_{2B} AR antagonists, was successfully condensed and precipitated. The carboxylic acid for the synthesis of **24** was not commercially available and was therefore prepared according to a literature procedure (Borrmann et al., 2009). To gain a purity of over 95% for **24**, an additional chromatographic purification procedure was required. Compound **25**, the precursor of the A₁ AR antagonist rolofylline (**4**), which contains an 8-noradamantanyl substituent, and propyl residues on *N*1 and *N*3, precipitated in high purity (99%); fractional precipitation after cooling to 0°C was required to give a final yield of 79%. The less bulky and less hydrophobic cyclopentanecarboxylic acid was reacted with 5,6-diamino-3-methyluracil to obtain amide **26** as a precursor for 8-cyclopentyltheophylline (CPX), and was isolated in 69% yield with 99% purity. The additional substituent on *N*1 can be easily introduced subsequently by alkylation according to literature procedures (Hockemeyer et al., 2004). The precursor **29** of the A₁ AR antagonist bamifylline (**11**), with methyl groups at both uracil nitrogen atoms, precipitated immediately in 85% yield and 99% purity. Compound **27**, the precursor of the

TABLE 1 | Formation of 6-amino-5-carboxamidouracil derivatives.

Precursors for xanthines with known bioactivity						
Compounds	R ¹	Precursor for (target)	R ²	R ³	Isolated yield (%)	Purity after precipitation (%)
21		13 (DPP-4 inhibitor)	H	Me	62	99
22		8b (A ₃ antagonist)	Me	H	78	96
23		9 (A ₁ antagonists)	Pr	Pr	87	98
24 ^d		7a, 7b, 7c (A _{2B} antagonists)	H	Et	78	99 ^a
25 ^c		4 (A ₁ antagonist)	Pr	Pr	79	99
26		CPX (A ₁ antagonist)	H	Me	69	99
27 ^d		5 (A _{2A} antagonist)	Et	Et	70	97
28 ^e		6a–c (A _{2A} antagonists)	H	Propargyl	83	98
29		11 (A ₁ antagonists)	Me	Me	84	99
Precursors for xanthines with various 8-substituents						
30 ^f		–	Pr	Pr	85	99
31		–	H	Et	80	94
32		–	H	Et	90	99

(Continued)

TABLE 1 | Continued

Compounds	R ¹	Precursor for (target)	R ²	R ³	Isolated yield (%)	Purity after precipitation (%)
33		–	H	Et	89	98
34		–	H	Et	88	99
35		–	H	Et	99	99
36 ^g		–	H	Et	87	99
37		–	H	Et	80	90
38		–	H	Et	81	99
39		–	H	Et	78	87

^aPurity after additional column chromatography. ^b(Borrmann et al., 2009); ^c(Moore et al., 1999); ^d(Rabasseda et al., 2001); ^e(Hockemeyer et al., 2004); ^f(Daly et al., 1985); ^g(Rodríguez-Borges et al., 2010).

A_{2A} AR antagonist and anti-Parkinson drug istradefylline (**5**), precipitated in 70% yield with 97% purity. Amide formation with 3-methoxycinnamic acid, carrying the styrene moiety, which is required for the preparation of the potent and selective A_{2A} AR antagonists of the MSX series (**6a-c**), gave the 6-amino-5-carboxamidouracil precursor **28** in 83% isolated yield after precipitation.

To investigate the impact of different carboxylic acid derivatives regarding precipitation of the product, we used 3-ethyldiaminouracil and various carboxylic acids as a test system for the formation of differently substituted 6-amino-5-carboxamidouracils (Table 1). Compound **32**, with a phenylpropionyl residue, was isolated in 90% yield. The analogous compound **33** containing a rigidified cyclopropyl ring gave a similar yield of 89%, as did the ether analog **34**. The presence of an α -methyl group in compound **35** resulted in quantitative product formation and precipitation. The 6-amino-5-carboxamidouracil **38** bearing an alkyl residue was isolated in 81% yield with 99% purity.

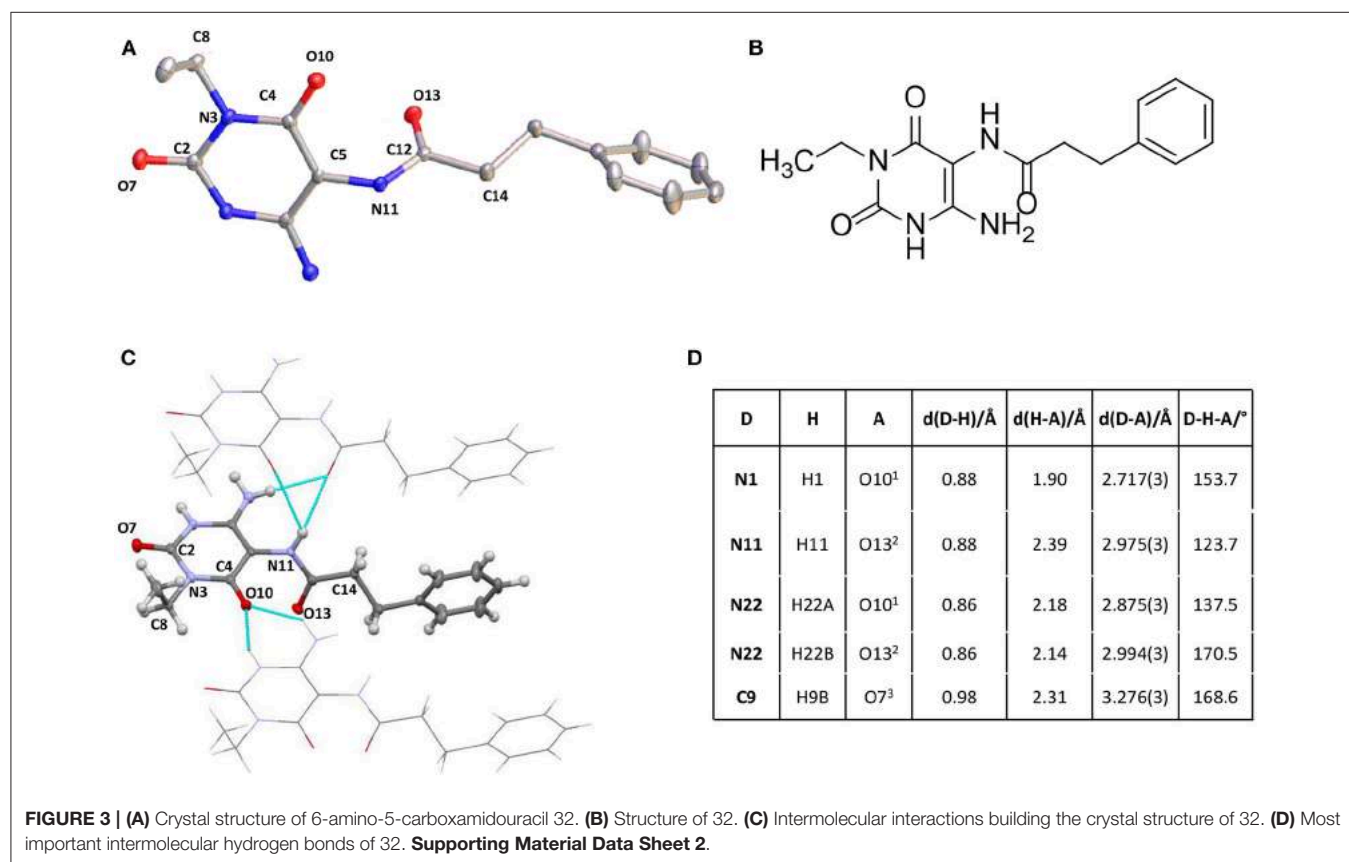
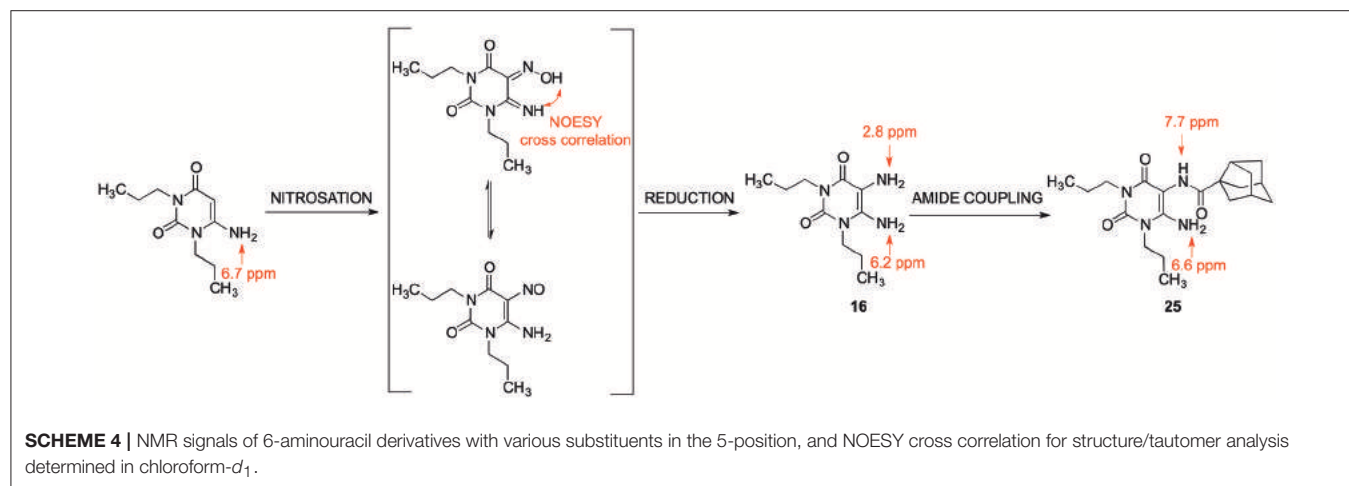
Comparing all reactions, we observed the following trends: 1,3-disubstituted uracils could be formed best in case of a bulky, hydrophobic carboxylic acid derivative, which favors precipitation from the DMF/H₂O solution. Reactions of

N1-unsubstituted diaminouracils generally gave higher product yields, and the products were easily precipitated. The melting points of those products were high indicating the formation of intermolecular hydrogen bonds in the solid state, which was confirmed by the crystal structure of **32** (see below).

Structural Studies and Regioselectivity

Since 5,6-diaminouracil carries two amino groups, the question arises, which one forms the amide bond (Yang et al., 2015). Due to literature reports, the 5-amino group is proposed to react (Sauer et al., 2000; Hayallah et al., 2002; Hockemeyer et al., 2004). We checked this assumption by NMR and small molecule X-ray crystallography, comparing the NMR signals of 6-aminouracil, 6-amino-5-nitrosouracil, 5,6-diaminouracil, and 5-amino-6-carboxamidouracil. We additionally applied 2-dimensional NMR spectroscopy, namely heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY), for determining the structure of amide **25**.

In literature, the product of the first reaction step has been described as a 5-nitroso derivative. Based on our NMR experiments, the 5-(hydroxyimino)-6-imino derivative is the tautomer that is present in chloroform employed as a solvent



(Scheme 4). The chemical shift of the 5-amino group in compound 16 indicates a magnetic shielding of the hydrogen atoms giving the nitrogen atom a more nucleophilic character, which is in accordance with our regioselectivity studies.

Finally, we tried to obtain a crystal structure of 25. Different crystallization experiments were performed but the crystallization of 25 has not been successful. Fortunately, compound 32, crystallized from DMSO solution at room temperature, yielding a crystal of the size $0.4 \times 0.2 \times 0.08$ mm.

Measurement and analysis of the resulting crystal structure using a Bruker X8-KappaApexII instrument showed a monocline crystal system within the space group $P2_1$. In accordance with the NMR experiment of 25 the crystal structure of 32 confirmed a regioselective amide coupling of the carboxylic acid with the 5,6-diamino-uracil derivative in position 5. The crystal is mainly formed by intermolecular hydrogen bonds. π -Stacking or interaction with the solvent could not be observed. The most important intermolecular hydrogen bonds are summarized in

Figure 3. All NH groups showed a donor functionalization and all oxygen atoms showed acceptor properties to surrounding molecules. **Figure 3** visualizes these intermolecular interactions. The surrounding molecules are shaded while the intermolecular interactions are shown in turquoise. All bond lengths were in the expected range.

CONCLUSIONS

In summary, we report on a new regioselective amide formation of 5,6-diaminouracil derivatives with carboxylic acids using the coupling reagent COMU which leads to the preparation of important precursors for xanthine derivatives. The reaction is completed after only 5–10 min of stirring at room temperature in DMF, followed by straightforward isolation of the formed amides by precipitation through the addition of water. After filtration, the 6-amino-5-carboxamidouracils were obtained in high isolated yields and showed in most cases purities of 90% or higher requiring no further chromatographic purification. The new procedure is advantageous with regard to reaction time and yields, and it avoids hazardous coupling or chlorinating reagents. In addition to several new derivatives, we synthesized the 6-amino-5-carboxamidouracil precursors of important, biologically active and literature-known xanthines utilizing the new method. The regioselectivity of the amide formation with the 5- rather than the 6-amino group of the uracil derivatives was proven by 2D-NMR spectroscopy and X-ray crystallography. The new regioselective amide coupling procedure allows the preparation of a variety of xanthine

precursors. Moreover, the procedure will be well-suitable for automated and parallel synthesis.

AUTHOR CONTRIBUTIONS

DM performed most of the experiments. CM supervised the experiments. LW and MS had the idea to use COMU for the synthesis of xanthine precursors, and performed initial experiments elaborating reaction and workup conditions. GS determined the X-ray crystal structure. MS supervised experiments performed by LW. All authors contributed to writing the manuscript.

ACKNOWLEDGMENTS

We thank Dr. Jörg Hockemeyer for helpful discussions, and Marion Schneider, Annette Reiner, and Sabine Terhart-Krabbe for recording NMR and MS data. MS and LW were supported by the German Research Foundation DFG (SCHO 1593/1-1).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2019.00056/full#supplementary-material>

For details of synthetic procedures, crystallographic parameters, and NMR and IR spectra see **Supporting Material Data Sheets 1 and 2**.

REFERENCES

- Alciato, P., Cantone, P. A., Fico, D., Gagliardini, R., and Petrella, V. (1990). Bamifylline in the therapy of asthmatic syndromes. Efficacy and side effects vs delayed-action theophylline anhydride. *Minerva Med.* 81, 93–97.
- Baraldi, P. G., Fruttarolo, F., Tabrizi, M. A., Romagnoli, R., and Preti, D. (2007). Novel 8-heterocyclyl xanthine derivatives in drug development—an update. *Expert Opin. Drug. Discov.* 2, 1161–1183. doi: 10.1517/17460441.2.9.1161
- Baraldi, P. G., Tabrizi, M. A., Gessi, S., and Borea, P. A. (2008). Adenosine receptor antagonists: translating medicinal chemistry and pharmacology into clinical utility. *Chem. Rev.* 108, 238–263. doi: 10.1021/cr0682195
- Baraldi, P. G., Tabrizi, M. A., Preti, D., Bovero, A., Romagnoli, R., Fruttarolo, F., et al. (2004). Design, synthesis, and biological evaluation of new 8-heterocyclic xanthine derivatives as highly potent and selective human A_{2B} adenosine receptor antagonists. *J. Med. Chem.* 47, 1434–1447. doi: 10.1021/jm0309654
- Basu, S., Barawkar, D. A., Ramdas, V., Patel, M., Waman, Y., Panmand, A., et al. (2017). Design and synthesis of novel xanthine derivatives as potent and selective A_{2B} adenosine receptor antagonists for the treatment of chronic inflammatory airway diseases. *Eur. J. Med. Chem.* 134, 218–229. doi: 10.1016/j.ejmech.2017.04.014
- Borrmann, T., Hinz, S., Bertarelli, D. C., Li, W., Florin, N. C., Scheiff, A. B., et al. (2009). 1-Alkyl-8-(piperazine-1-sulfonyl)phenylxanthines: development and characterization of adenosine A_{2B} receptor antagonists and a new radioligand with subnanomolar affinity and subtype specificity. *J. Med. Chem.* 52, 3994–4006. doi: 10.1021/jm900413e
- Chen, J. F., Eltzschig, H. K., and Fredholm, B. B. (2013). Adenosine receptors as drug targets—what are the challenges? *Nat. Rev. Drug. Disc.* 12, 265–286. doi: 10.1038/nrd3955
- Cheng, R. K. Y., Segala, E., Robertson, N., Deflorian, F., Doré, A. S., Errey, J. C., et al. (2017). Structures of human A₁ and A_{2A} adenosine receptors with xanthines reveal determinants of selectivity. *Structure* 25, 1275–1285. doi: 10.1016/j.str.2017.06.012
- Costante, R., Stefanucci, A., Carradori, S., Novellino, E., and Mollica, A. (2015). DPP-4 inhibitors: a patent review (2012–2014). *Expert Opin. Ther. Pat.* 25, 209–236. doi: 10.1517/13543776.2014.991309
- Crepaldi, G., Carruba, M., Comaschi, M., Del Prato, S., Frajese, G., and Paolisso, G. (2007). Dipeptidyl peptidase 4 (DPP-4) inhibitors and their role in type 2 diabetes management. *J. Endocrinol. Invest.* 30, 610–614. doi: 10.1007/bf03346357
- Daly, J. W., Padgett, W., Shamim, M. T., Butts-Lamb, P., and Waters, J. (1985). 1,3-Dialkyl-8-(p-sulfophenyl)xanthines: potent water-soluble antagonists for A₁- and A₂-adenosine receptors. *J. Med. Chem.* 28, 487–492. doi: 10.1021/jm00382a018
- Doré, A. S., Robertson, N., Errey, J. C., Ng, I., Hollenstein, K., Tehan, B., et al. (2011). Structure of the adenosine A_{2A} receptor in complex with ZM241385 and the xanthines XAC and caffeine. *Structure* 19, 1283–1293. doi: 10.1016/j.str.2011.06.014
- Dungo, R., and Deeks, E. D. (2013). Istradefylline: first global approval. *Drugs* 73, 875–882. doi: 10.1007/s40265-013-0066-7
- El-Faham, A., and Albericio, F. (2010). COMU: a third generation of uronium-type coupling reagents. *J. Pept. Sci.* 16, 6–9. doi: 10.1002/psc.1204
- El-Faham, A., and Albericio, F. (2011). Peptide coupling reagents, more than a letter soup. *Chem. Rev.* 111, 6557–6602. doi: 10.1021/cr100048w
- El-Faham, A., Subirós Funos, R., Prohens, R., and Albericio, F. (2009). COMU: a safer and more effective replacement for benzotriazole-based uronium coupling reagents. *Chem. Eur. J.* 15, 9404–9416. doi: 10.1002/chem.200900615
- El-Sabbagh, O. I., El-Sadek, M. E., El-Kalyoubi, S., and Ismail, I. (2007). Synthesis, DNA binding and antiviral activity of new uracil, xanthine, and pteridine derivatives. *Arch. Pharm.* 340, 26–31. doi: 10.1002/ardp.200600149

- Feoktistov, I., Biaggioni, I., Polosa, R., and Holgate, S. T. (1998). Adenosine A_{2B} receptors: a novel therapeutic target in asthma? *Trends. Pharmacol. Sci.* 19, 148–153. doi: 10.1016/S0165-6147(98)01179-1
- Franco, R., Oñatibia-Astibia, A., and Martínez-Pinilla, E. (2013). Health benefits of methylxanthines in cacao and chocolate. *Nutrients* 5, 4159–4173. doi: 10.3390/nu5104159
- Gallagher, M. (2004). *Method for Improving Cognitive Function by Co-administration of a GABA_B Receptor Antagonist and an Acetylcholinesterase Inhibitor* (Baltimore: Michela Gallagher), US20050267077A1.
- Glukhova, A., Thal, D. M., Nguyen, A. T., Vecchio, E. A., Jörg, M., Scammells, P. J., et al. (2017). Structure of the adenosine A₁ receptor reveals the basis for subtype selectivity. *Cell* 168, 867–877. doi: 10.1016/j.cell.2017.01.042
- Hayallah, A. M., Sandoval-Ramírez, J., Reith, U., Schobert, U., Preiss, B., Schumacher, B., et al. (2002). 1,8-Disubstituted xanthine derivatives: synthesis of potent A_{2B}-selective adenosine receptor antagonists. *J. Med. Chem.* 45, 1500–1510. doi: 10.1021/jm011049y
- Hinz, S., Alnouri, W. M., Pleiss, U., and Müller, C. E. (2018). Tritium-labeled agonists as tools for studying adenosine A_{2B} receptors. *Purinergic Signal.* 14, 223–233. doi: 10.1007/s11302-018-9608-5
- Hjørringgaard, C. U., Brust, A., and Alewood, P. F. (2012). Evaluation of COMU as a coupling reagent for *in situ* neutralization Boc solid phase peptide synthesis. *J. Pept. Sci.* 18, 199–207. doi: 10.1002/psc.1438
- Hockemeyer, J., Burbiel, J. C., and Müller, C. E. (2004). Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: potential drugs for Parkinson's disease. *J. Org. Chem.* 69, 3308–3318. doi: 10.1021/jo0358574
- Ilaš, J., Pečar, S., Hockemeyer, J., Euler, H., Kirfel, A., and Müller, C. E. (2005). Development of spin-labeled probes for adenosine receptors. *J. Med. Chem.* 48, 2108–2114. doi: 10.1021/jm049513x
- Jacobson, K. A. (2009). Functionalized congener approach to the design of ligands for G protein-coupled receptors (GPCRs). *Bioconjug. Chem.* 20, 1816–1835. doi: 10.1021/bc9000596
- Jacobson, K. A., Kiriasis, L., Barone, S., Bradbury, B. J., Kammula, U., Campagne, J. M., et al. (1989). Sulfur-containing 1,3-dialkylxanthine derivatives as selective antagonists at A₁-adenosine receptors. *J. Med. Chem.* 32, 1873–1879. doi: 10.1021/jm00128a031
- Jacobson, K. A., Shi, D., Gallo-Rodriguez, C., Manning, M., Müller, C., Daly, J. W., et al. (1993). Effect of trifluoromethyl and other substituents on activity of xanthines at adenosine receptors. *J. Med. Chem.* 36, 2639–2644. doi: 10.1021/jm00070a007
- Kim, Y. C., Ji, X., Melman, N., Linden, J., and Jacobson, K. A. (2000). Anilide derivatives of an 8-phenylxanthine carboxylic congener are highly potent and selective antagonists at human A_{2B} adenosine receptors. *J. Med. Chem.* 43, 1165–1172. doi: 10.1021/jm990421v
- Kondo, T., and Mizuno, Y. (2015). A long-term study of istradefylline safety and efficacy in patients with Parkinson's disease. *Clin. Neuropharmacol.* 38, 41–46. doi: 10.1097/wnf.0000000000000073
- Köse, M., Gollos, S., Karcz, T., Fiene, A., Heisig, F., Behrenswerth, A., et al. (2018). Fluorescent-labeled selective adenosine A_{2B} receptor antagonist enables competition binding assay by flow cytometry. *J. Med. Chem.* 61, 4301–4316. doi: 10.1021/acs.jmedchem.7b01627
- Leone, R. D., Lo, Y.-C., and Powell, J. D. (2015). A_{2A}R antagonists: next generation checkpoint blockade for cancer immunotherapy. *Comput. Struct. Biotechnol. J.* 13, 265–272. doi: 10.1016/j.csbj.2015.03.008
- LeWitt, P. A., Guttman, M., Tetrud, J. W., Tuite, P. J., Mori, A., Chaikin, P., et al. (2008). Adenosine A_{2A} receptor antagonist istradefylline (KW-6002) reduces “off” time in Parkinson's disease: a double-blind, randomized, multicenter clinical trial (6002-US-005). *Ann. Neurol.* 63, 295–302. doi: 10.1002/ana.21315
- Liu, W., Chun, E., Thompson, A. A., Chubukov, P., Xu, F., Katritch, V., et al. (2012). Structural basis for allosteric regulation of GPCRs by sodium ions. *Science* 337:232. doi: 10.1126/science.1219218
- MacMillan, D. S., Murray, J., Sneddon, H. F., Jamieson, C., and Watson, A. B. J. (2013). Evaluation of alternative solvents in common amide coupling reactions: replacement of dichloromethane and *N,N*-dimethylformamide. *Green Chem.* 15, 596–600. doi: 10.1039/c2gc36900a
- Maxwell, C. E., and Salivar, C. J. (1952). *Method of Preparing 4-Aminouracils*. Brooklyn, NY: Pfizer & Co., Inc.
- Moore, A. G., Schow, S. R., Lum, R. T., Nelson, M. G., and Melville, C. R. (1999). Convenient one-pot synthesis of 8-substituted xanthines from 6-amino-5-nitrosouracils. *Synthesis* 7, 1123–1126. doi: 10.1055/s-1999-3513
- Müller, C., and Jacobson, K. A. (2011). Xanthines as adenosine receptor antagonists. *Handb. Exp. Pharmacol.* 200, 151–199. doi: 10.1007/978-3-642-13443-2_6
- Müller, C. E., Baqi, Y., Hinz, S., and Namasivayam, V. (2018). “Medicinal chemistry of A_{2B} adenosine receptors,” in *The Adenosine Receptors*, eds. P. A. Borea, K. Varani, S. Gessi, S. Merighi, and F. Vincenzi (Cham: Springer International Publishing), 137–168.
- Müller, C. E., Diekmann, M., Thorand, M., and Ozola, V. (2002). [³H]8-Ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one ([³H]PSB-11), a novel high-affinity antagonist radioligand for human A₃ adenosine receptors. *Bioorg. Med. Chem. Lett.* 12, 501–503. doi: 10.1016/S0960-894X(01)00785-5
- Müller, C. E., Hockemeyer, J., Tzvetkov, N., and Burbiel, J. (2008). *New 8-Ethylxanthine Compounds Useful for Treating e.g. Parkinson's Disease, Catalepsy, Dystonia, Dyskinetic Syndrome, Restless Legs Syndrome, Migraine, Pain, Dementia, Neurodegenerative Disorders, Alcohol Withdrawal, and Stroke* (Monheim), WO002008077557A1.
- Müller, C. E., Shi, D., Manning Jr., M., and Daly, J. W. (1993). Synthesis of paraxanthine analogs (1, 7-disubstituted xanthines) and other xanthines unsubstituted at the 3-position: structure-activity relationships at adenosine receptors. *J. Med. Chem.* 36, 3341–3349. doi: 10.1021/jm00074a015
- Nieber, K. (2017). The impact of coffee on health. *Planta Med.* 83, 1256–1263. doi: 10.1055/s-0043-115007
- Oñatibia-Astibia, A., Franco, R., and Martínez-Pinilla, E. (2017). Health benefits of methylxanthines in neurodegenerative diseases. *Mol. Nutr. Food Res.* 61, 1–14. doi: 10.1002/mnfr.201600670
- Ozola, V., Thorand, M., Diekmann, M., Qurishi, R., Schumacher, B., Jacobson, K. A., et al. (2003). 2-Phenylimidazo[2,1-*i*]purin-5-ones: Structure-activity relationships and characterization of potent and selective inverse agonists at human A₃ adenosine receptors. *Bioorg. Med. Chem.* 11, 347–356. doi: 10.1016/s0968-0896(02)00456-x
- Park, A., and Stacy, M. (2012). Istradefylline for the treatment of Parkinson's disease. *Expert Opin. Pharmacother.* 13, 111–114. doi: 10.1517/14656566.2012.643869
- Rabasseda, X., Sorbera, L., Martin, L., Leeson, P., and Castañer, J. (2001). Monographs-KW-6002. Antiparkinsonian, antidepressant, adenosine A₂ antagonist. *Drug Fut.* 26, 20–24.
- Rodríguez-Borges, J. E., García-Mera, X., Balo, M. C., Brea, J., Caamaño, O., Fernández, F., et al. (2010). Synthesis and pharmacological evaluation of novel 1,3,8- and 1,3,7,8-substituted xanthines as adenosine receptor antagonists. *Bioorg. Med. Chem.* 18, 2001–2009. doi: 10.1016/j.bmc.2010.01.028
- Sauer, R., Maurinsh, J., Reith, U., Fülle, F., Klotz, K. N., and Müller, C. E. (2000). Water-soluble phosphate prodrugs of 1-propargyl-8-styrylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. *J. Med. Chem.* 43, 440–448. doi: 10.1021/jm9911480
- Scammells, P. J., Baker, S. P., Belardinelli, L., and Olsson, R. A. (1994). Substituted 1,3-dipropylxanthines as irreversible antagonists of A₁ adenosine receptors. *J. Med. Chem.* 37, 2704–2712. doi: 10.1021/jm00043a010
- Singh, S., and Yadav, R. (2016). Bronchial asthma: a global health problem. *Int. J. Pharm. Bio. Sci.* 7, 163–173. doi: 10.22376/ijpbs.2016.7.4.p163-173
- Slawsky, M. T., and Givertz, M. M. (2009). Rolofylline: a selective adenosine 1 receptor antagonist for the treatment of heart failure. *Expert Opin. Pharmacother.* 10, 311–322. doi: 10.1517/14656560802682213
- Sun, B., Bachhawat, P., Chu, M. L., Wood, M., Ceska, T., Sands, Z. A., et al. (2017). Crystal structure of the adenosine A_{2A} receptor bound to an antagonist reveals a potential allosteric pocket. *Proc. Natl. Acad. Sci. U.S.A.* 114, 2066–2071. doi: 10.1073/pnas.1621423114

- Twibanire, J. A. K., and Grindley, T. B. (2011). Efficient and controllably selective preparation of esters using uronium-based coupling agents. *Org. Lett.* 13, 2988–2991. doi: 10.1021/ol201005s
- Yan, L., Bertarelli, D. C. G., Hayallah, A. M., Meyer, H., Klotz, K. N., and Müller, C. E. (2006). A new synthesis of sulfonamides by aminolysis of *p*-nitrophenylsulfonates yielding potent and selective adenosine A_{2B} receptor antagonists. *J. Med. Chem.* 49, 4384–4391. doi: 10.1021/jm060277v
- Yang, S. M., Yasgar, A., Miller, B., Lal-Nag, M., Brimacombe, K., Hu, X., et al. (2015). Discovery of NCT-501, a potent and selective theophylline-based inhibitor of aldehyde dehydrogenase 1A1 (ALDH1A1). *J. Med. Chem.* 58, 5967–5978. doi: 10.1021/acsjmedchem.5b00577

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Marx, Wingen, Schnakenburg, Müller and Scholz. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Supplementary Material

Fast, efficient and versatile synthesis of 6-amino-5-carboxamidouracils as precursors for 8-substituted xanthines

Daniel Marx[‡], Lukas M. Wingen[‡], Gregor Schnakenburg, Christa E. Müller*, Matthias S. Scholz

* **Correspondence:** Christa E. Müller: christa.mueller@uni-bonn.de

[‡] These authors contributed equally to this work

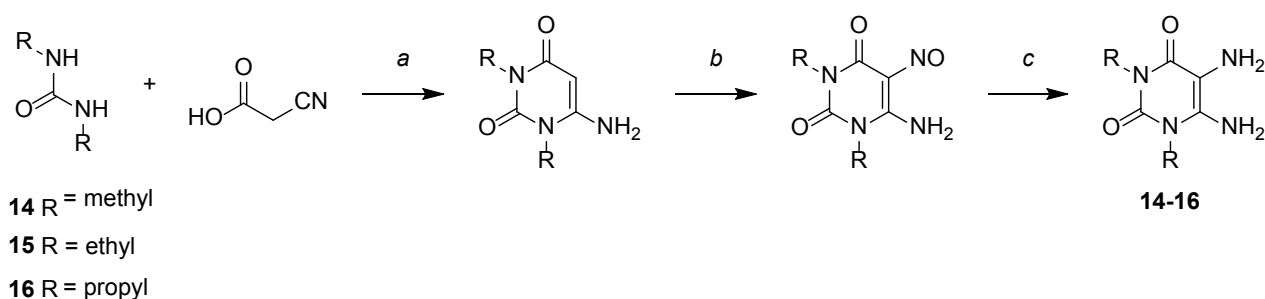
TABLE OF CONTENTS

Synthesis of diaminouracil derivatives.....	2
Crystallographic data.....	3
¹ H- and ¹³ C-NMR spectra of compound 21-39..	8
IR spectra of compound 21-39	28
References	37

Synthesis of diaminouracil derivatives

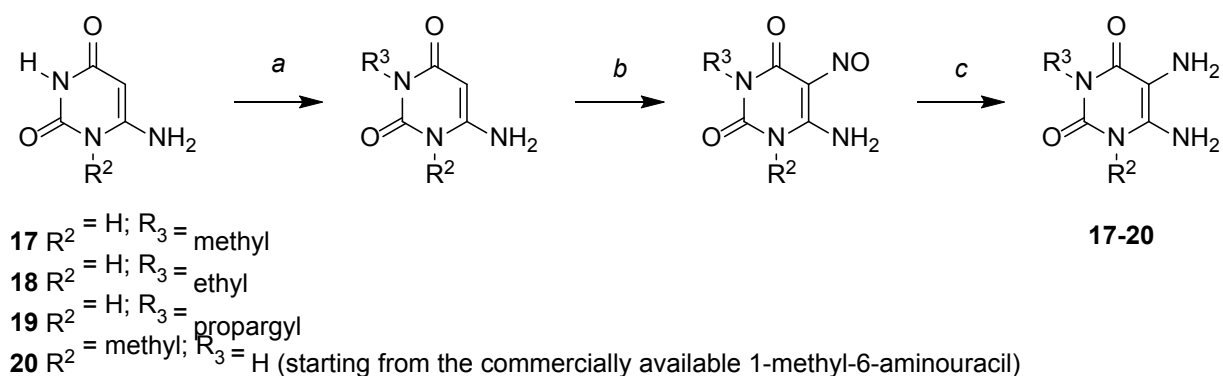
Different *N*1- and *N*3-substituted uracil derivatives required as precursors were synthesized according to literature procedures (Maxwell, et al., 1952; Müller et al., 1993; Hockemeyer et al., 2004). Due to the therapeutic potential of A_{2A} and A_{2B} adenosine receptor antagonists, we synthesized various 6-amino-5-carboxamidouracil precursors for xanthine derivatives containing ethyl and propargyl substituents at the uracil *N*1 atom, which are known to be beneficial for interaction with those adenosine receptor subtypes.

Scheme S1. Preparation of symmetrical *N*1,*N*3-dialkyl-5,6-diaminouracil derivatives.



Reagents and conditions: (a) Ac₂O, 60 °C, 3 h; (b) aq. AcOH, HNO₂, 50-60 °C; (c) sodium dithionite, NH₃/H₂O, 60 °C.

Scheme S2. Preparation of *N*1- or *N*3-substituted 5,6-diaminouracil derivatives.



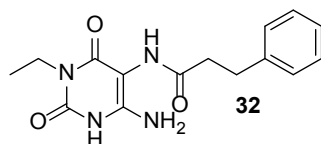
Reagents and conditions: (a) 2.1 equiv. of hexamethyldisilazane (HMDS), reflux, 60–70 °C, 1.7 equiv of methyl iodide for (**17**), ethyl iodide for (**18**) or 3-bromopropyne for (**19**); (b) aq. AcOH, HNO₂, 50-60 °C; (c) sodium dithionite, NH₃/H₂O, 60 °C.

Analytical data were in accordance with published data. For details see (Maxwell, et al., 1952; Müller et al., 1993; Hockemeyer et al., 2004).

Crystallographic data

CCDC 1878798 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

Table S1. Crystal data and structure refinement for compound 32.



Identification code	GPHARM62, 32 // GXray5353f
Crystal Habitus	clear colourless plate
Device Type	Bruker X8-KappaApexII
Empirical formula	C ₁₅ H ₁₈ N ₄ O ₃
Moiety formula	C ₁₅ H ₁₈ N ₄ O ₃
Formula weight	302.33
Temperature/K	100
Crystal system	monoclinic
Space group	P2 ₁
a/Å	4.6833(3)
b/Å	28.2023(18)
c/Å	5.4732(4)
α/°	90
β/°	98.690(3)

3

$\gamma/^\circ$	90
Volume/ \AA^3	714.60(8)
Z	2
$\rho_{\text{calc}}/\text{g/cm}^3$	1.405
μ/mm^{-1}	0.101
F(000)	320.0
Crystal size/ mm^3	$0.4 \times 0.2 \times 0.08$
Absorption correction	multi-scan
Tmin; Tmax	0.6046; 0.7460
Radiation	MoK α ($\lambda = 0.71073$)
2 Θ range for data collection/ $^\circ$	7.532 to 60.124 $^\circ$
Completeness to theta	0.995
Index ranges	$-2 \leq h \leq 6, -39 \leq k \leq 39, -7 \leq l \leq 7$
Reflections collected	11797
Independent reflections	4180 [$R_{\text{int}} = 0.0380, R_{\text{sigma}} = 0.0471$]
Data/restraints/parameters	4180/1/201
Goodness-of-fit on F^2	1.026
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0441, wR_2 = 0.1014$
Final R indexes [all data]	$R_1 = 0.0569, wR_2 = 0.1097$
Largest diff. peak/hole / $e \text{\AA}^{-3}$	0.32/-0.23
Flack parameter	0.2(5)

Table S2. Bond lengths for compound 32.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O7	C2	1.214(3)	C5	C6	1.375(3)
O10	C4	1.244(3)	C8	C9	1.523(4)
O13	C12	1.237(3)	C12	C14	1.511(3)
N1	C2	1.381(3)	C14	C15	1.522(3)
N1	C6	1.369(3)	C15	C16	1.515(3)
N3	C2	1.383(3)	C16	C17	1.381(4)
N3	C4	1.396(3)	C16	C21	1.386(4)
N3	C8	1.485(3)	C17	C18	1.391(4)
N11	C5	1.420(3)	C18	C19	1.355(5)
N11	C12	1.351(3)	C19	C20	1.384(5)
N22	C6	1.351(3)	C20	C21	1.387(4)
C4	C5	1.425(3)			

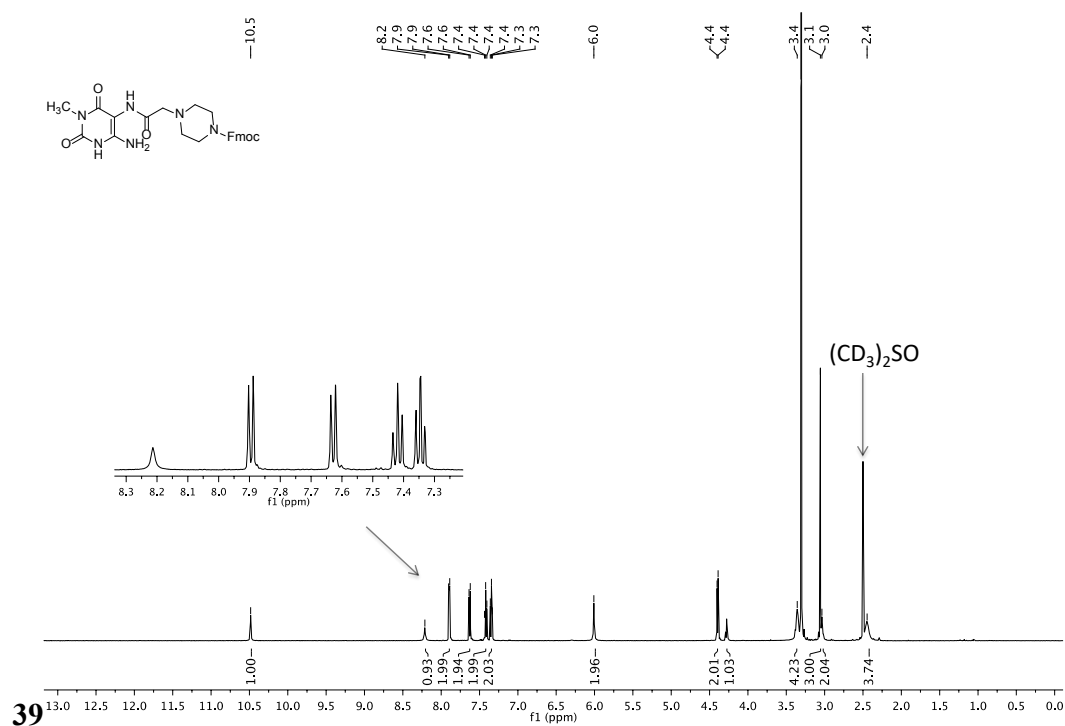
Table S3 Bond angles for compound 32.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C6	N1	C2	124.5(2)	N22	C6	C5	124.6(2)
C2	N3	C4	123.6(2)	N3	C8	C9	112.4(2)
C2	N3	C8	116.5(2)	O13	C12	N11	122.4(2)
C4	N3	C8	119.9(2)	O13	C12	C14	123.2(2)
C12	N11	C5	123.2(2)	N11	C12	C14	114.4(2)
O7	C2	N1	121.0(2)	C12	C14	C15	114.0(2)
O7	C2	N3	123.5(2)	C16	C15	C14	112.8(2)
N1	C2	N3	115.5(2)	C17	C16	C15	120.5(3)
O10	C4	N3	120.1(2)	C17	C16	C21	117.7(3)
O10	C4	C5	123.0(2)	C21	C16	C15	121.7(2)
N3	C4	C5	116.9(2)	C16	C17	C18	121.2(3)
N11	C5	C4	119.5(2)	C19	C18	C17	120.8(3)
C6	C5	N11	119.8(2)	C18	C19	C20	118.9(3)
C6	C5	C4	120.6(2)	C19	C20	C21	120.7(3)
N1	C6	C5	118.5(2)	C16	C21	C20	120.6(3)
N22	C6	N1	116.8(2)				

Table S4. Hydrogen bonds for compound 32.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N1	H1	O10 ¹	0.88	1.90	2.717(3)	153.7
N11	H11	O13 ²	0.88	2.39	2.975(3)	123.7
N22	H22A	O10 ¹	0.86	2.18	2.875(3)	137.5
N22	H22B	O13 ²	0.86	2.14	2.994(3)	170.5
C9	H9B	O7 ³	0.98	2.31	3.276(3)	168.6

¹-1+X,+Y,-1+Z; ²-1+X,+Y,+Z; ³+X,+Y,1+Z

¹H- and ¹³C-NMR spectra of compounds 21-**Figure S1. ¹H-NMR spectrum of 21 in (CD₃)₂SO at room temperature.**

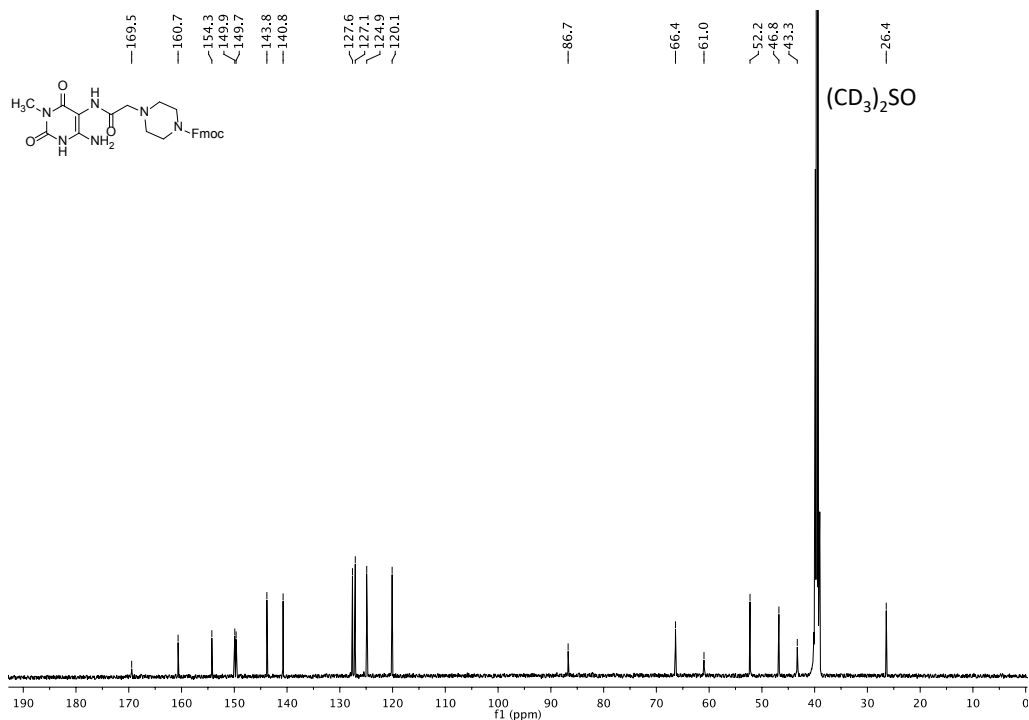


Figure S2. $^{13}\text{C-NMR}$ spectrum of 21 in $(\text{CD}_3)_2\text{SO}$ at room temperature

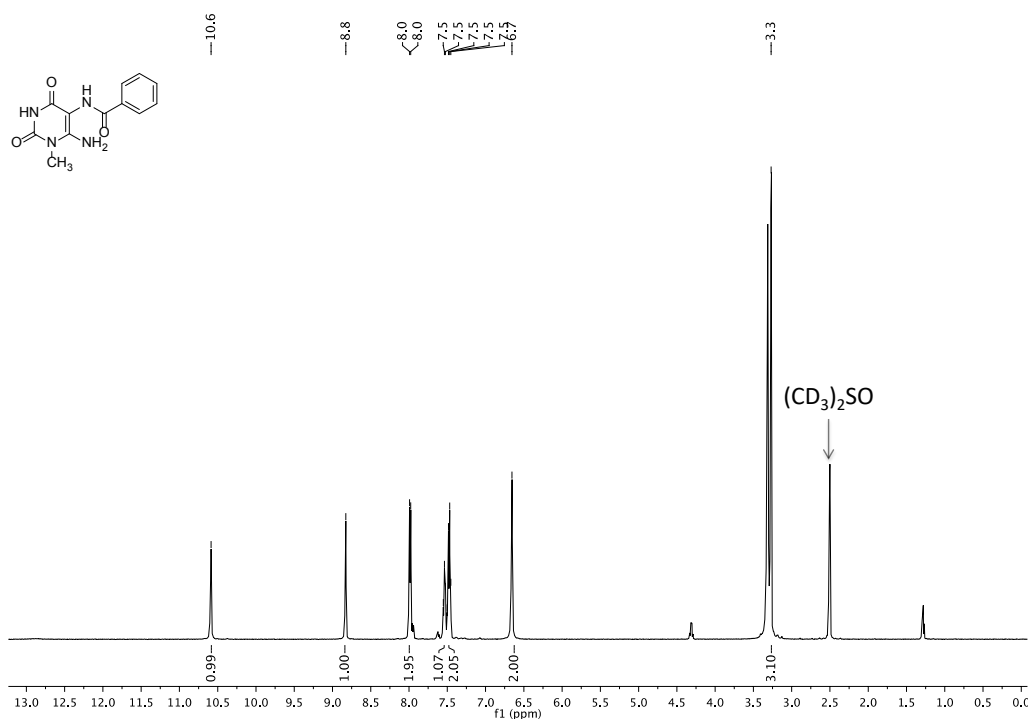


Figure S3. $^1\text{H-NMR}$ spectrum of 22 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

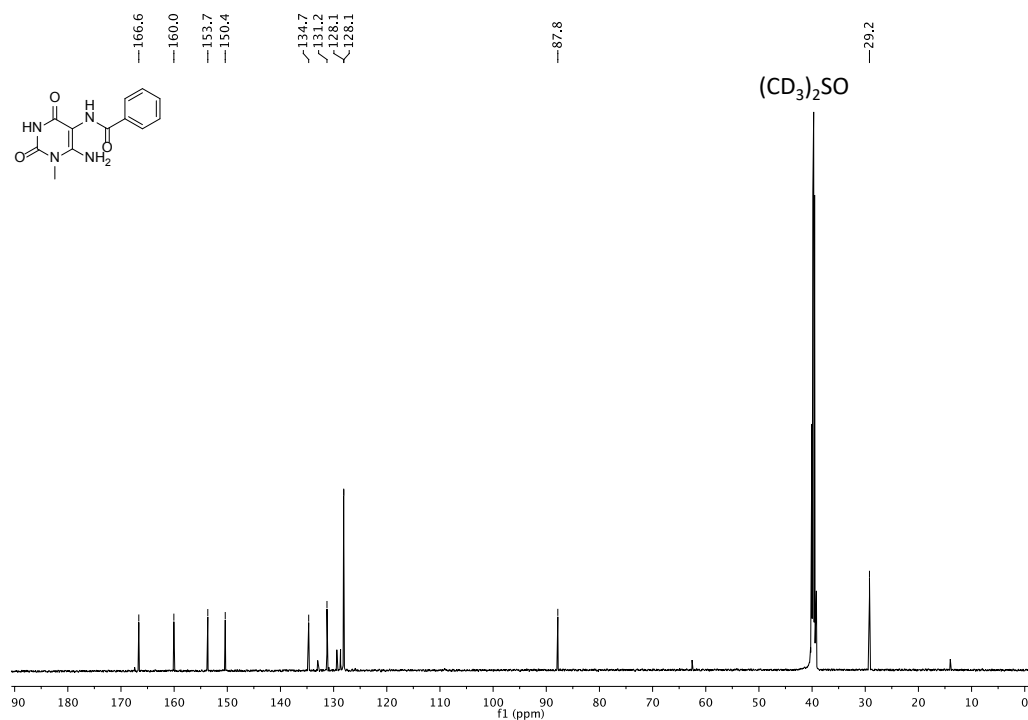


Figure S4. ^{13}C -NMR spectrum of 22 in (CD₃)₂SO at room temperature.

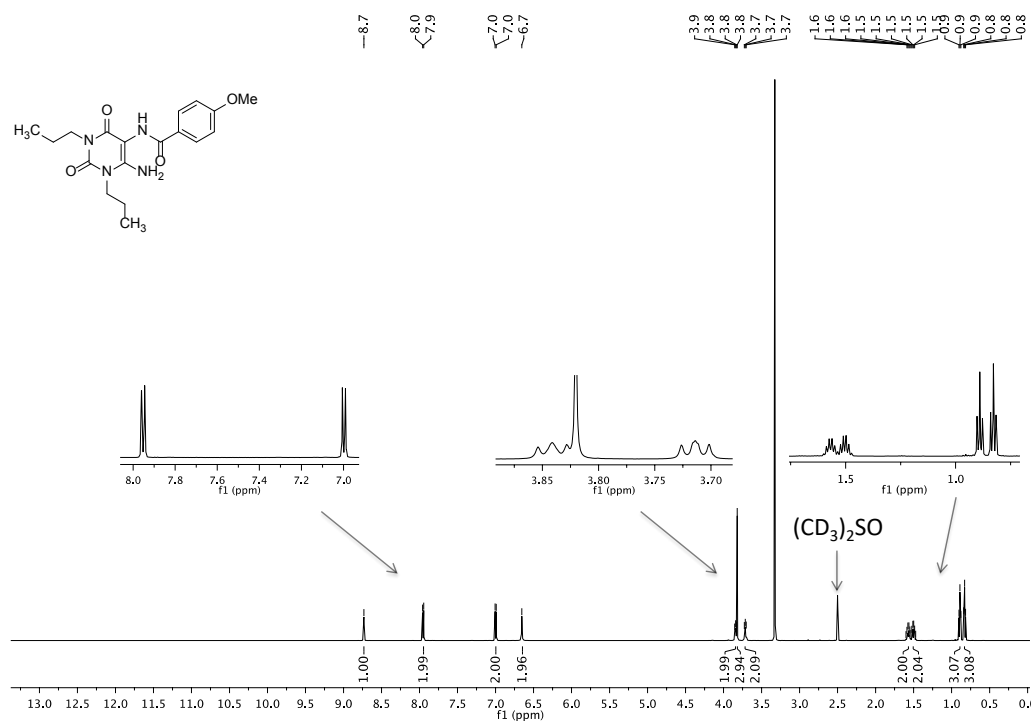


Figure S5. ^1H -NMR spectrum of 23 in (CD₃)₂SO at room temperature.

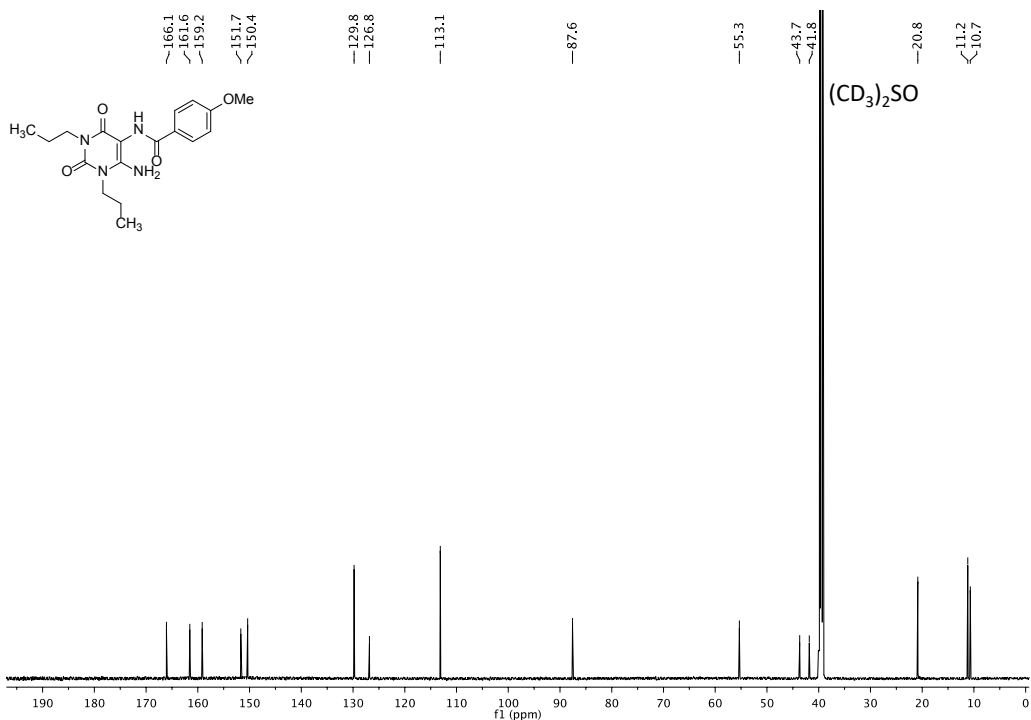


Figure S6. $^{13}\text{C-NMR}$ spectrum of 23 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

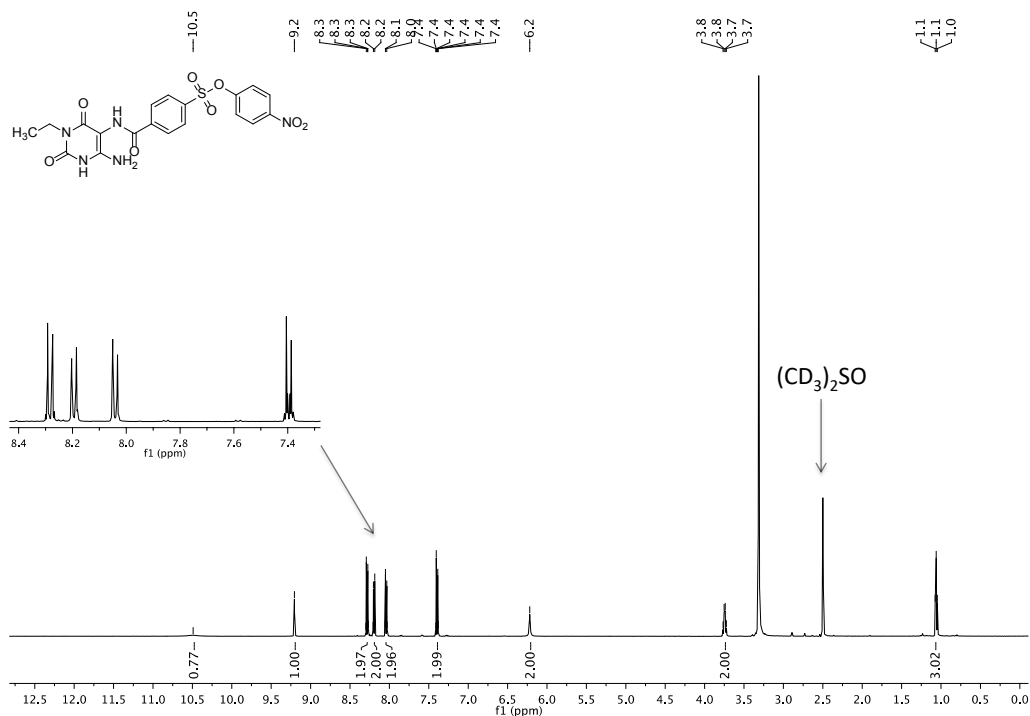


Figure S7. $^1\text{H-NMR}$ spectrum of 24 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

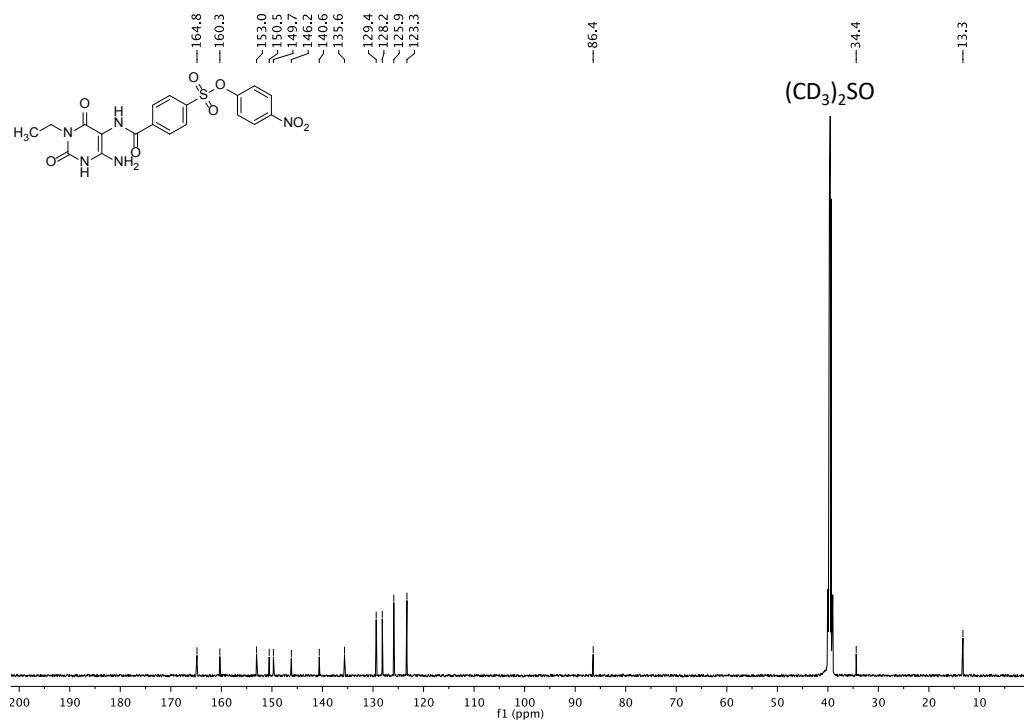


Figure S8. ^{13}C -NMR spectrum of 24 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

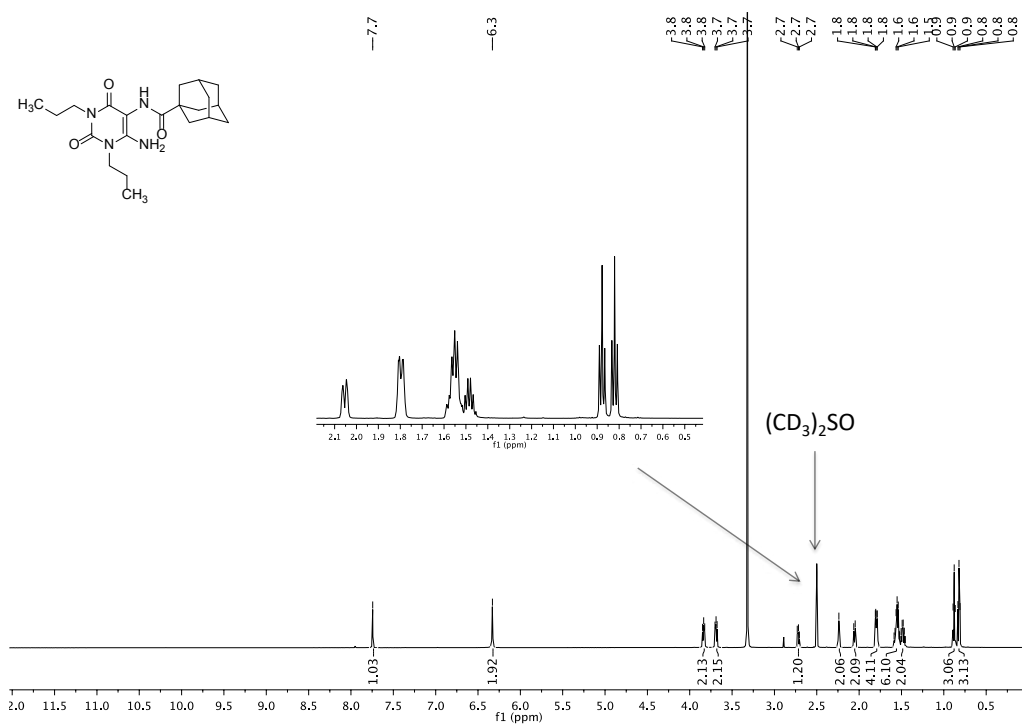


Figure S9. ^1H -NMR spectrum of 25 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

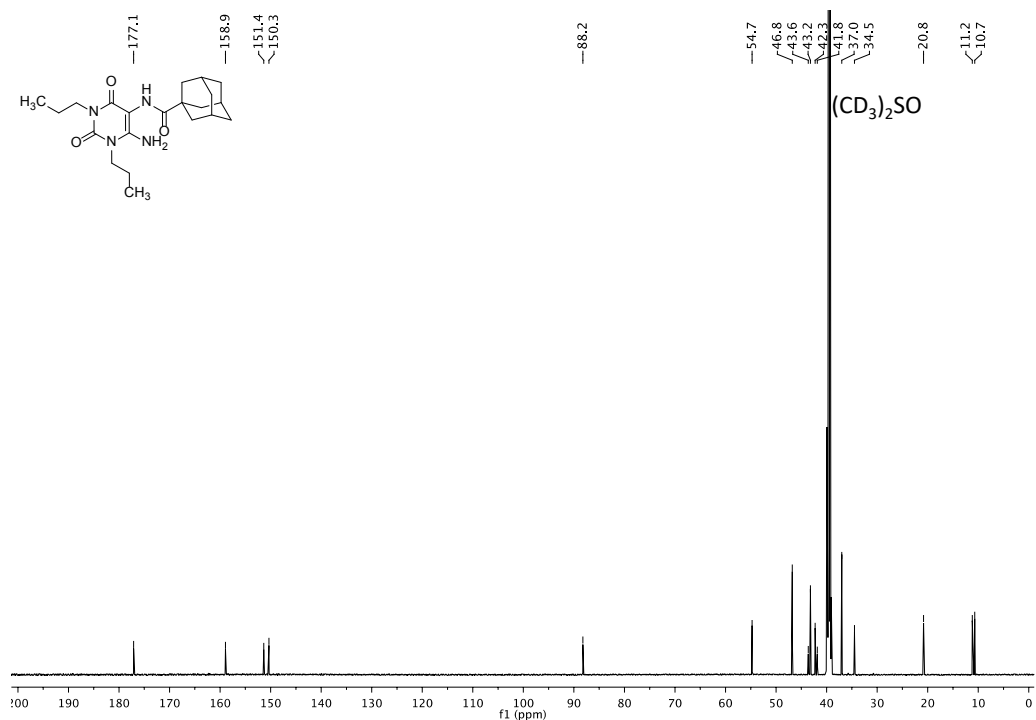


Figure S10. ¹³C-NMR spectrum of 25 in (CD₃)₂SO at room temperature.

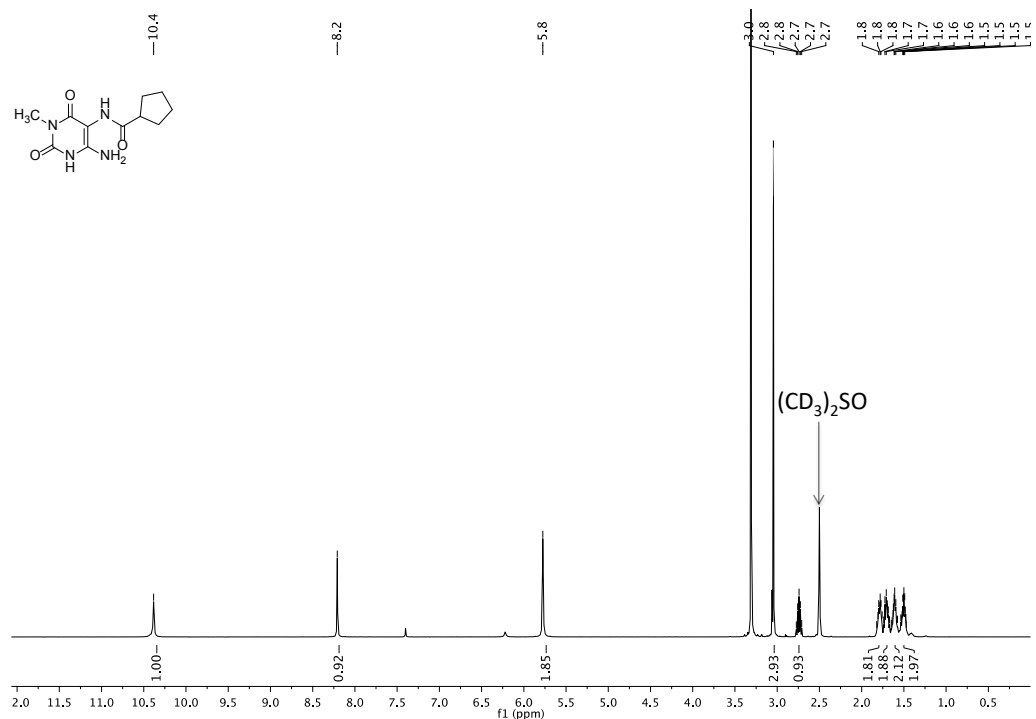


Figure S11. ¹H-NMR spectrum of 26 in (CD₃)₂SO at room temperature.

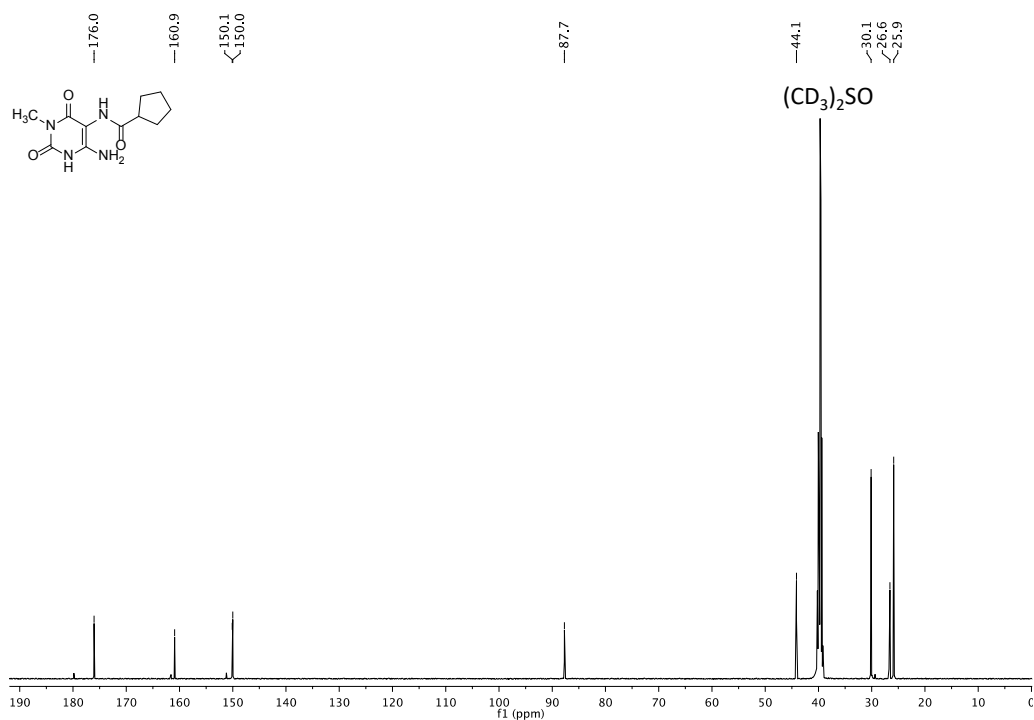


Figure S12. ^{13}C -NMR spectrum of 26 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

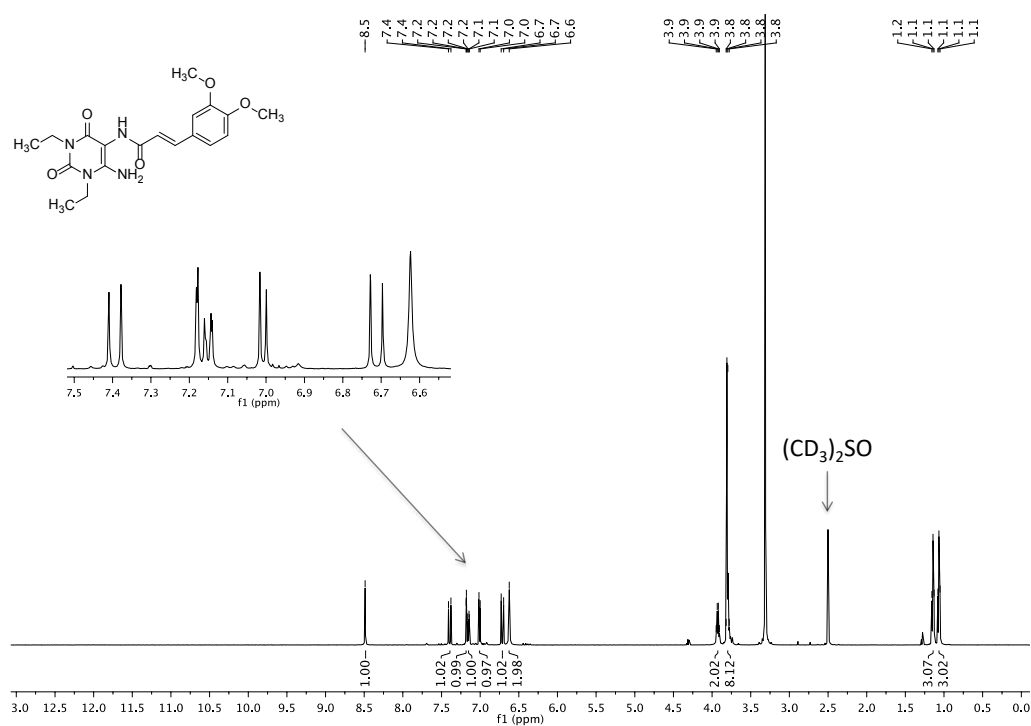


Figure S13. ^1H -NMR spectrum of 27 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

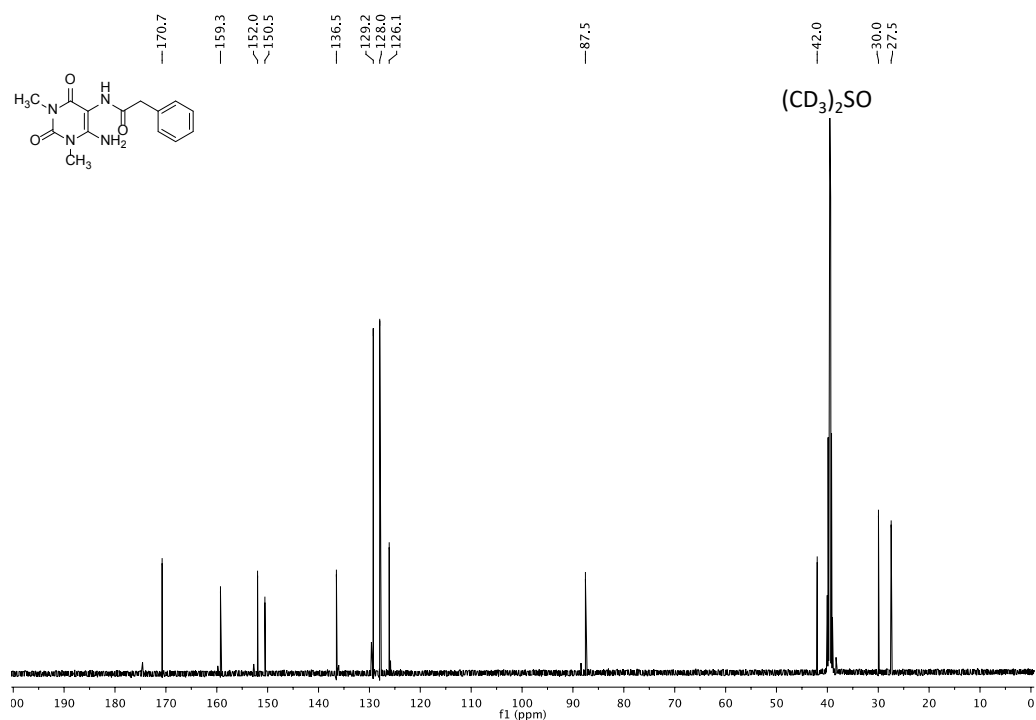


Figure S18. $^{13}\text{C-NMR}$ spectrum of 29 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

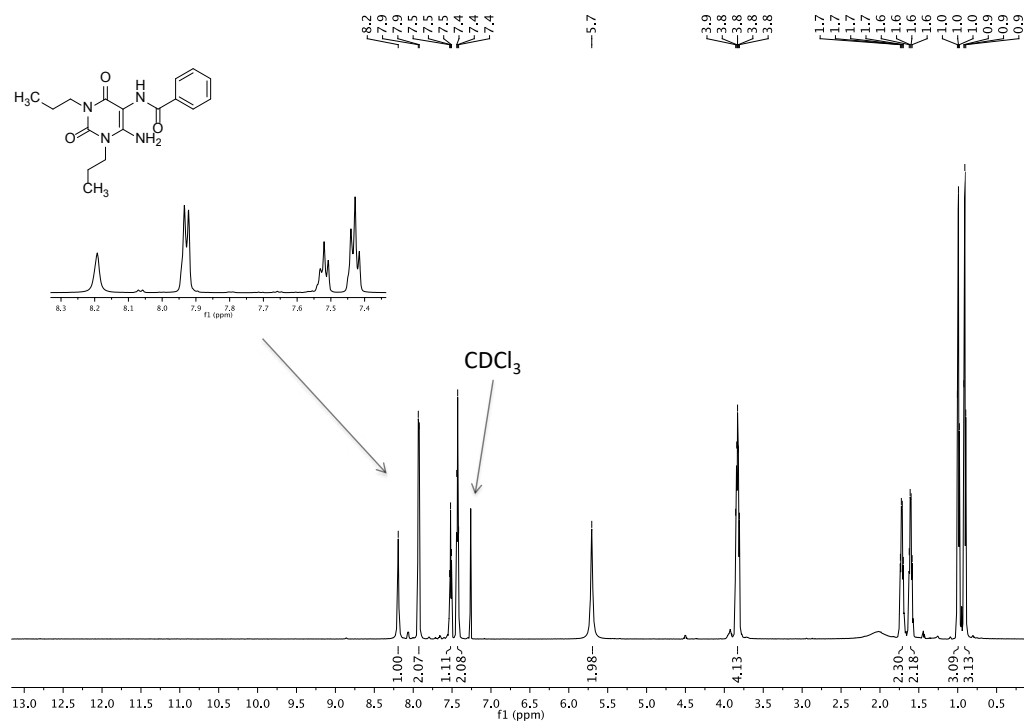


Figure S19. $^1\text{H-NMR}$ spectrum of 30 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

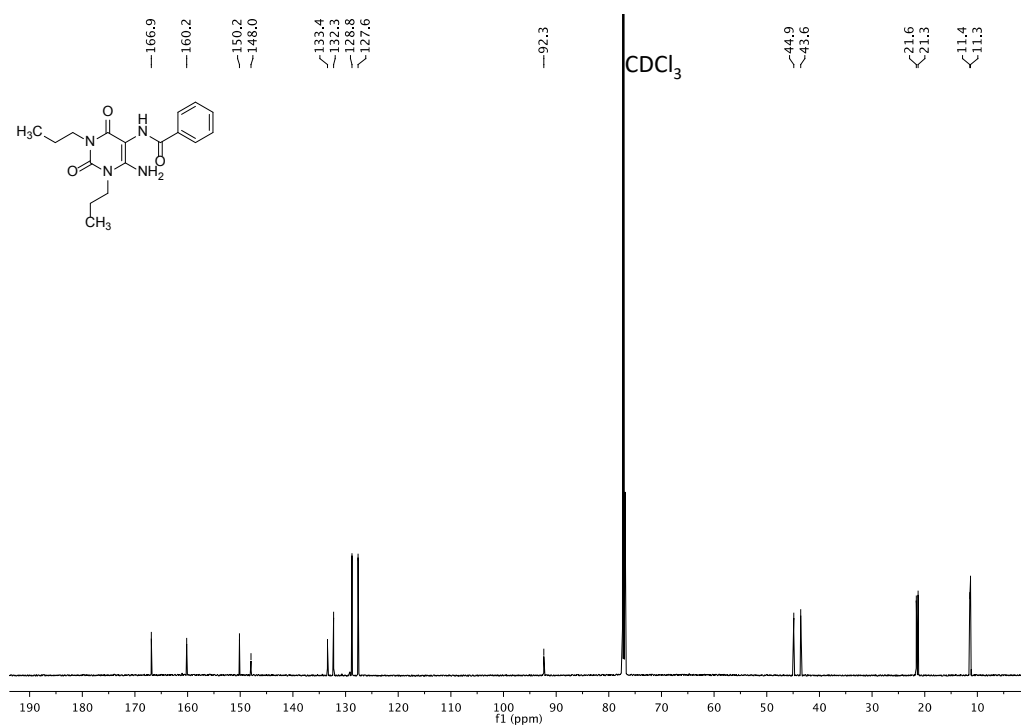


Figure S20. ^{13}C -NMR spectrum of 30 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

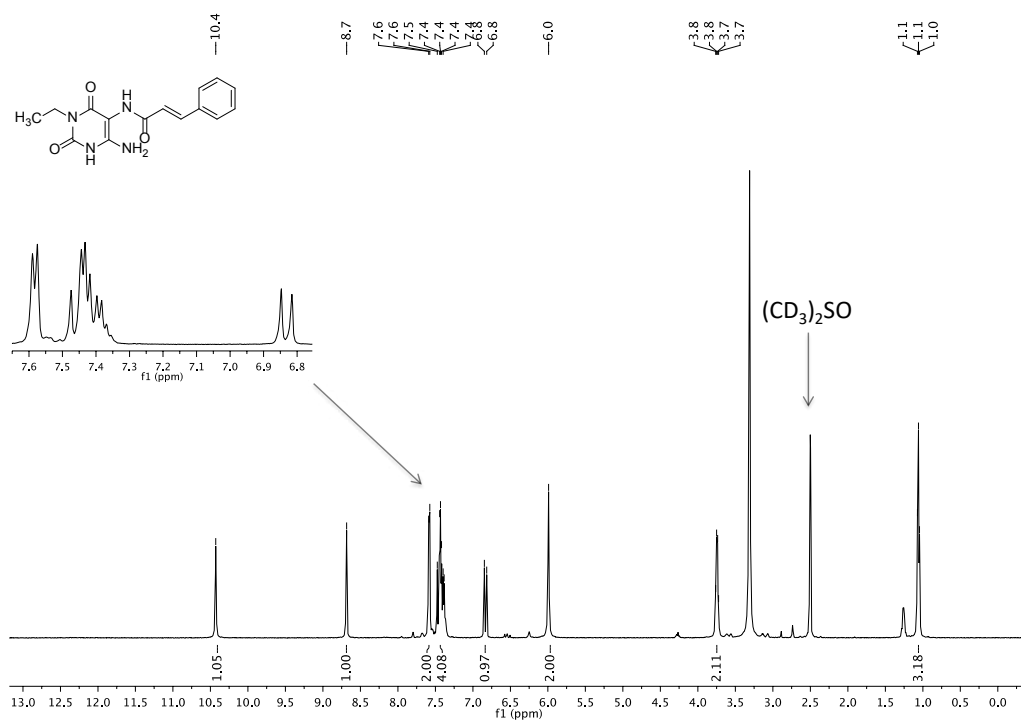


Figure S21. ^1H -NMR spectrum of 31 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

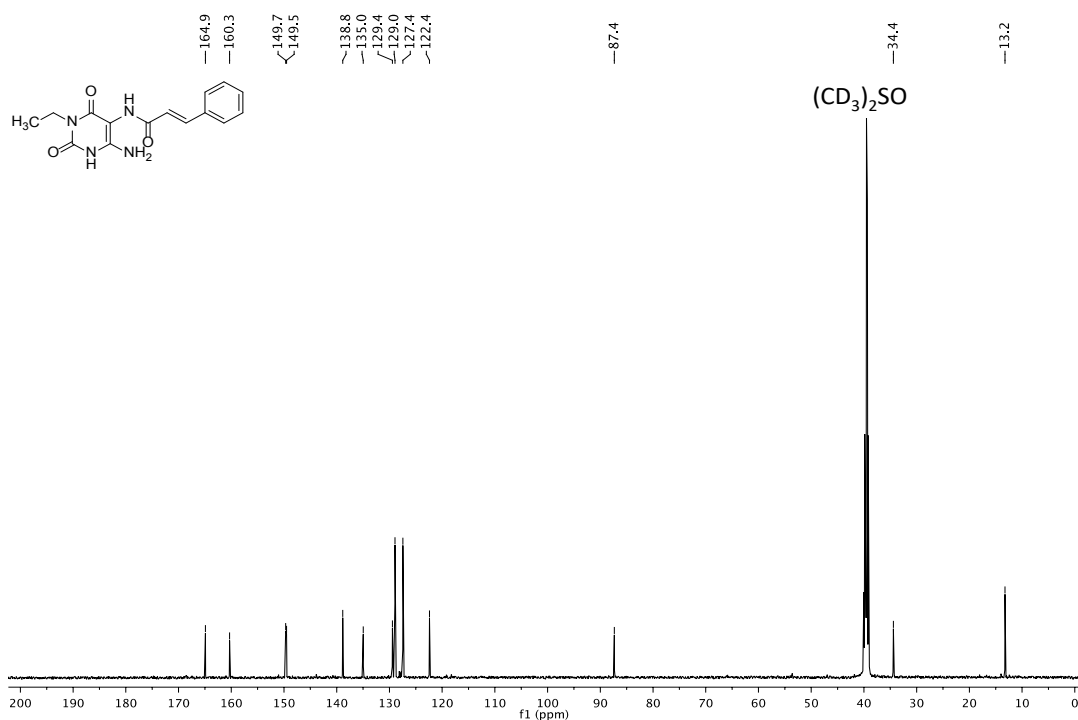


Figure S22. ^{13}C -NMR spectrum of 31 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

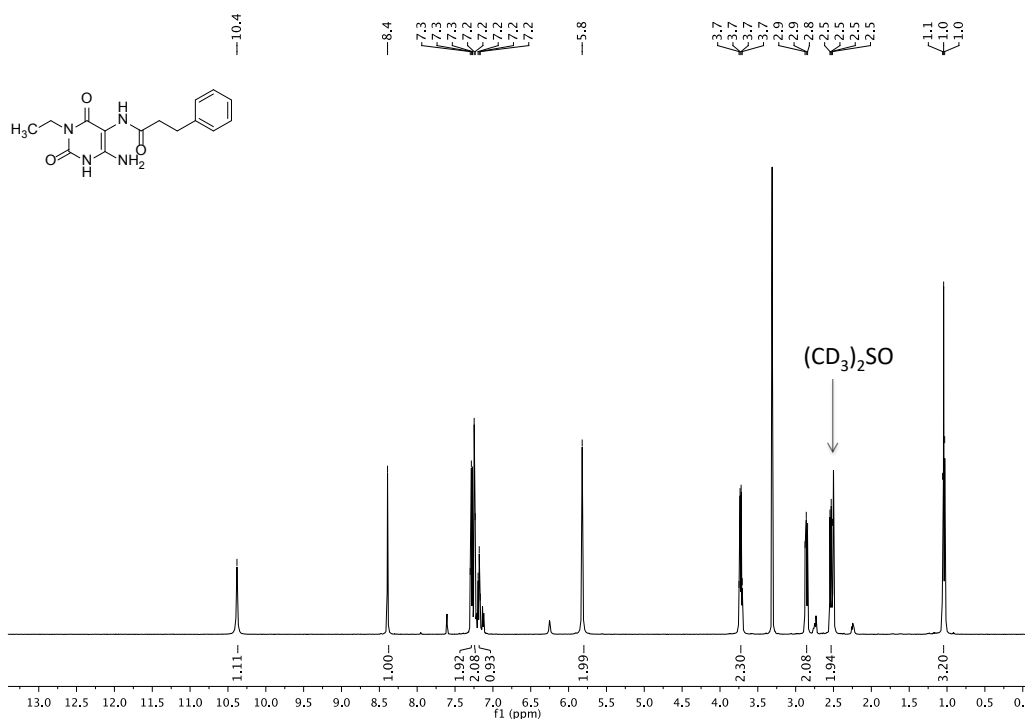


Figure S23. ^1H -NMR spectrum of 32 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

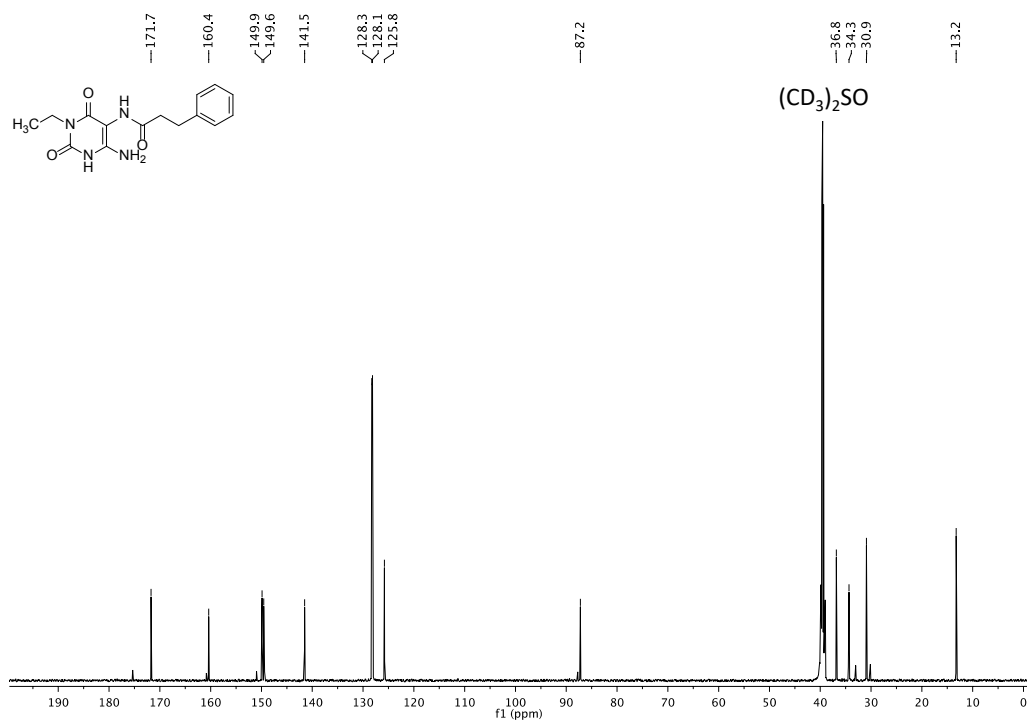


Figure S24. ^{13}C -NMR spectrum of 32 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

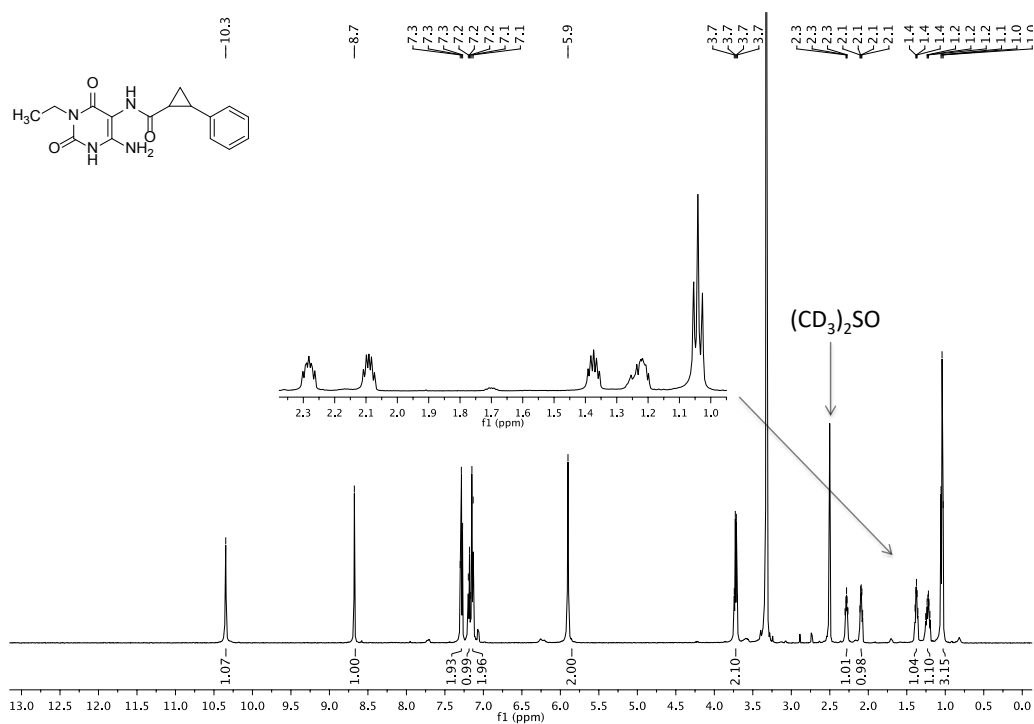


Figure S25. ^{13}C -NMR spectrum of 33 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

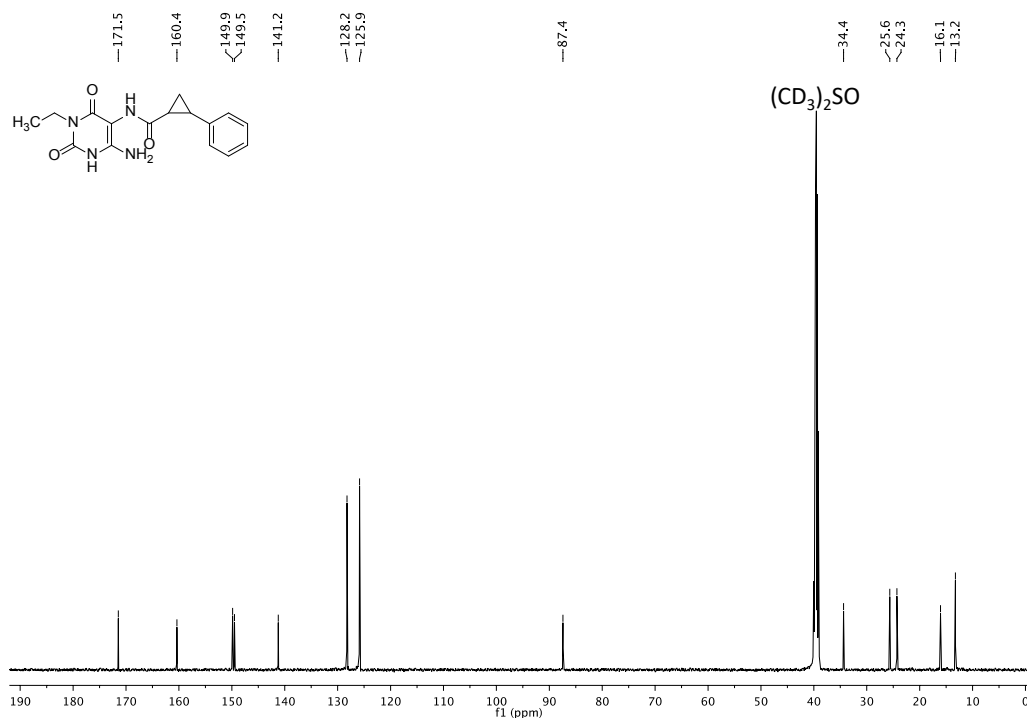


Figure S26. $^{13}\text{C-NMR}$ spectrum of 33 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

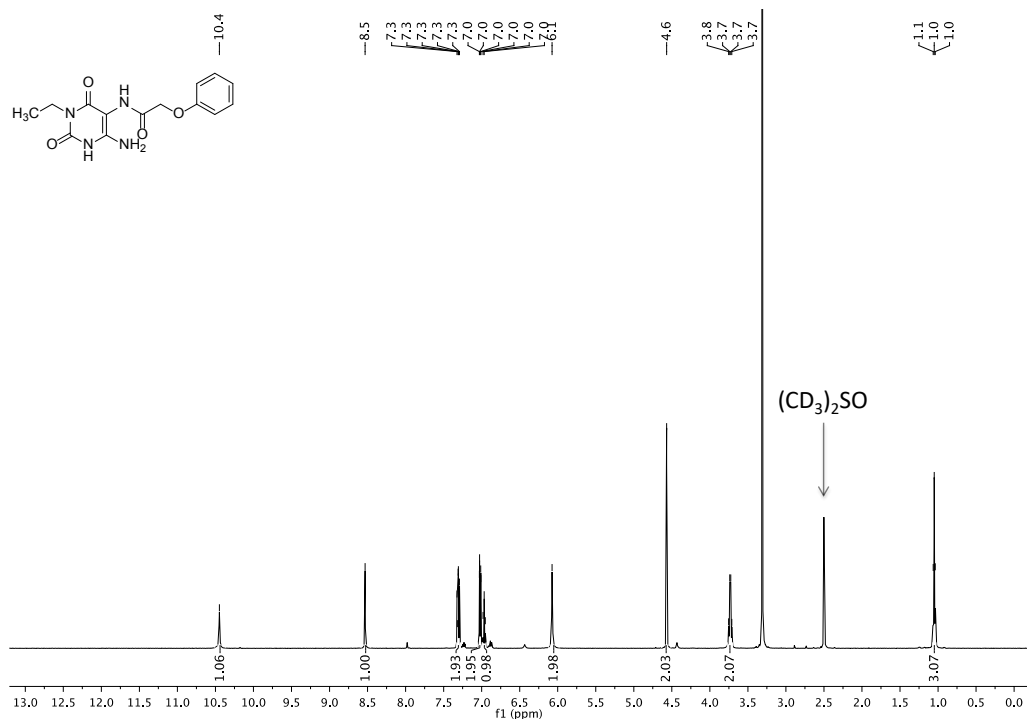


Figure S27. $^1\text{H-NMR}$ spectrum of 34 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

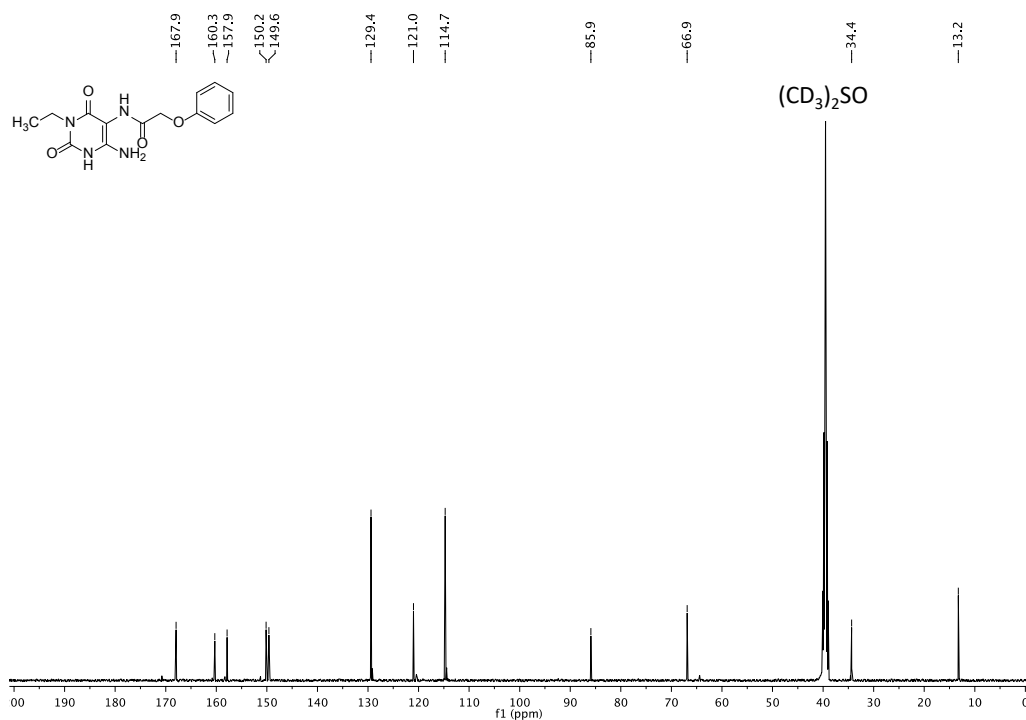


Figure S28. ^{13}C -NMR spectrum of 34 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

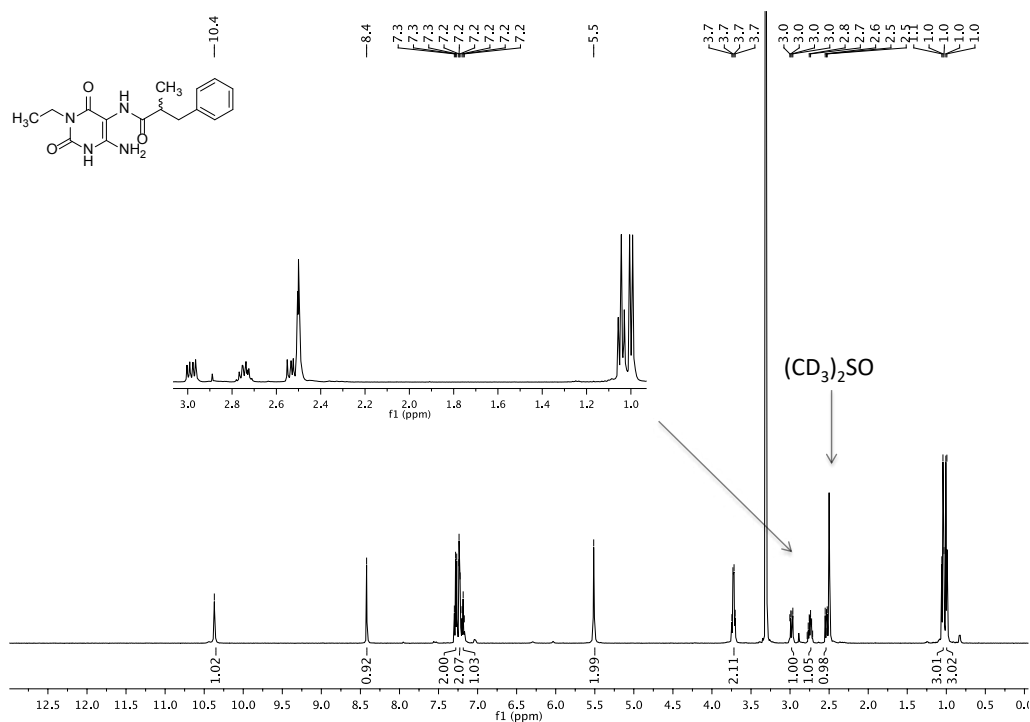


Figure S29. ^1H -NMR spectrum of 35 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

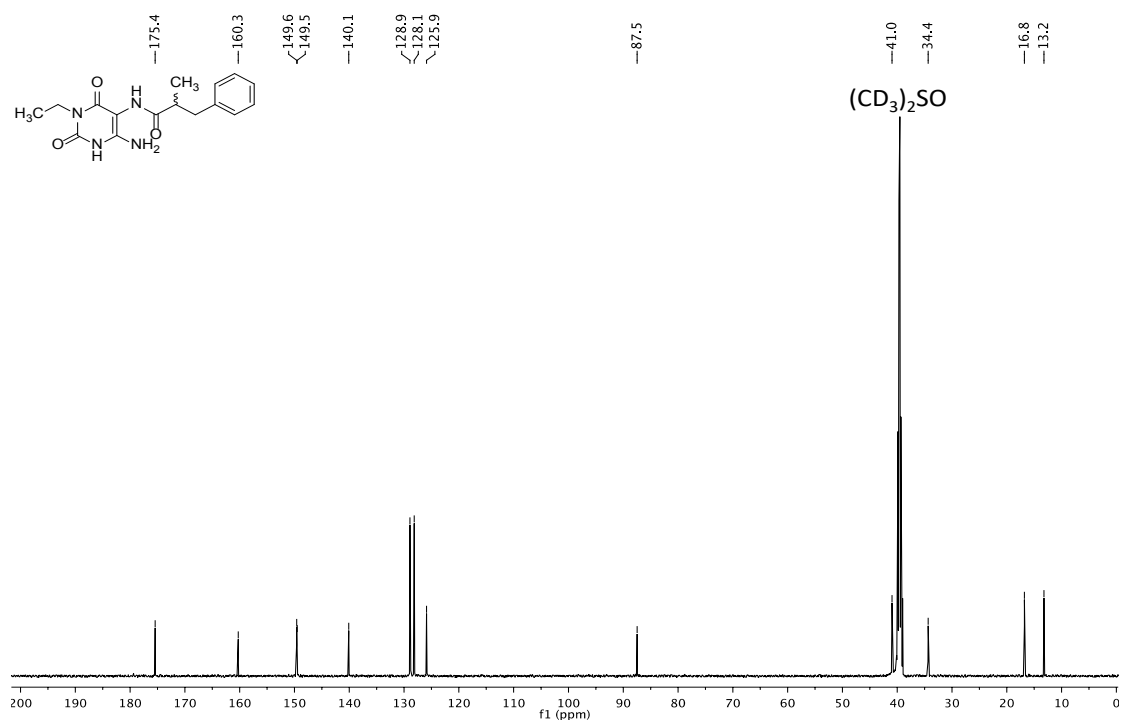


Figure S30. ^{13}C -NMR spectrum of 35 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

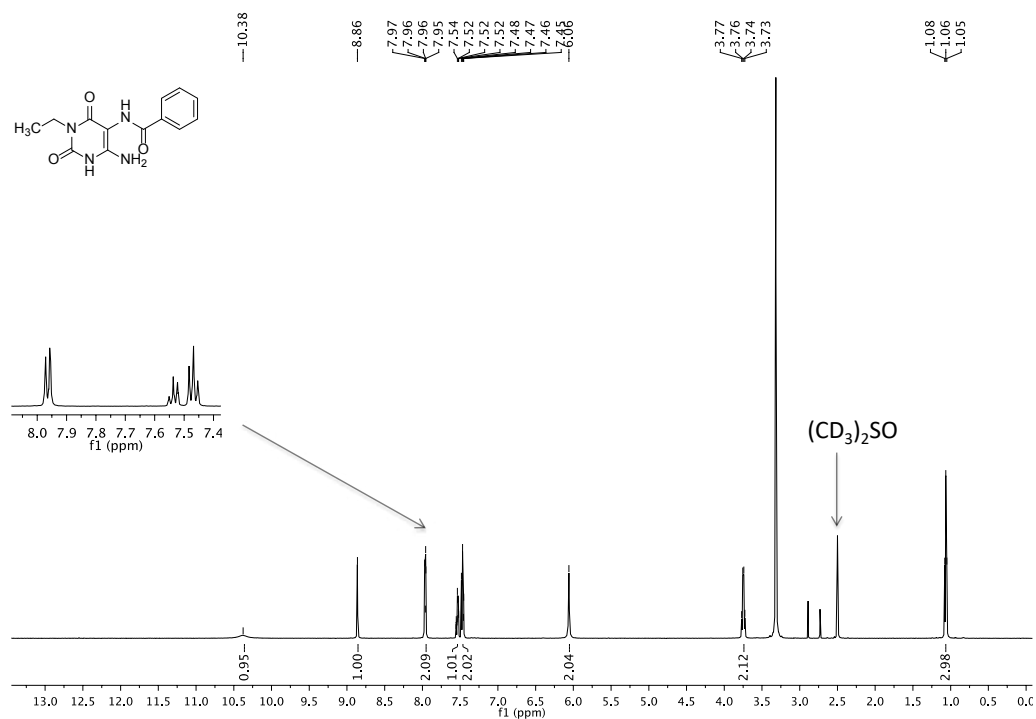


Figure S31. ^1H -NMR spectrum of 36 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

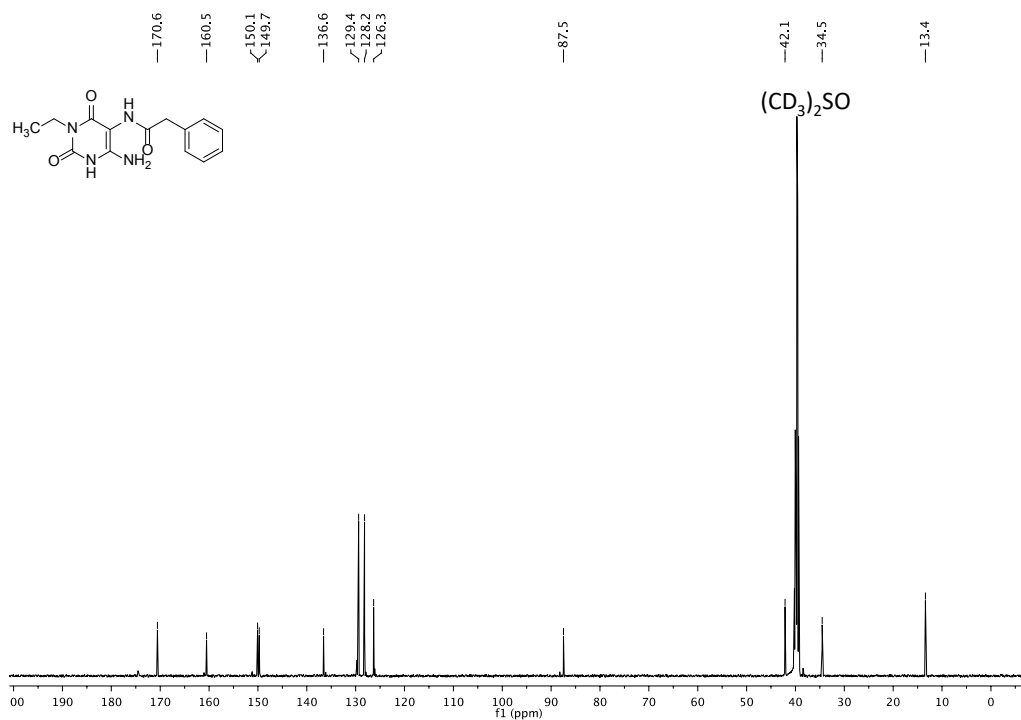


Figure S34. $^{13}\text{C-NMR}$ spectrum of 37 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

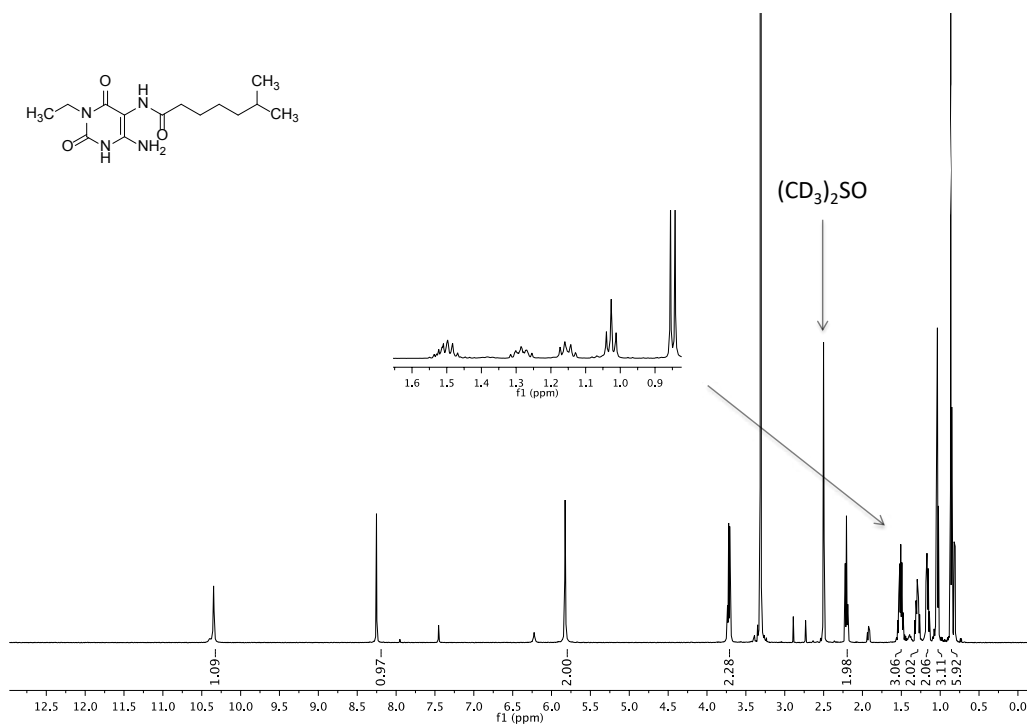


Figure S35. $^1\text{H-NMR}$ spectrum of 38 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

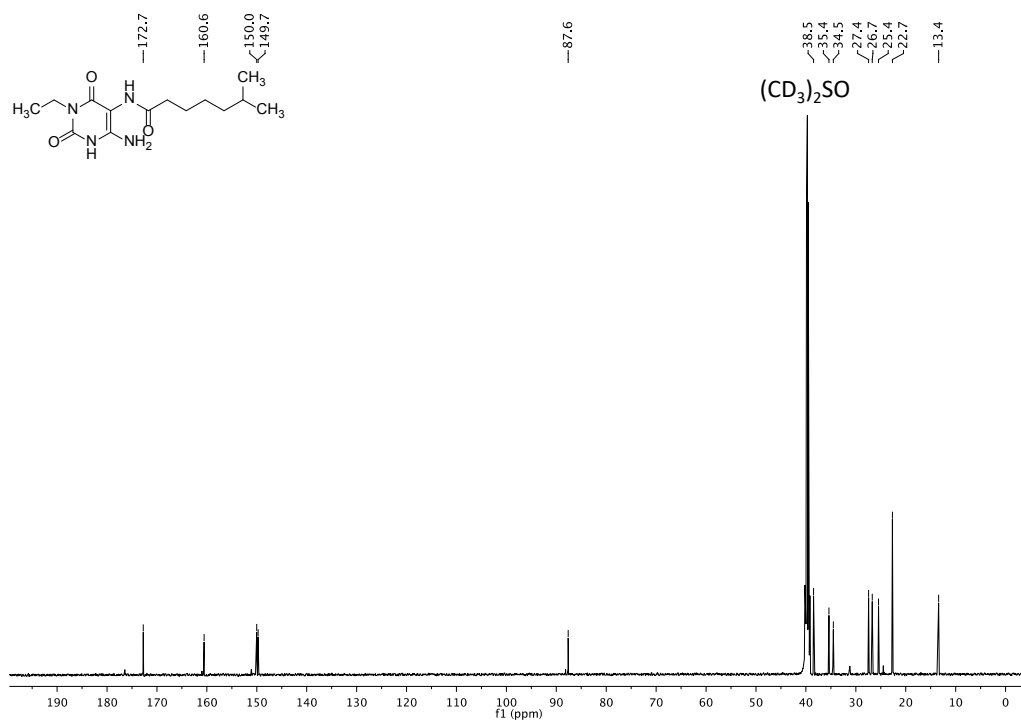


Figure S36. ^{13}C -NMR spectrum of 38 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

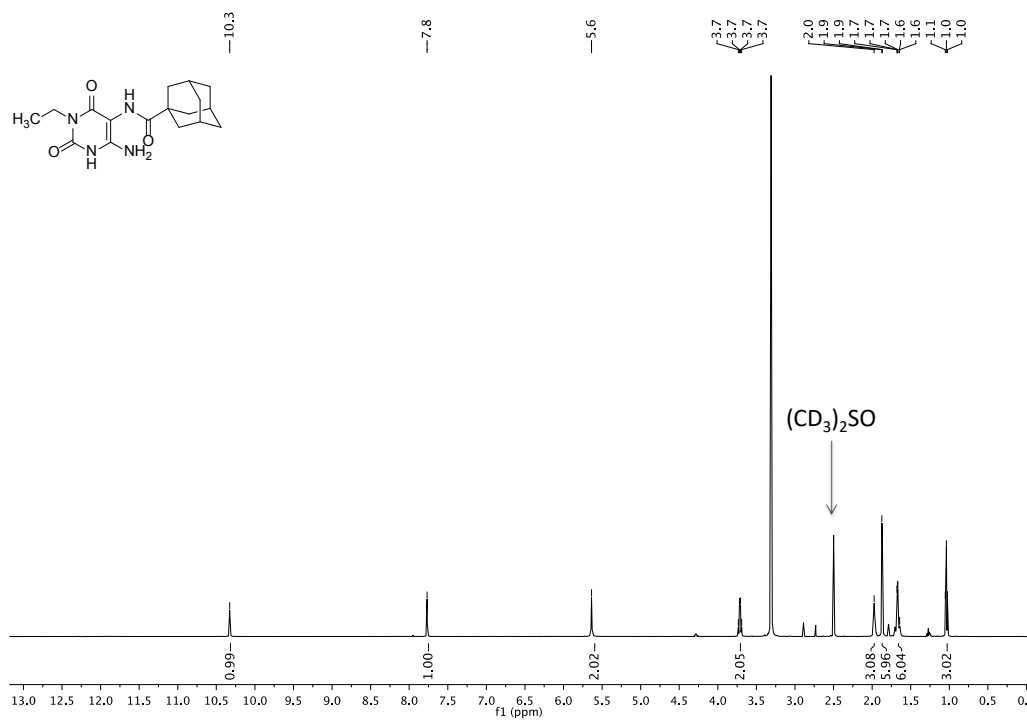


Figure S37. ^1H -NMR spectrum of 39 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

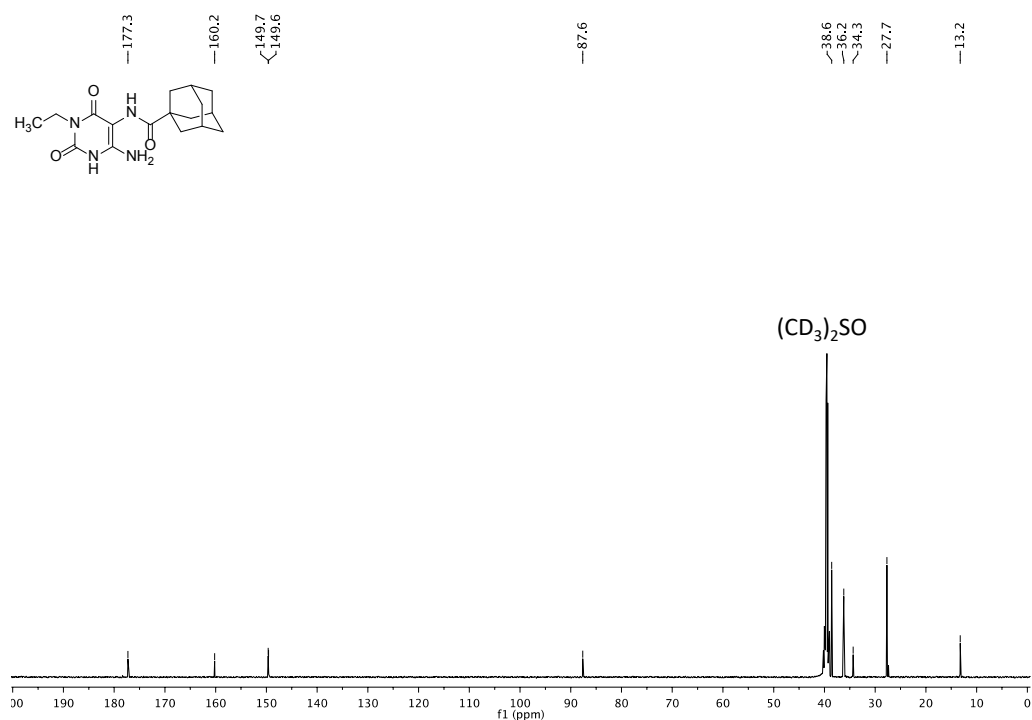
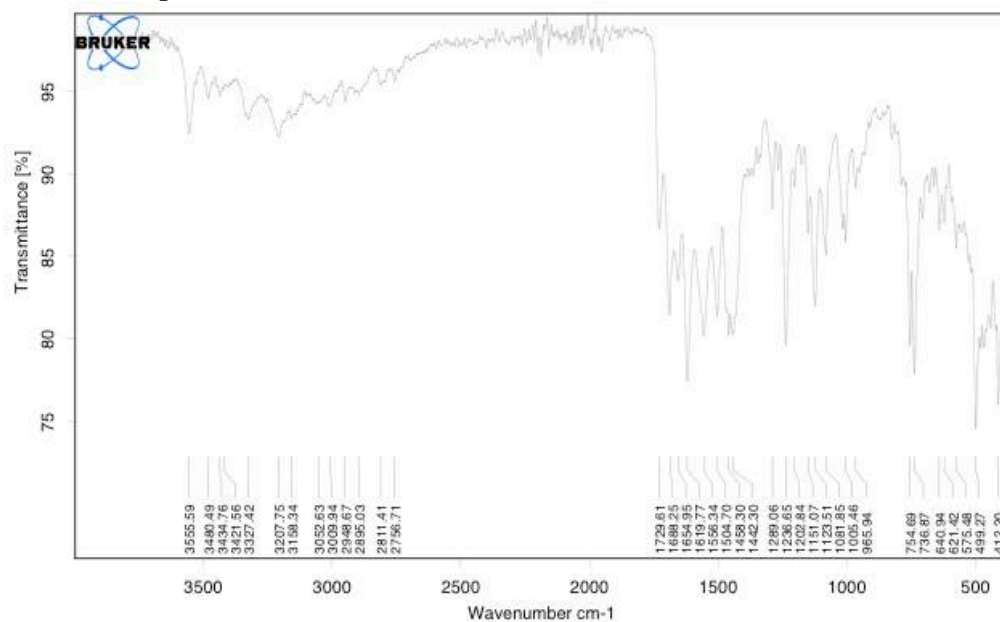


Figure S38. ^{13}C -NMR spectrum of 39 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

IR-spectra of compounds 21-



39

Figure S39. IR-spectrum of 21.

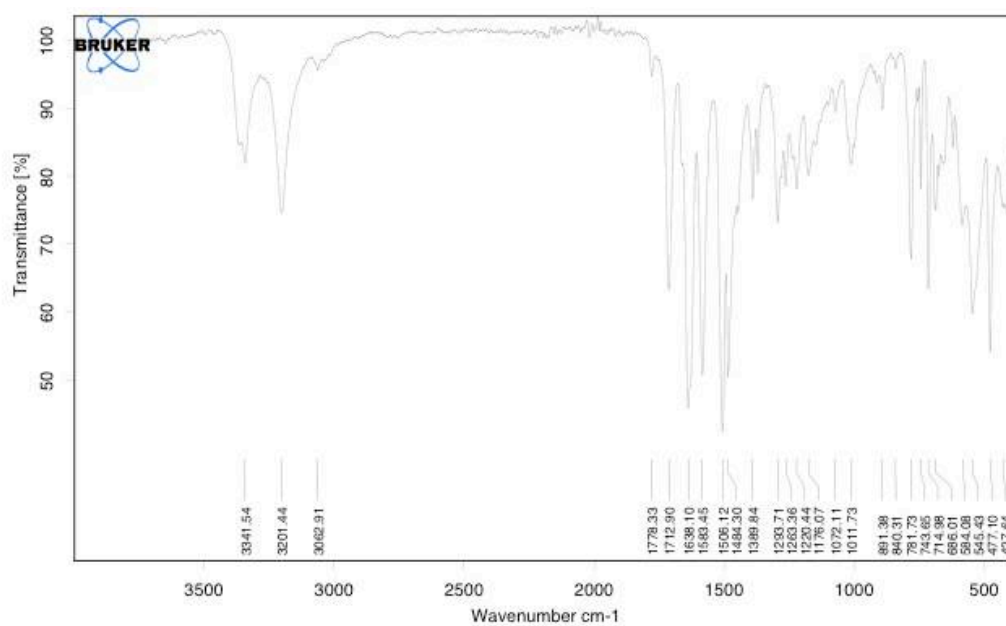


Figure S40. IR-spectrum of 22.

28

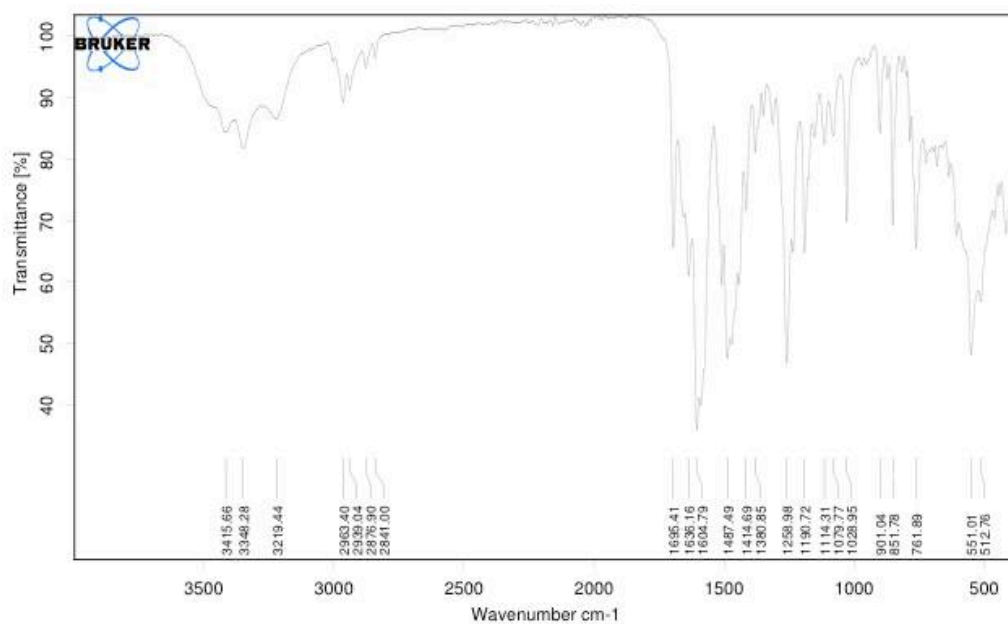


Figure S41. IR-spectrum of 23.

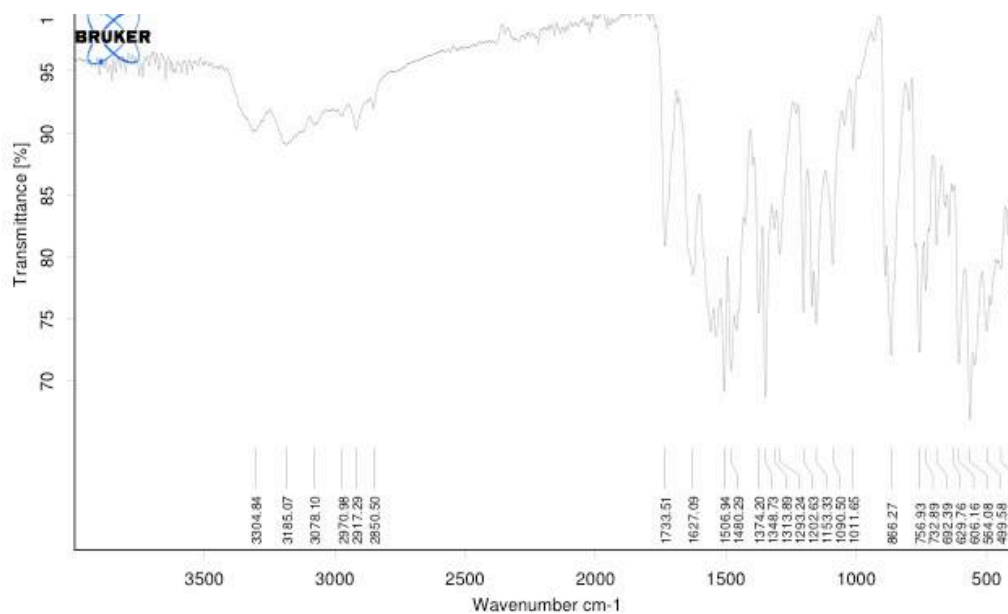


Figure S42. IR-spectrum of 24.

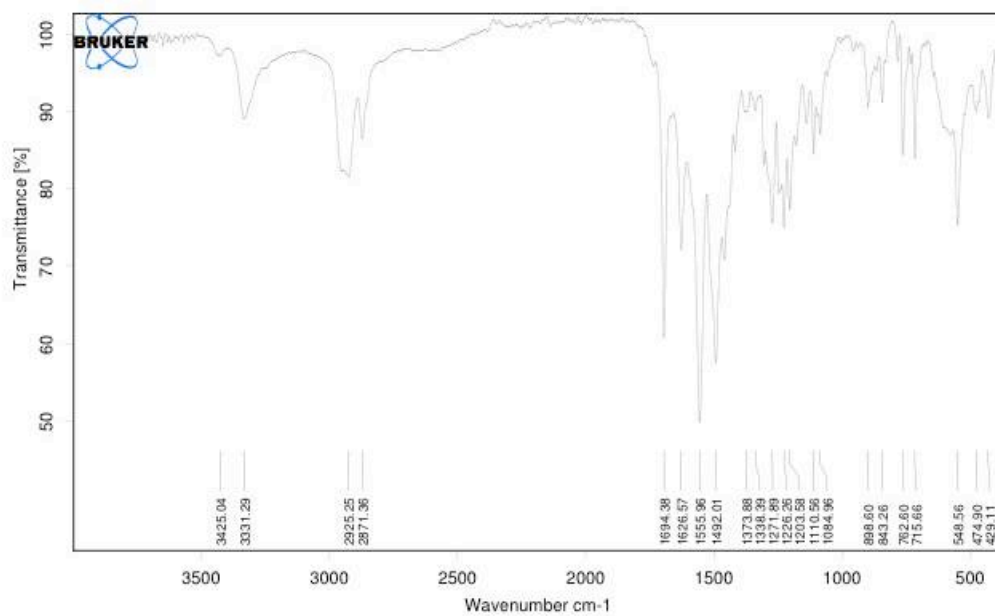


Figure S43. IR-spectrum of 25.

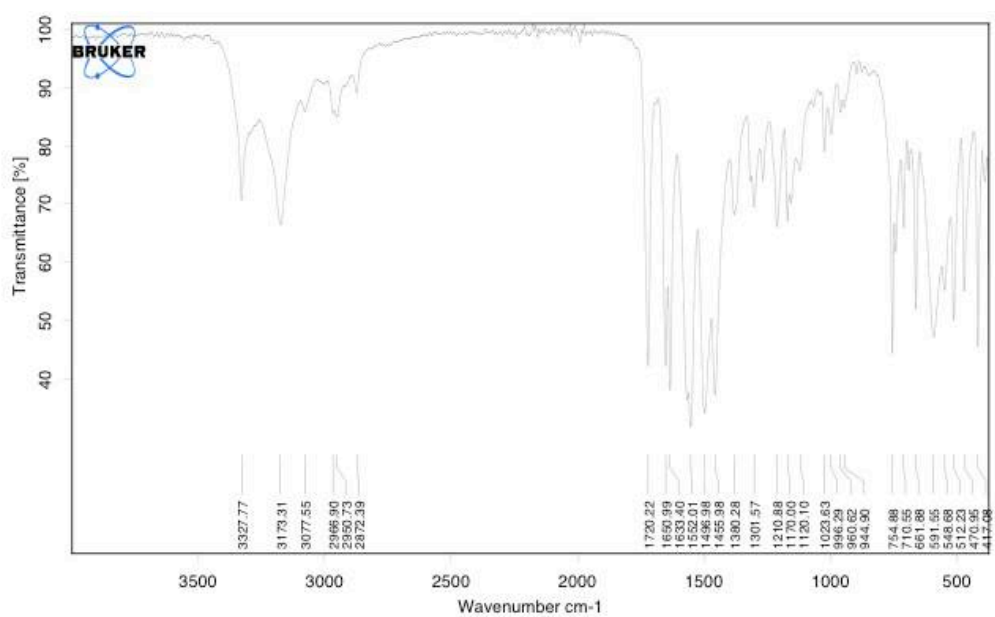


Figure S44. IR-spectrum of 26

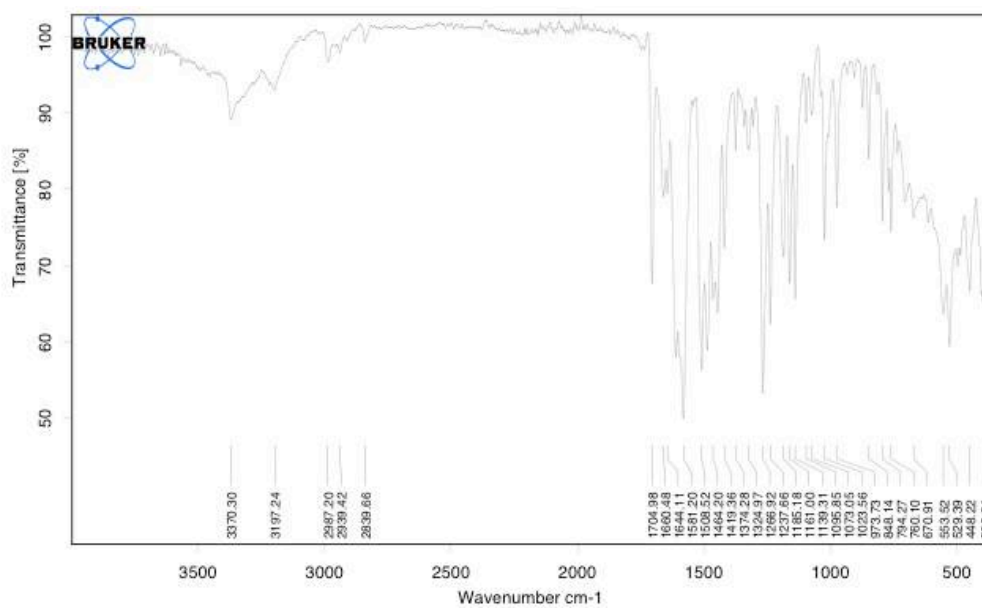


Figure S45. IR-spectrum of 27.

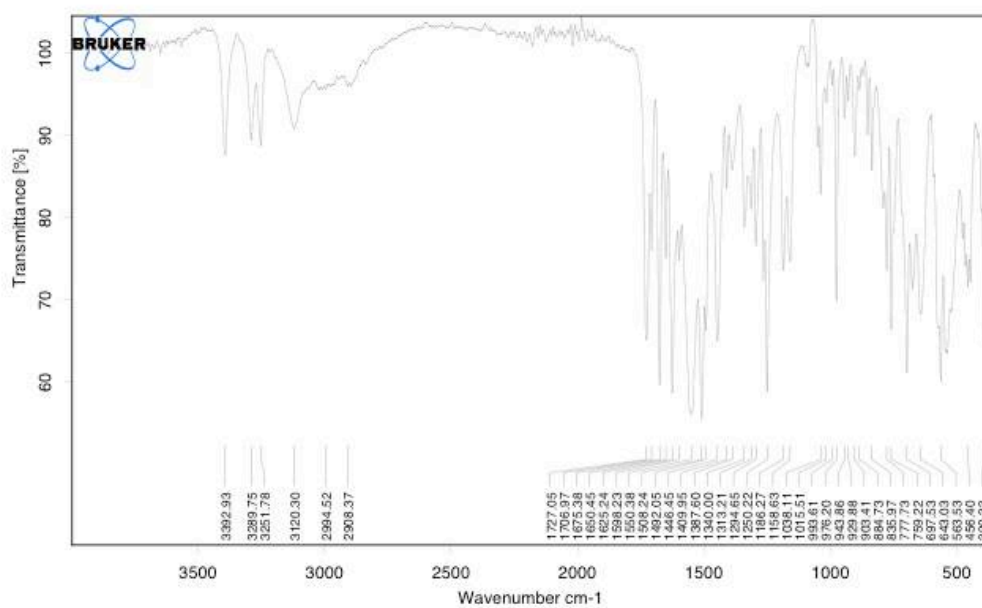


Figure S46. IR-spectrum of 28.

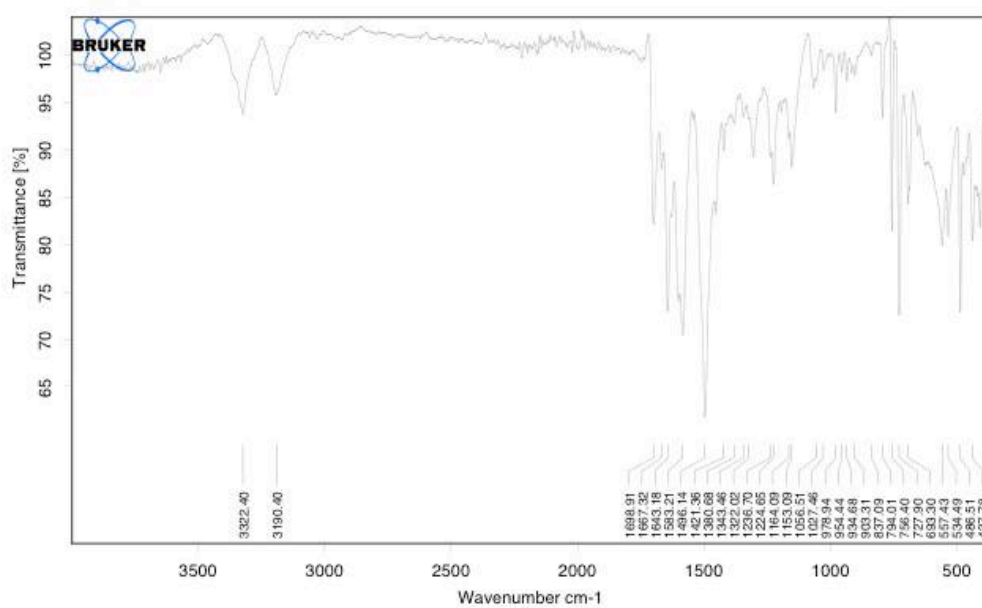


Figure S47. IR-spectrum of 29.

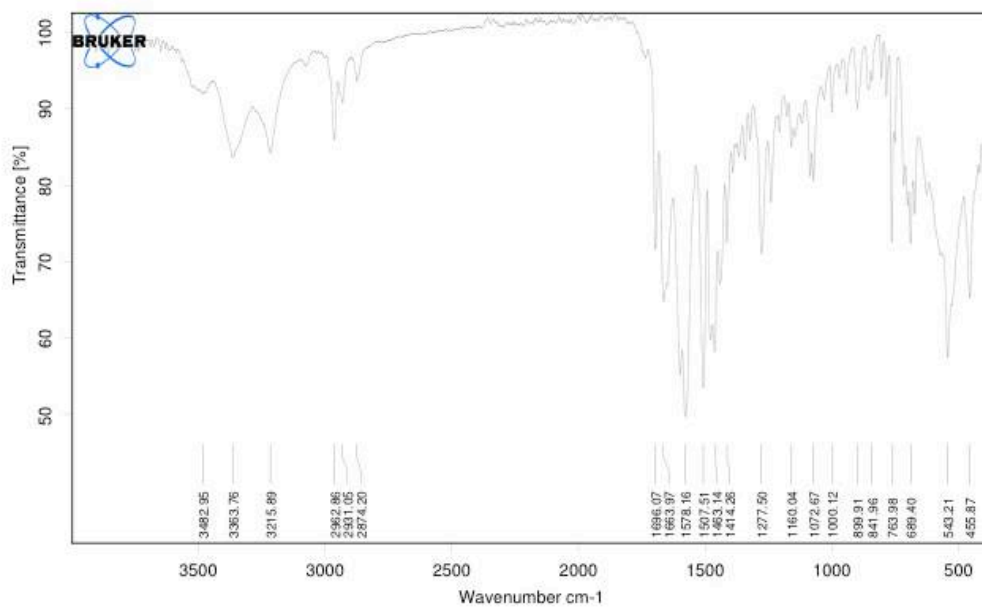


Figure S48. IR-spectrum of 30.

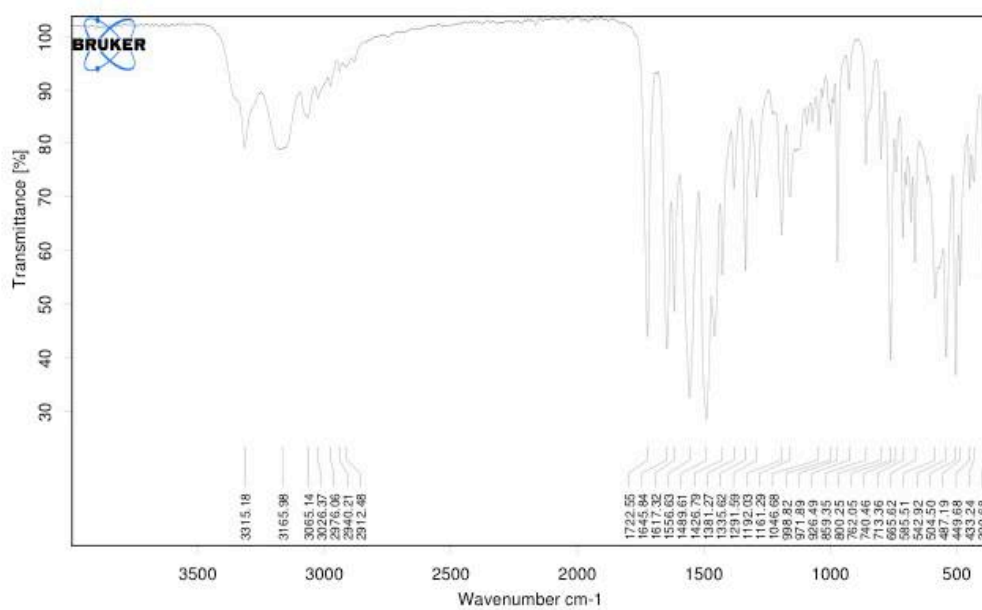


Figure S49. IR-spectrum of 31.

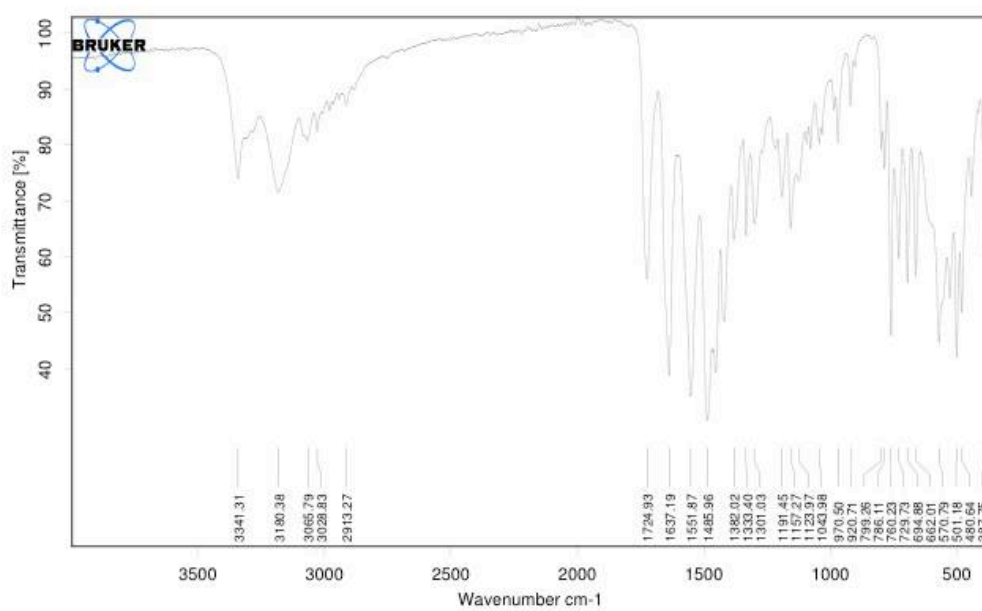


Figure S50. IR-spectrum of 32.

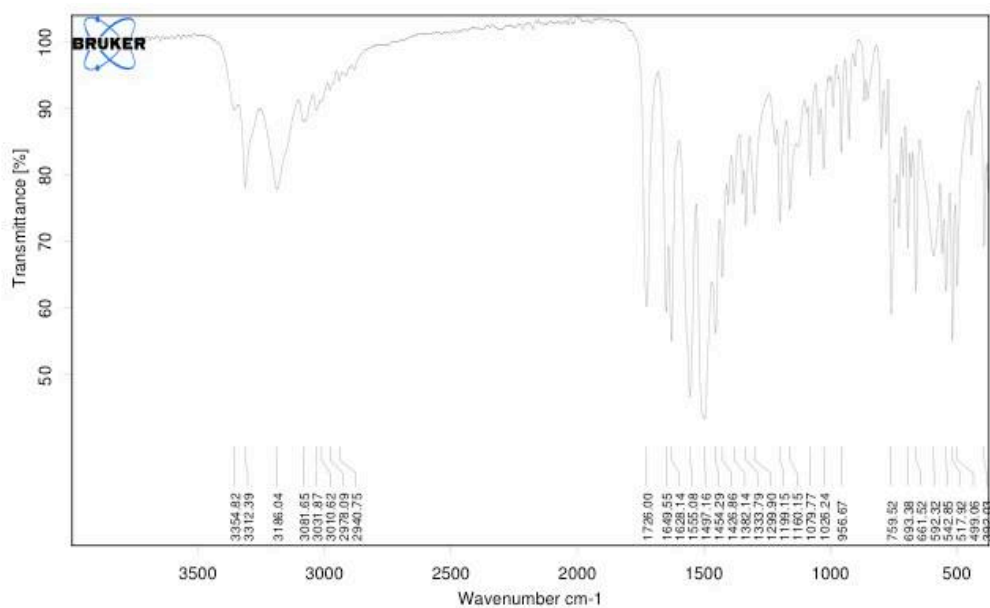


Figure S51. IR-spectrum of 33.

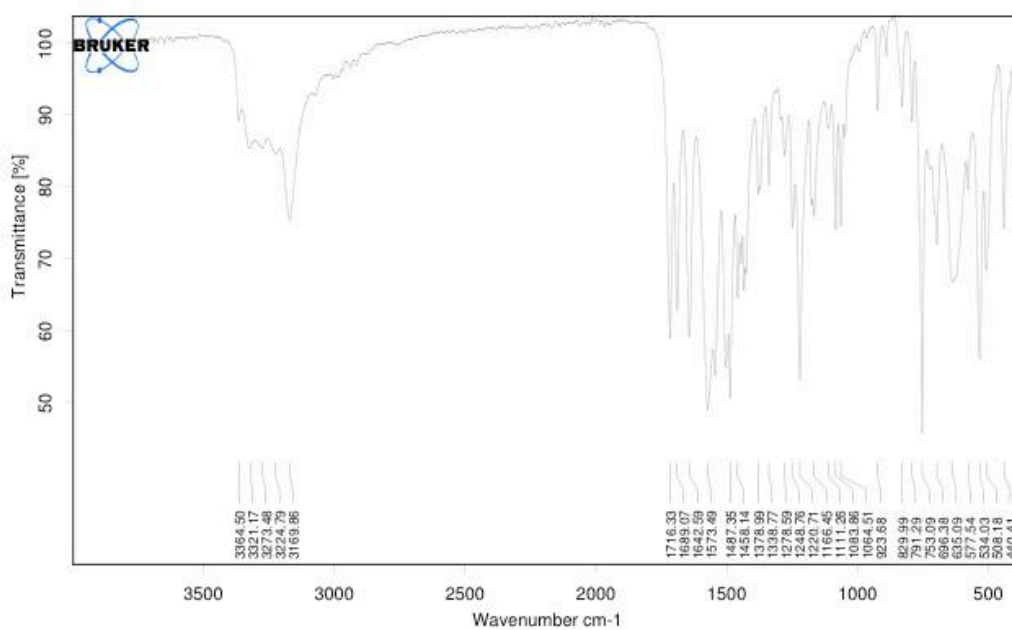


Figure S52. IR-spectrum of 34.

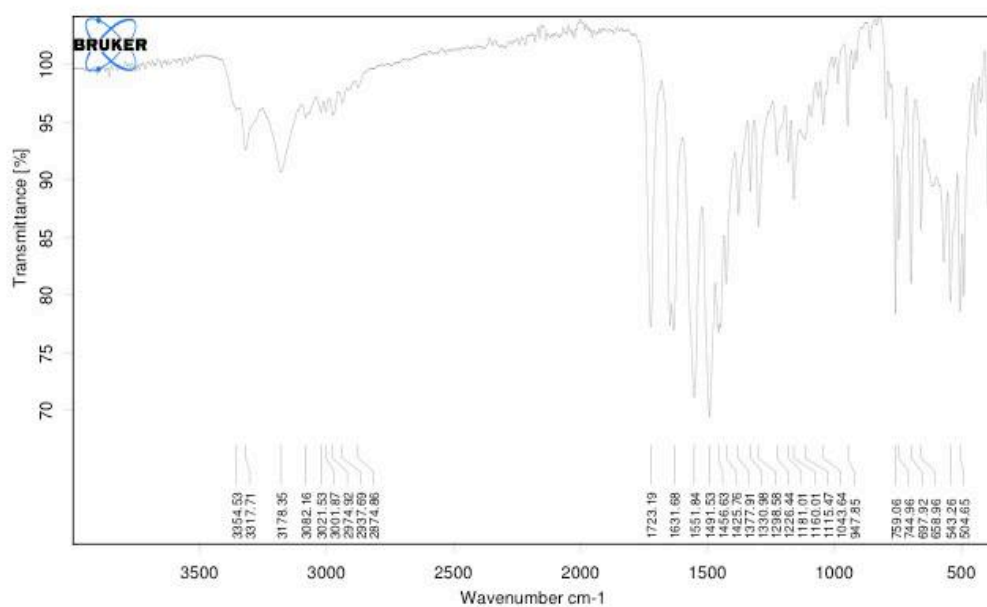


Figure S53. IR-spectrum of 35.

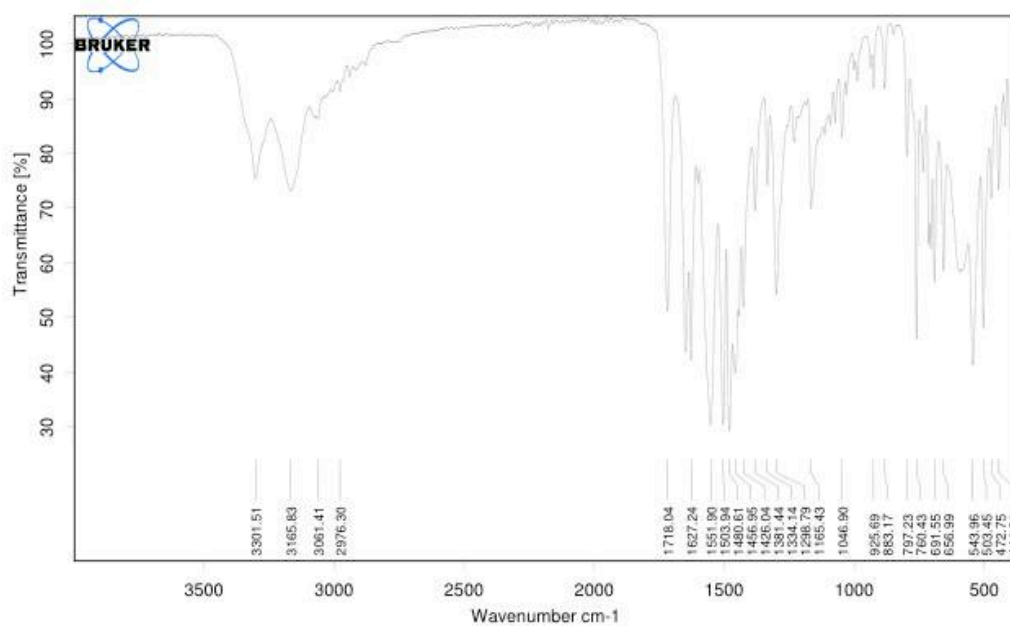


Figure S54. IR-spectrum of 36.

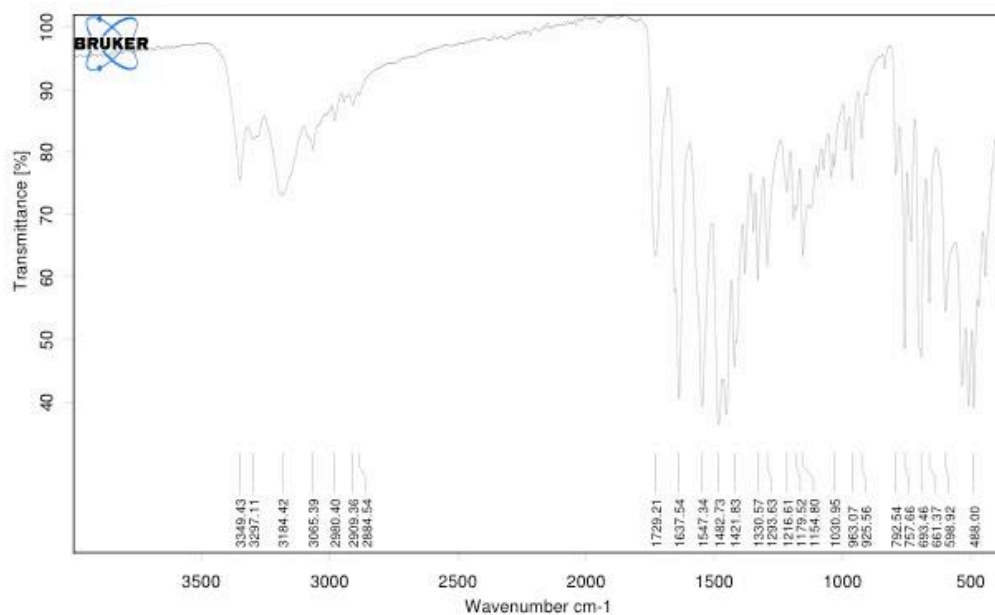


Figure S55. IR-spectrum of 37.

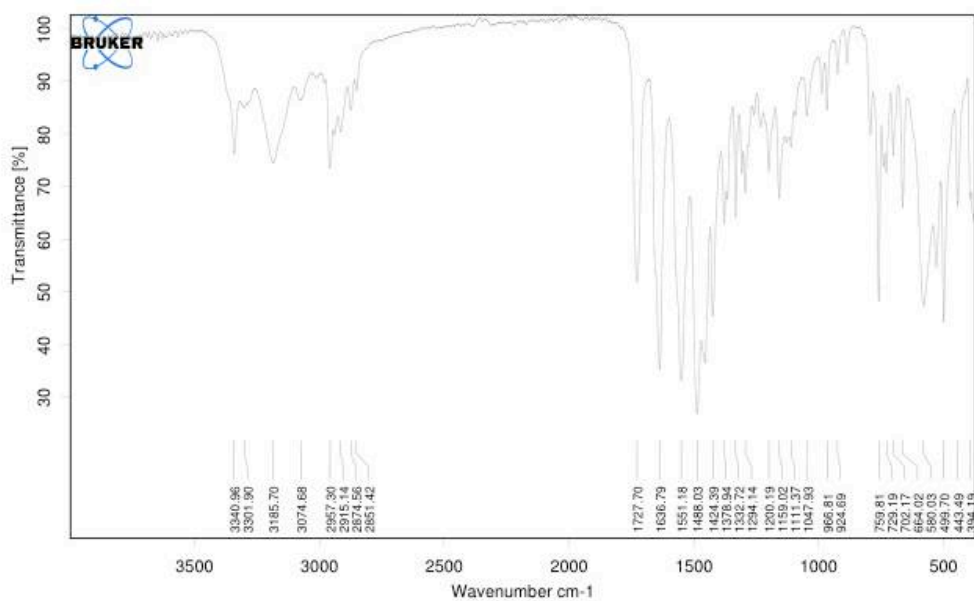


Figure S56. IR-spectrum of 38.

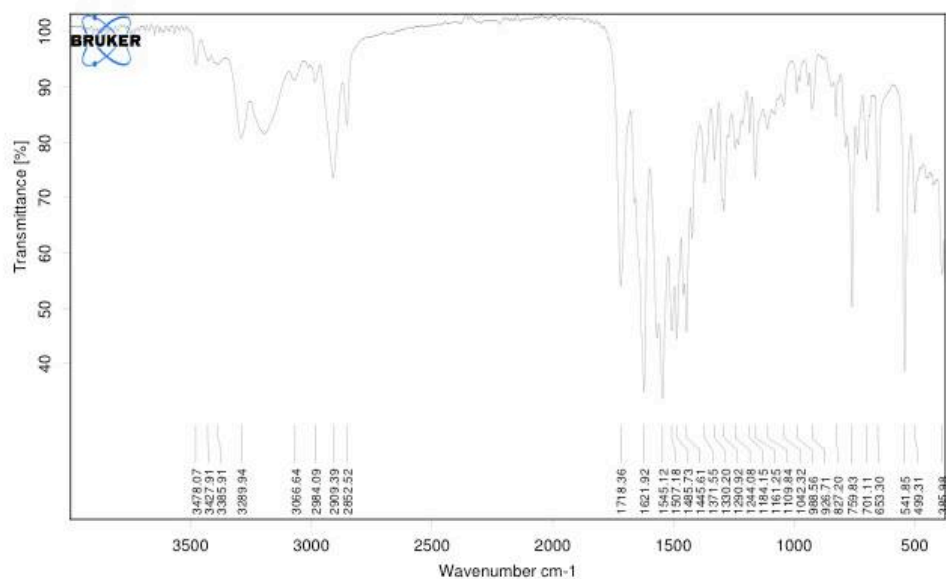


Figure S57. IR-spectrum of 39.

References

Hockemeyer, J., Burbiel, J.C., and Müller, C.E. (2004). Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: Potential drugs for Parkinson's disease. *J. Org. Chem.* 69, 3308-3318. doi: 10.1021/jo0358574.

Maxwell, L.C.E., and Salivar, C.J. (1952). Method of preparing 4-aminouracils. *US2715625A*.

Müller, C.E., Shi, D., Manning Jr, M., and Daly, J.W. (1993). Synthesis of paraxanthine analogs (1, 7-disubstituted xanthines) and other xanthines unsubstituted at the 3-position: Structure-activity relationships at adenosine receptors. *J. Med. Chem.* 36, 3341-3349.

● Alert level C

PLAT220 ALERT 2 C	Non-Solvent Resd 1 C Ueq(max)/Ueq(min) Range	3.1 Ratio
PLAT340 ALERT 3 C	Low Bond Precision on C-C Bonds	0.00417 Ang.
PLAT911 ALERT 3 C	Missing FCF ReFl Between Thmin & STh/L= 0.600	5 Report

● Alert level G

PLAT007 ALERT 5 G	Number of Unrefined Donor-H Atoms	4 Report
PLAT032 ALERT 4 G	Std. Uncertainty on Flack Parameter Value High .	0.500 Report
PLAT910 ALERT 3 G	Missing # of FCF Reflection(s) Below Theta(Min).	2 Note
PLAT912 ALERT 4 G	Missing # of FCF Reflections Above STh/L= 0.600	6 Note
PLAT933 ALERT 2 G	Number of OMIT Records in Embedded .res File ...	6 Note
PLAT978 ALERT 2 G	Number C-C Bonds with Positive Residual Density.	8 Info

- 0 **ALERT level A** = Most likely a serious problem - resolve or explain
0 **ALERT level B** = A potentially serious problem, consider carefully
3 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
6 **ALERT level G** = General information/check it is not something unexpected

- 0 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
3 ALERT type 2 Indicator that the structure model may be wrong or deficient
3 ALERT type 3 Indicator that the structure quality may be low
2 ALERT type 4 Improvement, methodology, query or suggestion
1 ALERT type 5 Informative message, check
-

It is advisable to attempt to resolve as many as possible of the alerts in all categories. Often the minor alerts point to easily fixed oversights, errors and omissions in your CIF or refinement strategy, so attention to these fine details can be worthwhile. In order to resolve some of the more serious problems it may be necessary to carry out additional measurements or structure refinements. However, the purpose of your study may justify the reported deviations and the more serious of these should normally be commented upon in the discussion or experimental section of a paper or in the "special_details" fields of the CIF. checkCIF was carefully designed to identify outliers and unusual parameters, but every test has its limitations and alerts that are not important in a particular case may appear. Conversely, the absence of alerts does not guarantee there are no aspects of the results needing attention. It is up to the individual to critically assess their own results and, if necessary, seek expert advice.

Publication of your CIF in IUCr journals

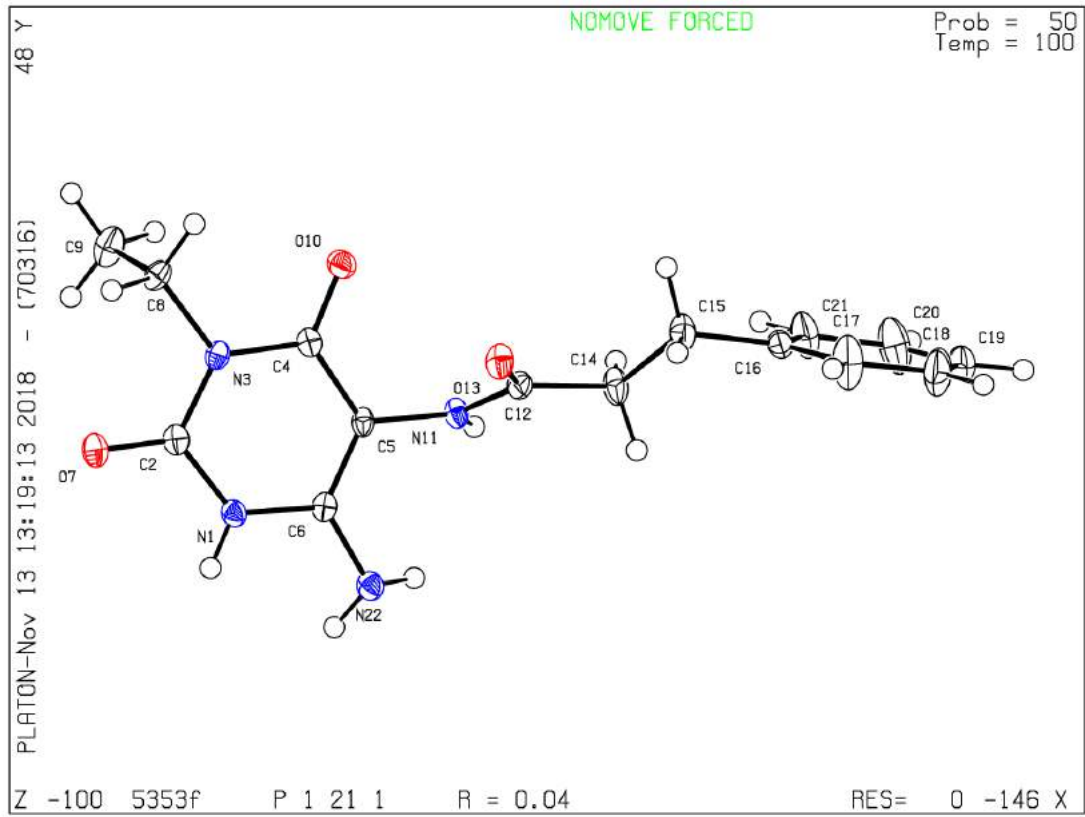
A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E* or *IUCrData*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

Publication of your CIF in other journals

Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF submission.

PLATON version of 19/10/2018; check.def file version of 15/10/2018

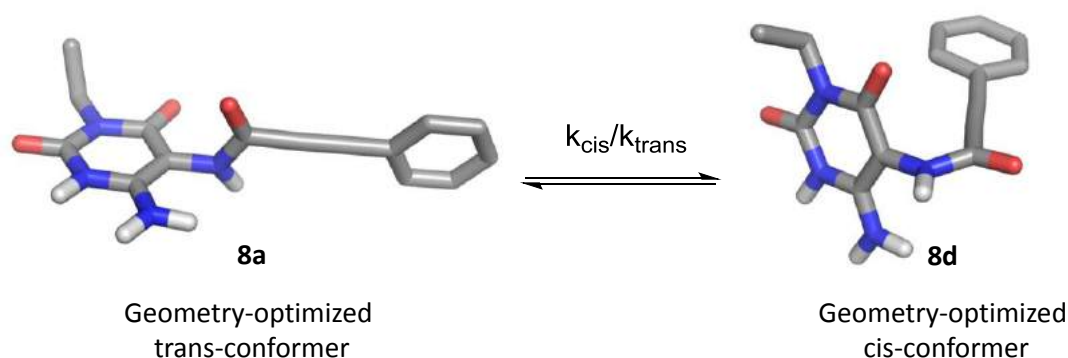
Datablock 5353f - ellipsoid plot



2. Article II: Structural and Conformational Studies on Carboxamides of 5,6-Diaminouracils—Precursors of Biologically Active Xanthine Derivatives

Daniel Marx, Gregor Schnakenburg, Stefan Grimme and Christa E. Müller*

The 5-amido-6-aminouracil derivatives represents important intermediates in the xanthine synthesis. If these compounds are examined by NMR spectroscopy, a duplication of the signals is noticeable, which was analyzed in some detail in the following article. Different analytical experiments such as dynamic and two-dimensional NMR spectroscopy, density functional theory (DFT) calculations, X-ray analysis and dynamic high-performance liquid chromatography (HPLC) were performed in order to explain these unexpected properties of the 6-amino-5-carboxamidouracil derivatives.



The data indicated a low rotational barrier of the $C_{sp^2}-N$ bond of the described amides. Thus, rotation around the amide bond is slow on the NMR timescale and the observation of two conformers is permitted. If this rotation is accelerated e.g. by temperature increase, a coalescence of the two NMR signals can be observed. This effect varies depending on the substituent on the carboxamide residue.

Article

Structural and Conformational Studies on Carboxamides of 5,6-Diaminouracils—Precursors of Biologically Active Xanthine Derivatives

Daniel Marx ^{1,2}, Gregor Schnakenburg ³, Stefan Grimme ⁴  and Christa E. Müller ^{1,2,*} 

¹ Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, D-53121 Bonn, Germany; dmarx@uni-bonn.de

² Pharma Center Bonn, University of Bonn, D-53121 Bonn, Germany

³ Department of Chemistry, Institute of Inorganic Chemistry, University of Bonn, D-53121 Bonn, Germany; gregor.schnakenburg@uni-bonn.de

⁴ Mulliken Center for Theoretical Chemistry, Institute of Physical and Theoretical Chemistry, University of Bonn, D-53115 Bonn, Germany; grimme@thch.uni-bonn.de

* Correspondence: christa.mueller@uni-bonn.de; Tel.: +49-0228-732-301

Academic Editor: F. Javier Luque

Received: 2 May 2019; Accepted: 4 June 2019; Published: 9 June 2019



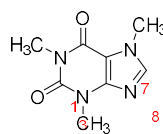
Abstract: 8-Arylethynylxanthine derivatives are potent, selective adenosine A_{2A} receptor antagonists, which represent (potential) therapeutics for Parkinson's disease, Alzheimer's dementia, and the immunotherapy of cancer. 6-Amino-5-amidouracil derivatives are important precursors for the synthesis of such xanthines. We noticed an unexpected duplication of NMR signals in many of these uracil derivatives. Here, we present a detailed analytical study of structurally diverse 6-amino-5-carboxamidouracils employing dynamic and two-dimensional NMR spectroscopy, density functional theory calculations, and X-ray analysis to explain the unexpected properties of these valuable drug intermediates.

Keywords: amide; DFT calculation; dynamic NMR; rotamers/conformers; uracil; xanthine; X-ray crystallography

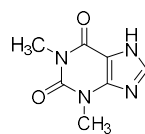
1. Introduction

The nucleoside adenosine is an important (patho)physiological modulator in the brain as well as in peripheral tissues and organs [1]. It activates G protein-coupled adenosine receptors (ARs) which comprise A₁-, A_{2A}-, A_{2B}-, and A₃AR subtypes. The A_{2A}AR subtype has become a major (potential) drug target. A_{2A}AR antagonists have been developed for neurodegenerative diseases such as Parkinson's [2] and Alzheimer's disease [3], and, recently, their great potential for the immunotherapy of cancer has been discovered [4]. The first identified AR antagonists were the natural alkaloids caffeine (1,3,7-trimethylxanthine, **1**) and theophylline (1,3-dimethylxanthine, **2**) (Figure 1) [5]. After discovering these alkylxanthines as moderately potent, non-selective AR antagonists, several groups tried to modify the xanthine scaffold to obtain more potent and selective compounds. N7-methylation, as in caffeine, combined with coplanar aromatic substitution at the C8-position of xanthines as in the styrylxanthines **3** and **4** (Figure 1) led to high affinity and selectivity for the A_{2A}AR [6–8]. A disadvantage of the styrylxanthine structure is its photosensitivity in solid form as well as in dilute solutions [9].

First generation: Natural, nonselective adenosine receptor antagonists

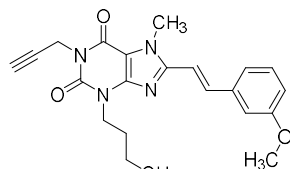


Caffeine
1

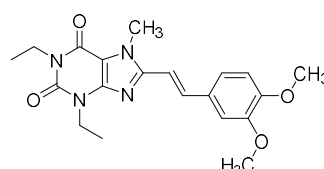


Theophylline
2

Second generation: 8-styrylxanthines - selective but photosensitive A_{2A}AR antagonists

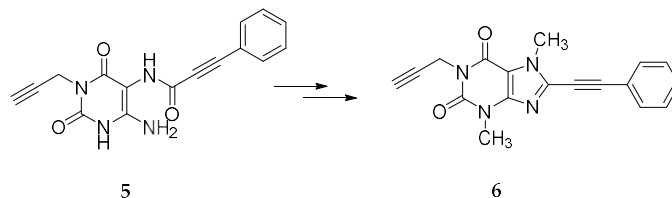


MSX-2
3



Istradefylline
4

Third generation: Selective A_{2A}AR antagonist **6** showing increased stability, obtained by cyclization of amide precursor **5**

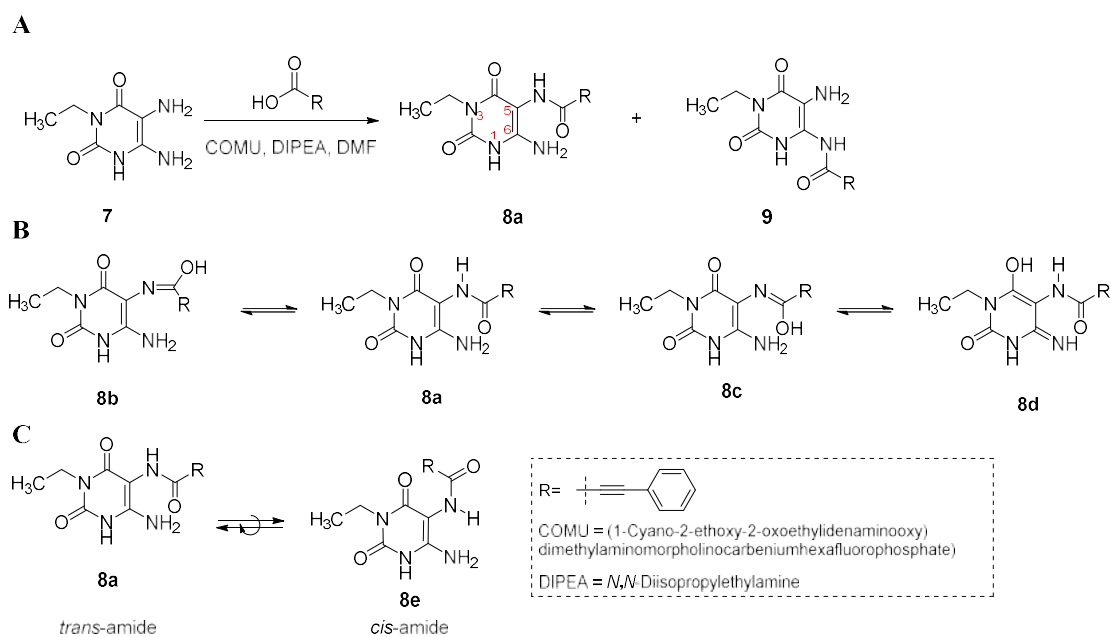


5

6

Figure 1. Examples of adenosine receptor antagonists with a xanthine scaffold. Shown is the development of the xanthine derivatives caffeine and theophylline towards potent and selective adenosine A_{2A} receptor antagonists.

Replacement of the styryl moiety present in **3** and **4**, by an arylethynyl residue, as in **6**, resulted in a new class of photochemically stable A_{2A}AR antagonists (Figure 1) [10]. Their 6-amino-5-carboxamidouracil precursors (e.g., compound **5**, Figure 1) were often not fully characterized, but directly converted to the final xanthine derivatives [11]. Upon close examination of ¹H- and ¹³C-NMR spectra of 6-amino-5-arylethynylcarboxamidouracil **8a** (Scheme 1), obtained by coupling of 5,6-diamino-3-ethyl uracil **7** with phenylethynyl carboxylic acid, we observed a duplication of almost all signals (see Figure 2). However, this phenomenon was not observed for its styryl analog containing a double bond instead of the triple bond in **8a**. Thus, we performed a comprehensive analytical study of 6-amino-5-arylethynylcarboxamidouracil (**8a**) in order to rationalize these unexpected observations. Additionally, different *N*1,*N*3-substituted 6-amino-5-carboxamidouracil derivatives were synthesized and analyzed.



Scheme 1. Potential products obtained by coupling of 5,6-diamino-3-ethyluracil **7** with phenylethynylcarboxylic acid. **(A)** Possible regioisomers **8a** and **9** that could result from an amide coupling reaction of **7** with phenylethynylcarboxylic acid. **(B)** Possible formation of tautomers **8b**, **8c**, and **8d** of **8a**. **(C)** Possible *cis*- and *trans*-amide bond conformers **8a** and **8e**.

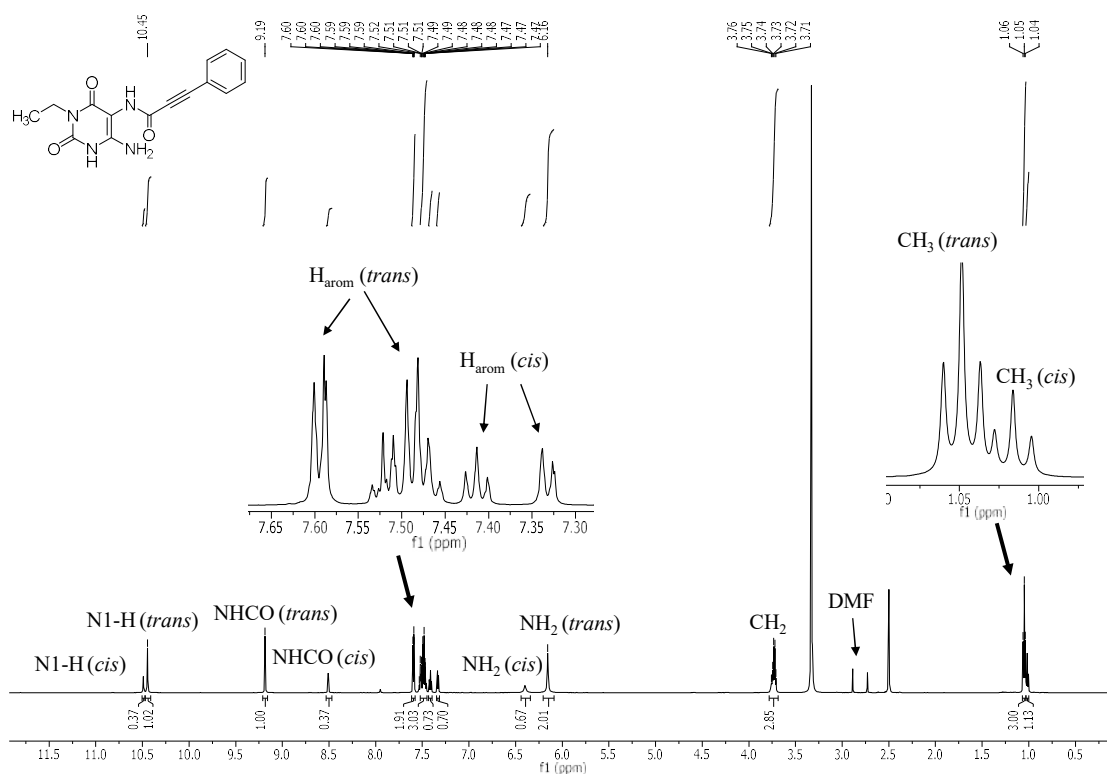


Figure 2. $^1\text{H-NMR}$ spectra of compound **8a** in $\text{DMSO-}d_6$.

Different explanations for the duplication of peaks in the NMR spectra of 6-amino-5-arylethynylcarboxamides are conceivable. A mixture of regioisomers **8a** and **9** appeared to be likely due to possible amide coupling with the 5- or the 6-amino group of 5,6-diamino-3-ethyluracil (**7**, Scheme 1A). On the other hand, the presence of amide–iminol tautomers might be possible (Scheme 1B,

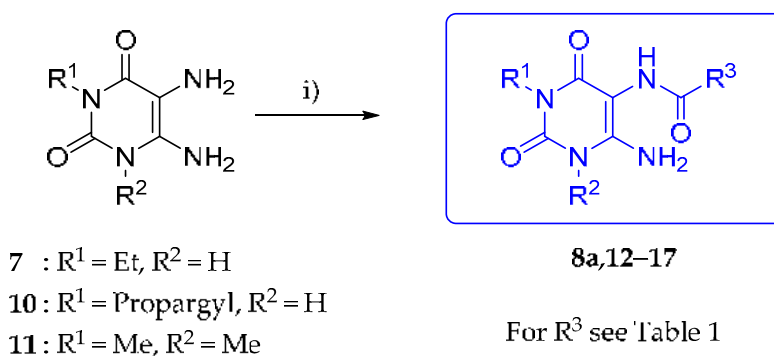
structures **8a**, **8b**, **8c**, and **8d**). Moreover, amides can exist as different stereoisomers with *s-cis* (cisoid) or *s-trans* (transoid) conformation (see Scheme 1C), a phenomenon that is well known for peptides [12–16].

In the present study, we performed dynamic and 2D-NMR studies and X-ray crystallography, and additionally employed computational methods to elucidate the structures of 5/6-amino-5/6-carboxamidouracil derivatives such as **8/9** (Scheme 1). Different analogs of compound **8a** were synthesized and analyzed in detail in order to explore if different *N1,N3*-substituents or different linkers affect the structures leading to a duplication of NMR signals, as observed for compound **8**.

2. Results and Discussion

2.1. Chemistry

Condensation of 5,6-diaminouracil derivatives **7**, **10**, and **11** with different carboxylic acid derivatives in the presence of (1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholinocarbenium hexafluorophosphate (COMU) yielded the desired 6-amino-5-carboxamido derivatives **8** and **12–17** (Scheme 2). The amide coupling reaction was performed by the recently described optimized procedure using COMU as a coupling reagent in the presence of diisopropylethylamine (DIPEA) as a base in dimethylformamide (DMF) at room temperature [17]. The products were precipitated by the addition of water, filtered off, and dried; no further purification was required. These precursors can be further converted to the corresponding xanthine derivatives by a dehydrative cyclization reaction [9,18,19].



Scheme 2. Synthesis of 6-amino-5-carboxamidouracil derivatives. Reagents and conditions: (i) $\text{R}^3\text{-COOH}$, COMU, DIPEA, DMF, rt, 5 min, H_2O (79%–90%). The synthesis of the starting material was performed according to procedures described in the literature (Scheme S1 and S2, Supplementary Information) [9].

2.2. Analytical Studies

In order to explain the duplication of signals in the NMR spectra of compound **8a** (Figure 2) different analytical experiments were performed. Yang et al. [20] described the formation of two regioisomers during the condensation reaction of 5,6-diamino-1,3-dimethyluracil with 2-hydroxyacetic acid. Furthermore, Poulsen et al. [21] proposed the formation of a 6-carboxamidouracil derivative during the condensation reaction of 5,6-diamino-1,3-dimethyluracil with glutamic anhydride. However, unambiguous evidence for the acylation of the 6-amino group was not provided by these authors.

High performance liquid chromatography (HPLC) of **8a** pointed to only a single product, a first indication that different regioisomers were likely not present, and the formation of **9** could be excluded (Scheme 1A). Additionally, compound **12** was selected for dynamic HPLC (DHPLC) experiments to explore the compound's behavior at different temperatures. DHPLC measurements were performed at 5, 15, 25, and 40 °C, but in all cases one single peak was observed. Only a slight shift in the retention times and peak broadening was visible upon decreasing of the temperature (Figure S16, Supporting Information). To further elucidate whether regioisomers, tautomers, or conformational isomers were

responsible for the signal duplication in the NMR spectra, dynamic NMR and 2D-NMR experiments were performed.

2.2.1. Dynamic NMR Experiments of 8a at High Temperatures

The most deshielded signal, that of the N1-H, resonates as a broad singlet at 10.43 ppm measured in DMSO- d_6 , and the second signal of the corresponding isomer is slightly shifted downfield to 10.48 ppm. The amide proton resonates at 9.17 ppm as a broad singlet, and the second signal shows a large upfield shift to 8.50 ppm. The amino group at position six resonates as a broad singlet at 6.14 ppm with an integration of two, while the corresponding second signal is shifted downfield to 6.39 ppm, showing an integration of 0.67. Dynamic NMR experiments were performed to study whether different tautomers of **8a** were present. The temperature should have a measurable influence on the ratio between tautomers. At a higher temperature, a higher percentage of the thermodynamically more stable tautomer would be expected [22]. ^1H - and ^{13}C -NMR spectra were recorded in DMF- d_7 and DMSO- d_6 from 223 to 378 K with 5–10 K intervals. The ratio of the isomers determined by integration of the ^1H -NMR signals was found to be 73:27. Unexpectedly, in both solvents, no change in the ratio between the two species could be observed upon raising of the temperature (Figure 3). Since the dynamic NMR experiments showed that the existence of two tautomers next to each other in solution could be excluded (Scheme 1B), a third explanation was investigated in more detail. To confirm the third hypothesis of different conformers, 2D-NMR experiments in DMSO- d_6 and DMF- d_7 were performed.

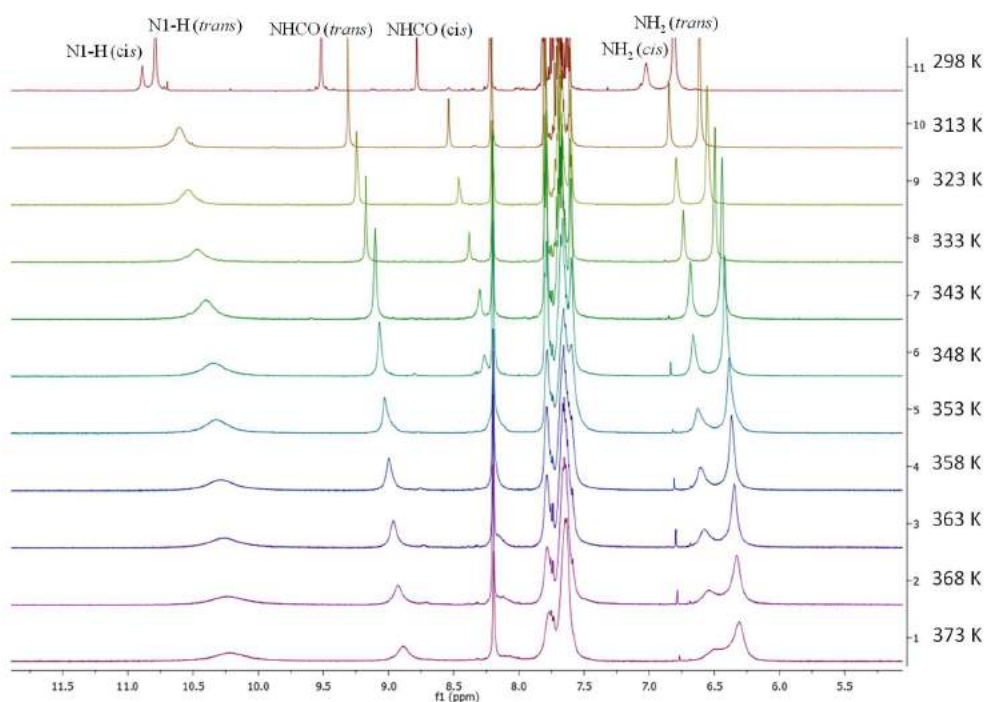


Figure 3. Dynamic NMR experiments of carboxamidouracil **8a** at high temperatures. The ^1H -NMR spectra from 5.0 to 12.0 ppm of **8a** were measured in DMSO- d_6 at different temperatures, between 298 and 373 K, to analyze the signals of the 6-amino group, the amide NH, and the N1-H in detail.

2.2.2. Two-Dimensional NMR Studies

Nuclear Overhauser enhancement and exchange spectroscopy (NOESY and EXSY) experiments, showed the presence of cross peaks with the same phase as the diagonal peaks (experimental mixing time D8 of 300 ms, Figure 4) for almost all ^1H signals, which points to a chemical exchange mechanism [12,23]. This takes place if a nucleus is transferred from one magnetic environment to another. As an example, the EXSY cross peaks of the *cis*-amide NH at 8.50 ppm and the *trans*-amide NH at 9.20 ppm are shown in Figure 4 in the same sign of the diagonal peaks (shown in red). Chemical

exchange with the solvent could be excluded by the fact that we observed a cross-correlation also for non-exchangeable protons. Cross-correlation in phase of the signal of one conformer with the corresponding signal from the other conformer was observed as a proof for conformer equilibrium (Figure 4).

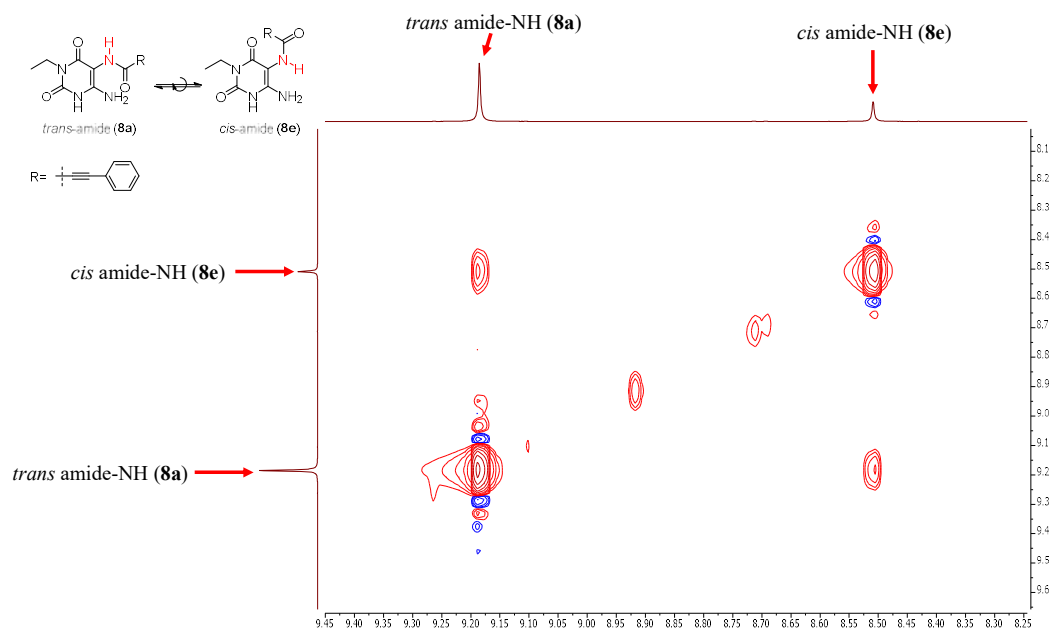


Figure 4. Exchange spectroscopy (EXSY)-NMR spectrum of **8a** in DMSO- d_6 indicating cross-correlation in phase between the two amide-NH protons of conformer **8a** and **8e**. EXSY cross peaks of the *cis*-amide NH at 8.50 ppm and the *trans*-amide NH at 9.20 ppm are shown.

These results clearly showed that the duplication of peaks in the NMR spectra of **8a** (and related amidouracil derivatives) was due to the presence of different conformational isomers, such as **8a** and **8e** (Scheme 1C).

2.2.3. Analysis of Differently Substituted 5-Amino-6-Carboxamidouracil Derivatives

We subsequently synthesized and investigated different 6-amino-5-carboxamidouracil derivatives with various carboxylic acid residues (Table 1) by dynamic and multidimensional NMR experiments. The equilibrium of their conformers and the energy of their rotational barrier were analyzed. In order to explore if the *N1*-substitution or the *N3*-substitution has any influence on the conformer equilibrium, compounds **12** (3-propargyl) and **13** (1,3-dimethyl) were synthesized (Table 1). Neither the change of the substitution pattern at the *N1*- or *N3*-position nor diethoxy substitution at the phenylethynyl residue changed the *cis*–*trans* ratio significantly (Table 1; compare **8a**, **12**, and **13**).

Table 1. Synthesized 6-amino-5-carboxamidouracil derivatives, yields, and experimental *trans/cis* ratios from ¹H-NMR in DMSO-*d*₆.

Compound	Structure	Yield ^a	<i>trans/cis</i> % Ratio ^b
8a		85%	73:27
12		79%	72:28
13		88%	77:23
14		80%	95:5
15		90%	89:11
16		87%	100:0
17		80%	91:9

^a Isolated yields of the amide coupling reaction of 5,6-diaminouracil derivatives (7, 10, and 11) with different carboxylic acid derivatives. ^b The ratio of the *cis*- and *trans*-amide conformers of 8a and 12–17 were determined by integration of their ¹H-NMR signals.

Finally, derivatives containing different linkers were prepared and analyzed. In the case of 8-arylethynyl derivatives the amount of the less stable conformer was between 20% and 30% in all three investigated cases (8a, 12, and 13). Mainly a single conformer was observed for the styryl-substituted derivative 14, while with a single-bond linker in phenylethyl derivative 15 the ratio of *trans*- and

cis-amide was approximately 89:11. A shorter linker shifted the equilibrium to a single conformer (**16**, **17**). However, this might also be due to a fast equilibrium between these two isomers, which cannot be distinguished by NMR spectroscopy. Due to the crystal structure and density functional theory (DFT) calculation of compound **8a** and the NOESY spectra of compound **17**, the *trans*-amide conformer is concluded to be the more stable rotational conformer.

2.2.4. NMR Studies at Low Temperature

When further dynamic NMR experiments were performed for amide **8** at low temperature (213–283 K), a different conformer equilibrium was observed. NOESY spectra at 223 K showed the separation of the amino groups of both amide conformers **8a** and **8e** (Figure 5). A cross-correlation for the amino group of conformer A (compound **8a**) was found between two peaks at 6.76 and 7.89 ppm. The same cross peak was found for the amino group of conformer B (compound **8e**) at 6.58 and 7.58 ppm. The peak corresponding to the amino group of conformer B could only be observed with NOESY NMR because it was covered by the aromatic signals. The latter was expected to slow rotation of the $C_{sp^2}-N$ bond [24]. The coalescence temperature of this amino group could be observed between 228 and 233 K (Figure 6). Using the simplified Eyring equation (Equation (4)), compound **8a** showed a rotational barrier for the amino group, $\Delta G^\ddagger = 46.4 \pm 0.42 \text{ kJ}\cdot\text{mol}^{-1}$.

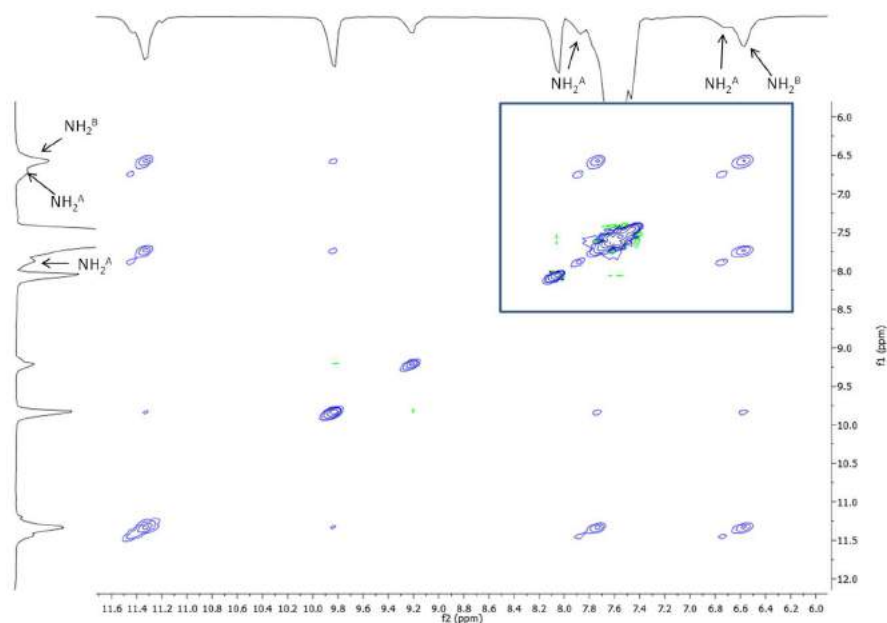


Figure 5. EXSY cross-correlation in $\text{DMSO-}d_6$ suggesting a low rotational barrier of the $C_{sp^2}-N$ bond in amide **8a** and **8e**. Cross-correlations for the amino groups of conformer A and conformer B were found at 6.76 and 7.89 ppm, and at 6.58 and 7.58 ppm, respectively.

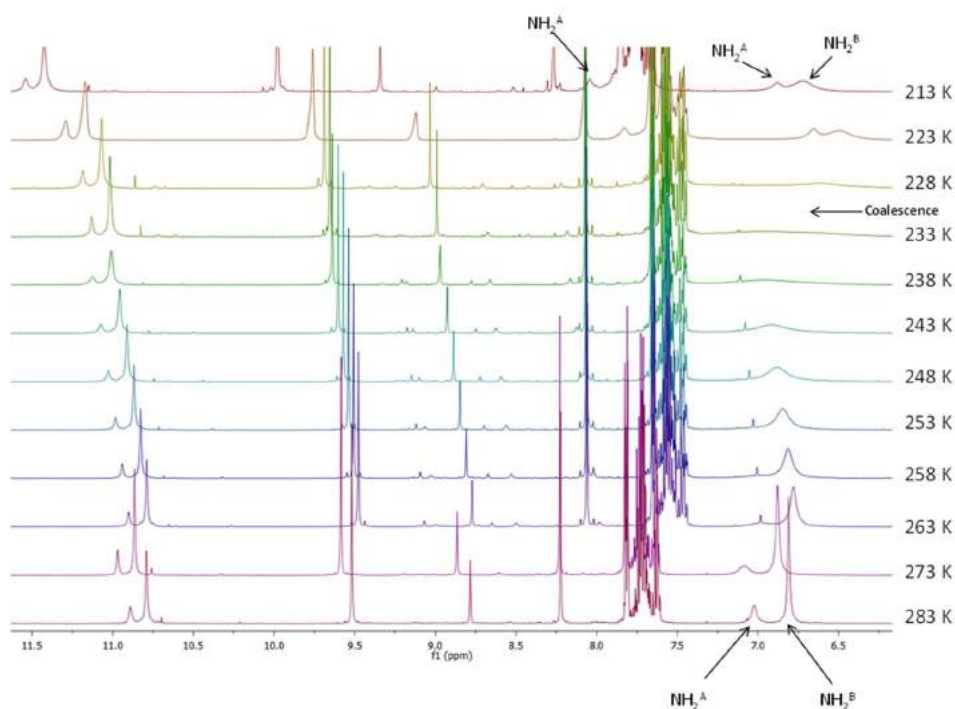


Figure 6. Proton dynamic NMR experiments of the carboxamidouracil isomers **8a** and **8e** in DMF- d_7 at low temperature. Different NMR spectra for amide **8** were recorded from 213–283 K, with 5–10 K intervals. Coalescence of the 6-amino group was detected between 228 K and 233 K.

However, the Eyring equation to calculate ΔG^\ddagger can only be used if the thermodynamic stability of the two conformers is equal. To determine ΔG^\ddagger , quantum chemical calculations are required. A line-shape analysis was not possible due to the fact that the coalescence temperature was above 105 °C (see Figure 3). In order to predict the 3D structures of these conformers, density functional theory (DFT) calculations in liquid phase and X-ray crystallography for the solid state of **8a** were subsequently performed (Figures 7 and 8).

2.2.5. DFT Calculations

The DFT computed free energy difference between the two conformers is $\Delta G^\ddagger = -1$ kcal, which corresponds to a $k_{\text{trans}}/k_{\text{cis}}$ ratio of 84:16 (Figure 7) at room temperature. This is consonant with the NMR results, which showed a ratio of 73:27 both indicating a higher stability of the *s-trans* conformer. The rotational barrier in DMSO solution is calculated to be $\Delta G^\ddagger = 20.0$ kcal·mol $^{-1}$, which is in accordance with the results from the NMR experiments.

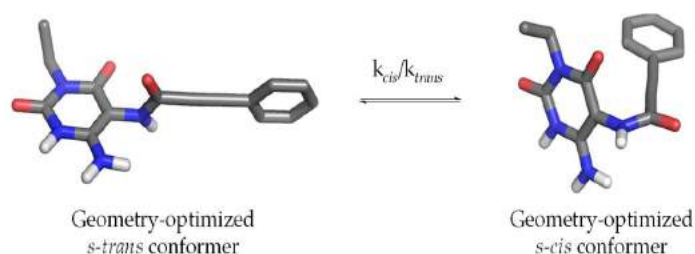


Figure 7. Geometry-optimized amide bond rotational conformers **8a** and **8e**. The conformers are shown in stick models (carbon atoms colored grey, oxygen atoms in red, the nitrogen atoms in blue, and the hydrogen atoms connected to nitrogen in white).

2.2.6. X-ray Crystal Structure

Several solvents were tried to obtain single crystals, but finally we only obtained suitable crystals (triclinic space group P1) of compound **8a** from DMSO solution. The resulting crystal structure showed exclusively the more stable regioisomer **8a** (Figure 8). No intramolecular hydrogen bonding and no π -stacking between the molecules was observed. The crystals were found to be mainly formed by intermolecular hydrogen bonding as shown in Figure 8.

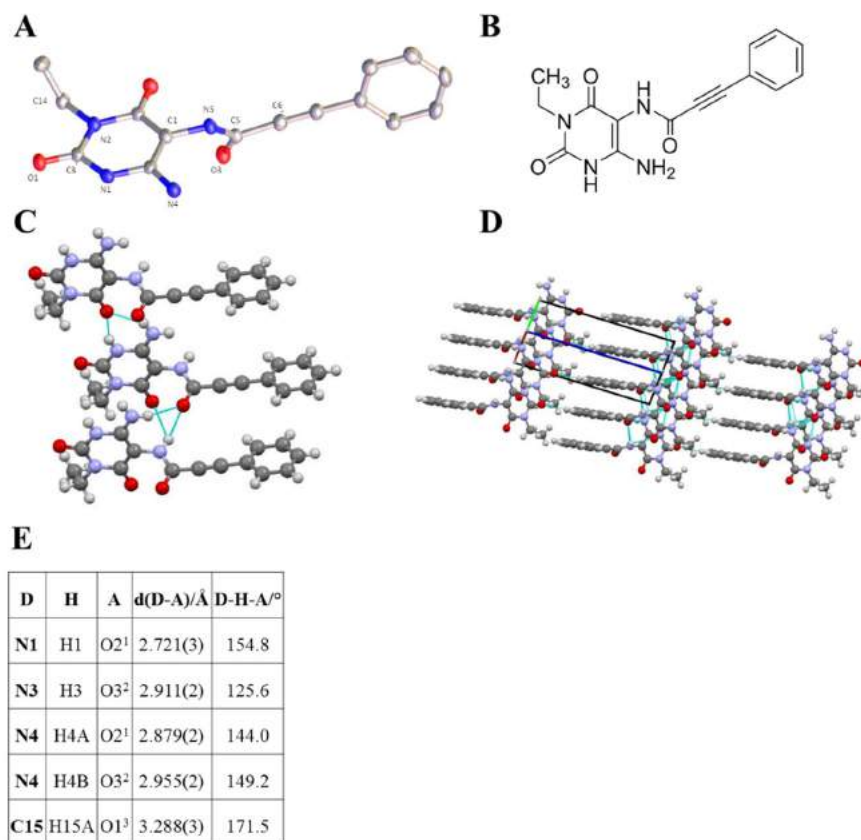


Figure 8. Results of the single X-ray crystallography of compound **8**. X-ray crystal structure (**A**) and chemical structure (**B**) of compound **8a**. (**C**) Intermolecular hydrogen bond interactions in the crystal (colored in cyan). (**D**) Crystal unit cell of the P1 space group. (**E**) Distances of intermolecular interactions in the crystal structure. For color coding see Figure 7.

3. Conclusions

6-Amino-5-carboxamidouracil derivatives, which are important intermediates in the synthesis of pharmaceutically important 8-substituted xanthine derivatives, were observed to show a more or less pronounced duplication of NMR signals, depending on their carboxylic acid residue. In order to understand this phenomenon, selected 6-amino-5-carboxamidouracils were analyzed using dynamic and 2D NMR-experiments, DFT calculations, and single-molecule X-ray crystallography. The duplication of NMR signals could be correlated with a partial double bond character of the amide bond and a low rotational barrier of this bond depending on the carboxylic acid residue. According to DFT calculations, in the case of 5-ethynylcarboxamidouracils, the triple bond appears to stabilize the thermodynamically less stable *cis* conformer. This could be observed in solution, while the obtained crystal structure consisted solely of the more stable *trans* conformer.

4. Materials and Methods

Chemicals were purchased from Merck (Darmstadt, Germany), ABCR (Karlsruhe, Germany), or TCI (Eschborn, Germany). Thin layer chromatography (TLC) was performed on TLC plates F₂₅₄ (Merck) and analyzed using UV light. High-resolution mass spectra (HR-MS) were recorded on a micrOTOF-Q mass spectrometer (Bruker, Billerica, MA, USA), further mass spectra were performed on an API 2000 (Applied Biosystems, Foster City, CA, USA) mass spectrometer. ¹H- and ¹³C-NMR spectra were recorded in CDCl₃, DMSO-*d*₆, or DMF-*d*₇ on a Bruker Ascend 600 MHz NMR-spectrometer (Bruker) operating at 600.18 MHz (¹H) or 150.93 MHz (¹³C), respectively. Chemical shifts (δ) are reported in ppm and are referenced to the chemical shifts of the residual solvent proton(s) present in CHCl₃ (7.26 ppm for the ¹H-NMR spectra and 77.16 ppm for the ¹³C-NMR spectra), in DMSO (2.50 ppm for the ¹H-NMR spectra and 39.52 ppm for the ¹³C-NMR spectra), and in DMF (2.75 ppm for the ¹H-NMR spectra and 29.76 ppm for the ¹³C-NMR spectra). Multiplicity: s, singlet; d, doublet; q, quartet; m, multiplet. Coupling constants (*J*) are shown in Hertz (Hz). Dynamic HPLC analyses were performed on “Knauer GmbH” (Berlin, Germany) systems of the “PLATINblue” series with a P-1 pump, a column oven, a PDA-1 diode array UV-detector, and a Knauer Eurospher II 100-2 C18, (2 mm, 100 × 2.0 mm; number 19040303147) column. The solvents were of HPLC grade.

4.1. Eyring Equation

If rotation around an amide bond is slow on the NMR timescale, and the rotational barrier is between 3 and 19 kcal/mol, the observation of two conformers is permitted due to the partial double bond character of the amide C–N bond [25]. Under this assumption, one sharp peak for the duplicated signals of **8** should be observed at the coalescence temperature in the ¹H-NMR spectrum [12,26–28]. Thus, the rotational barrier could be determined using the Eyring equations. Before the coalescence temperature is reached, the interconversion rate constant corresponds to the following equation:

$$k = \frac{1}{t} \ll \pi \frac{\Delta\nu}{\sqrt{2}} \quad (1)$$

where *k* is interconversion rate constant (s^{−1}), *t* is interconversion time (s), and Δ*ν* is the NMR shift (Hz) separation of the signals at low temperatures when exchange does not occur.

Heating leads to a faster exchange rate relative to the NMR timescale and only one averaged signal becomes detectable. At the coalescence temperature, the equation of the interconversion rate constant is

$$k_{Tc} = \pi \frac{\Delta\nu}{\sqrt{2}} \quad (2)$$

where *k*_{Tc} is the rate constant (s^{−1}).

The free Gibbs activation energy Δ*G*[#] of rotation can be calculated using the following Eyring equations:

$$-\Delta G^\# = K \frac{k_B \times T}{h} e^{\frac{-\Delta G^\#}{RT}} \quad (3)$$

$$\Delta G^\# = 4.57 \times 10^{-3} T \left(9.972 + \log \frac{T_c}{\Delta\nu} \right) \quad (4)$$

where *R* is the universal gas constant ($\frac{1.0872 \text{ cal}}{\text{K}\cdot\text{mol}}$), *k*_B is the Boltzmann constant ($3.2998 \times 10^{-24} \frac{\text{cal}}{\text{K}}$), *h* is the Planck constant ($1.584 \times 10^{-34} \text{ cal}\cdot\text{s}$), and *T*_c is the coalescence temperature (K) [12].

4.2. DFT Calculation

Most of the quantum chemical calculations were carried out with the TURBOMOLE and ORCA programs [29,30]. All structures were fully optimized at the dispersion-corrected DFT level using the PBEh-3c DFT [31]. This composite method contains a modified Perdew–Burke–Ernzerhof (PBE)-based hybrid function together with an efficient valence double-zeta AO basis set. The method also involves

an approximate counterpoise correction for basis set superposition errors (BSSE), as well as three-body dispersion effects [31]. Conformational searches [32], pre-optimizations, as well as the calculation of the Hessian to start transition state searches were conducted at the semi-empirical tight-binding level (GFN-xTB method) [33]. The GFN-xTB-optimized structures were used as input for subsequent full PBEh-3c optimizations. Reaction paths were obtained with the growing-string method (GSM) of Zimmerman [34] which was interfaced to our in-house XTB code [35]. Single-point gas phase energies were computed with the large polarized triple-zeta (def2-TZVPP) sets by Weigend et al. [36] in combination with the very accurate DSD-BLYP double hybrid functional [37]. The atom pairwise D3 correction with Becke–Johnson (BJ) damping to account for intra- and intermolecular London dispersion interactions was included in all treatments [38]. Note, that the original D2 treatment in DSD-BLYP is replaced by the D3 version (with damping parameters $s_6 = 0.57$, $a_1 = 0$, $s_8 = 0$, $a_2 = 5.4$) [39]. This functional performs excellently on the huge GMTKN55 thermochemical database (i.e., is practically the best out of 200+ tested DFT approximations) [40]. The combined level of theory used for electronic gas phase energies is denoted DSD-BLYP/TZ//PBEh-3c in standard notation. In all DFT treatments, the resolution-of-the-identity approximation [41] has been used for the two-electron integrals to speed up the computations. The numerical quadrature grid m4 (grid 5 in ORCA) was employed for the integration of the exchange-correlation contribution. Gibbs free energies at 298.15 K in DMSO were reported as a solvent (termed ΔG). The ro-vibrational corrections to the free energy are obtained from a modified rigid rotor, harmonic oscillator statistical treatment [42] based on scaled harmonic frequencies obtained at the (gas phase) HF-3c [43] level. For the entropy, all frequencies with wavenumbers below 100 cm^{-1} were treated as mixed rigid rotors and harmonic oscillators. Solvent effects on the thermochemical properties have been obtained by the COSMO-RS method [44] (COSMOtherm software package [44], parametrization from 2016) based on BP86/TZVP [36,45] single-point calculations. The PBEh-3c as well as GFN-xTB optimizations were run consistently in a continuum solvent. For GFN-xTB the built-in GBSA solvation model 23 is employed; while for PBEh-3c, the DCOSMO-RS method is used [46]. The solvation contributions to free energies at 298.15 K in DMSO solution are computed at those structures (i.e., PBEh-3c[DCOSMO-RS]) The computed free energies are obtained by $\Delta G = \Delta E + \Delta GRRHO + \Delta \delta GCOSMO-RS$, where the last two terms refer to the above mentioned ro-vibrational/translational and solvation contributions, respectively, to the free energy. The final theory level is denoted as DSD-BLYP/TZ[COSMO-RS(BP86/TZVP)]//PBEh-3c[DCOSMO-RS] where the abbreviations in square brackets denote the level of the solvation treatment.

4.3. General Procedures

4.3.1. Synthesis of 5,6-Diaminouracil Derivatives

The different *N*-substituted 5,6-diaminouracils were synthesized in analogy to procedures described in the literature [9].

4.3.2. General Procedure for Amide Formation

To a solution of the respective carboxylic acid (1.0 equiv.) and COMU (1.1 equiv.), dissolved in a minimum amount of DMF, a mixture of diaminouracil derivative (1.1 equiv.) and DIPEA (2.0 equiv.), dissolved in a minimum amount of DMF, was added dropwise. The reaction was stirred for 5–10 min at room temperature. Then, water was added, and the resulting precipitate was filtered off, washed with water, and dried under reduced pressure.

4.3.3. NMR Spectra

N-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-phenylpropiolamide (8). Yield: 85% (white solid); mp = 270–272 °C. Major isomer: $^1\text{H-NMR}$ (600 MHz, DMSO- d_6) δ 10.45 (s, 1H, N1-H), 9.16 (s, 1H, CONH), 7.62–7.56 (m, 2H, H_{arom}), 7.53–7.45 (m, 3H, H_{arom}), 6.14 (s, 2H, NH_2), 3.79–3.67 (m, 2H, N1- CH_2), 1.04 (t, $J = 7.0\text{ Hz}$, 2H, CH_2CH_3). $^{13}\text{C-NMR}$ (126 MHz, DMSO- d_6) δ 160.0 (CON),

152.7 (C6), 150.2 (CO), 149.5 (CO), 132.0 (2C, C_{arom}), 130.2 (C_{arom}), 129.0 (2C, C_{arom}), 120.0 (C_{arom}), 85.7 (C5 or C_{alkyne}), 84.9 (C5 or C_{alkyne}), 83.6 (C5 or C_{alkyne}), 34.4 (N1-CH₂), 13.2 (CH₂CH₃). Minor isomer: ¹H-NMR (600 MHz, DMSO-*d*₆) δ 10.49 (s, 1H, N1-H), 8.51 (s, 1H, CONH), 7.54–7.51 (m, 1H, H_{arom}), 7.43–7.39 (m, 2H, H_{arom}), 7.35–7.32 (m, 2H, H_{arom}), 6.40 (s, 2H, NH₂), 3.74–3.78 (m, 2H, N1-CH₂), 1.01 (t, *J* = 7.0 Hz, 3H, CH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ 162.3 (CON), 157.6 (C6), 151.5 (CO), 149.5 (CO), 132.0 (2C, C_{arom}), 130.3 (C_{arom}), 129.0 (2C, C_{arom}), 120.0 (C_{arom}), 87.5 (C5 or C_{alkyne}), 85.8 (C5 or C_{alkyne}), 83.2 (C5 or C_{alkyne}), 34.4 (N1-CH₂), 13.3 (CH₂CH₃). High resolution mass spectra (HRMS) (electrospray ionization-quadrupole-time-of-flight) (ESI-QTOF) calculated for C₁₅H₁₄N₄O₃ [M + H]⁺: 299.1139; found: 299.1139.

N-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3,4-diethoxyphenyl) propiolamide (**12**). Yield: 79% (white solid); mp = 185–183 °C. Major isomer: ¹H-NMR (600 MHz, DMSO-*d*₆) δ 10.64 (s, 1H, N1-H), 9.09 (s, 1H, CONH), 7.14 (dd, *J* = 8.4, 1.9 Hz, 1H, H_{arom}), 7.09 (d, *J* = 2.1 Hz, 1H, H_{arom}), 7.02 (d, *J* = 8.3 Hz, 1H, H_{arom}), 6.25 (s, 2H, NH₂), 4.41 (d, *J* = 2.4 Hz, 2H, H_{propargyl}), 4.09–4.01 (m, 4H, 2 × OCH₂), 3.02 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 1.34–1.31 (m, 6H, 2 × OCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ 159.3, 153.0, 150.7, 150.2, 149.1, 147.9, 125.8 (C_{arom}), 116.2 (C_{arom}), 113.1 (C_{arom}), 111.5 (C_{arom}), 85.5 (C5 or C_{alkyne}), 84.7 (C5 or C_{alkyne}), 83.7 (C5 or C_{alkyne}), 79.9 (C_{propargyl}), 72.5 (C_{propargyl}), 63.8 (2C, OCH₂), 28.9 (N1-CH₂), 14.6 (2C, OCH₂CH₃). Minor isomer: ¹H-NMR (600 MHz, DMSO-*d*₆) δ 10.72 (s, 1H, N1-H), 8.45 (s, 1H, CONH), 6.97–6.90 (m, 2H, H_{arom}), 6.82 (d, *J* = 1.8 Hz, 1H, H_{arom}), 6.50 (s, 2H, NH₂), 4.51–4.44 (m, 2H, H_{propargyl}), 4.05–4.02 (m, 2H, OCH₂) 3.98 (q, *J* = 7.4 Hz, 2H, OCH₂), 3.02–3.01 (m, 1H, H_{propargyl}), 1.32–1.28 (m, 6H, OCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ 160.3, 157.8, 157.8, 151.9, 150.2, 149.1, 147.8, 126.0 (C_{arom}), 116.2 (C_{arom}), 113.0 (C_{arom}), 111.4 (C_{arom}), 87.4 (C5 or C_{alkyne}), 87.1 (C5 or C_{alkyne}), 81.8 (C5 or C_{alkyne}), 79.9 (C_{propargyl}), 72.4 (C_{propargyl}), 63.9 (2C, OCH₂), 28.9 (N1-CH₂), 14.5 (2C, OCH₂CH₃). HRMS (ESI-QTOF) calculated for C₂₀H₂₀N₄O₅ [M + H]⁺: 397.1504; found: 397.1506.

N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3,4-diethoxyphenyl) propiolamide (**13**). Yield: 88% (white solid); mp = 220–223 °C. Major isomer: ¹H-NMR (600 MHz, DMSO-*d*₆) δ 9.08 (s, 1H, CONH), 7.16 (dd, *J* = 8.3, 2.0 Hz, 1H, H_{arom}), 7.11 (d, *J* = 1.9 Hz, 1H, H_{arom}), 7.03 (d, *J* = 8.8 Hz, 2H, H_{arom}), 6.76 (s, 2H, NH₂), 4.07 (dq, *J* = 16.9, 7.0 Hz, 4H, 2 × OCH₂), 3.31 (s, 3H, CH₃), 3.12 (s, 3H, CH₃), 1.34 (td, *J* = 7.0, 2.7 Hz, 6H, 2 × OCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ 158.9, 153.3, 152.1, 150.5, 150.2, 147.9, 125.8 (C_{arom}), 116.2 (C_{arom}), 113.1 (C_{arom}), 111.6 (C_{arom}), 86.1 (C5 or C_{alkyne}), 84.5 (C5 or C_{alkyne}), 83.9 (C5 or C_{alkyne}), 63.9 (2C, OCH₂), 30.0 (CH₃), 27.5 (CH₃), 14.6 (2C, OCH₂CH₃). Minor isomer: ¹H-NMR (600 MHz, DMSO-*d*₆) δ 8.46 (s, 1H, CONH), 7.04 (s, 1H, H_{arom}), 6.96 (d, *J* = 8.4 Hz, 1H, H_{arom}), 6.85 (dd, *J* = 8.3, 1.9 Hz, 1H, H_{arom}), 6.75 (s, 2H, NH₂), 4.07–4.05 (m, 2H, OCH₂), 3.95 (q, *J* = 6.9 Hz, 2H, OCH₂), 3.34 (s, 3H, CH₃), 3.15 (s, 3H, CH₃), 1.32–1.28 (m, 6H, 2 × OCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ 160.0, 158.0, 153.2, 150.5, 150.2, 147.9, 125.8 (C_{arom}), 116.1 (C_{arom}), 113.1 (C_{arom}), 111.5 (C_{arom}), 88.1 (C5 or C_{alkyne}), 86.9 (C5 or C_{alkyne}), 82.1 (C5 or C_{alkyne}), 63.8 (2C, OCH₂), 30.1 (CH₃), 27.6 (CH₃), 14.5 (2C, OCH₂CH₃). HRMS (ESI-QTOF) calculated for C₁₉H₂₂N₄O₅ [M + H]⁺: 387.1663; found: 387.1668.

N-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)cinnamamide (**14**). Yield: 80% (off-white solid); mp > 320 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 10.43 (s, 1H, N1-H), 8.68 (s, 1H, CONH), 7.58 (d, *J* = 7.4 Hz, 2H, H_{arom}), 7.50–7.37 (m, 4H, H_{arom} + H_{vinyl}), 6.83 (d, *J* = 15.9 Hz, 1H, H_{vinyl}), 5.99 (s, 2H, NH₂), 3.74 (q, *J* = 6.5 Hz, 2H, CH₂), 1.06 (t, *J* = 6.7 Hz, 3H, CH₃). ¹³C-NMR (DMSO-*d*₆, 126 MHz) δ 164.9 (CON), 160.3 (C6), 149.7 (CO), 149.5 (CO), 138.8 (C_{vinyl} or C_{arom}), 135.0 (C_{vinyl} or C_{arom}), 129.4 (C_{vinyl} or C_{arom}), 129.0 (2C, C_{arom}), 127.4 (2C, C_{arom}), 122.4 (C_{vinyl} or C_{arom}), 87.4 (C5), 34.4 (N3-CH₂), 13.2 (CH₃). HRMS (ESI-QTOF) calculated for C₁₅H₁₆N₄O₃ [M + H]⁺: 301.1295; found: 301.1294.

N-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-phenylpropanamide (**15**). Yield: 90% (white solid); mp > 320 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 10.38 (s, 1H, N1-H), 8.39 (s, 1H, CONH), 7.28 (t, *J* = 7.4 Hz, 2H, H_{arom}), 7.24 (d, *J* = 6.9 Hz, 2H, H_{arom}), 7.18 (t, *J* = 7.1 Hz, 1H, H_{arom}),

5.82 (s, 2H, NH₂), 3.73 (q, *J* = 6.9 Hz, 2H, N3-CH₂), 2.91–2.80 (m, 2H, CH₂), 2.53 (dd, *J* = 9.2, 7.0 Hz, 2H, CH₂), 1.04 (t, *J* = 7.0 Hz, 3H, CH₃). ¹³C-NMR (DMSO-*d*₆, 126 MHz) δ 171.7 (CON), 160.4 (C₆), 149.9 (CO), 149.6 (CO), 141.5 (C_{arom}), 128.3 (2C, C_{arom}), 128.1 (2C, C_{arom}), 125.8 (C_{arom}), 87.2 (C5), 36.8 (CH₂), 34.3 (N3-CH₂), 30.9 (CH₂), 13.2 (CH₃). HRMS (ESI-QTOF) calculated for C₁₅H₁₈N₄O₃ [M + H]⁺: 303.1452; found: 303.1454.

N-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)benzamide (**16**). Yield: 87% (yellowish solid; mp > 320 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 10.38 (s, 1H, N1-H), 8.86 (s, 1H, CONH), 7.99–7.91 (m, 2H, H_{arom}), 7.56–7.51 (m, 1H, H_{arom}), 7.47 (t, *J* = 7.5 Hz, 2H, H_{arom}), 6.06 (s, 2H, NH₂), 3.75 (q, *J* = 7.0 Hz, 2H, N3-CH₂), 1.06 (t, *J* = 7.0 Hz, 3H, CH₃). ¹³C-NMR (126 MHz, DMSO-*d*₆) δ 166.4 (CON), 160.5 (C₆), 150.4 (CO), 149.7 (CO), 134.5 (C_{arom}), 131.1 (C_{arom}), 128.0 (C_{arom}), 127.8 (C_{arom}), 87.1 (C5), 34.4 (N3-CH₂), 13.3 (CH₃). HRMS (ESI-QTOF) calculated for C₁₃H₁₄N₄O₃ [M + H]⁺: 275.1139; found: 275.1142.

N-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-phenylacetamide (**17**). Yield: 80% (white solid); mp > 320 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 10.39 (s, 1H, N1-H), 8.58 (s, 1H, CONH), 7.35–7.31 (m, 2H, H_{arom}), 7.28 (m, 2H, H_{arom}), 7.23–7.19 (m, 1H, H_{arom}), 5.90 (s, 2H, NH₂), 3.71 (q, *J* = 7.0 Hz, 2H, N3-CH₂), 3.56 (s, 2H, CH₂), 1.03 (t, *J* = 7.0 Hz, 3H, CH₃). ¹³C-NMR (DMSO-*d*₆, 126 MHz) δ 170.6 (CON), 160.5 (C₆), 150.1 (CO), 149.7 (CO), 136.6 (C_{arom}), 129.4 (C_{arom}), 128.2 (C_{arom}), 126.3 (C_{arom}), 87.5 (C5), 42.1 (COCH₂), 34.5 (N3-CH₂), 13.4 (CH₃). HRMS (ESI-QTOF) calculated for C₁₄H₁₆N₄O₃ [M + H]⁺: 289.1295; found: 289.1304.

Supplementary Materials: The following data are available online. Figure S1–S15: NMR spectra, Figure S16: DHPLC analyses of **12** at 205 nm, Figure S17: UV-spectrum of compound **12**. Table S1–S3: Crystal data and structure refinement for compound **8**. Scheme S1–S2: Synthesis of diaminouracil derivatives.

Author Contributions: D.M. synthesized the compounds and analyzed the NMR spectra. S.G. performed the DFT calculations. G.S. measured the X-ray crystal structure. C.E.M. supervised the experiments. D.M. and C.E.M. wrote the manuscript, all coauthors contributed to writing.

Funding: D.M. and C.E.M. were supported by the BMBF (German Federal Ministry for Education and Research) within the “Neuroallianz” consortium of the BioPharma initiative.

Acknowledgments: We thank Andreas Schneider for the DHPLC measurements, Jörg Hockemeyer for helpful discussions, and Senada Nozinovic, Marion Schneider, Sabine Terhart-Krabbe, and Annette Reiner for recording NMR and MS data.

Conflicts of Interest: No conflicts of interest.

References

1. Boison, D. Adenosine as a neuromodulator in neurological diseases. *Curr. Opin. Pharmacol.* **2008**, *8*, 2–7. [[CrossRef](#)] [[PubMed](#)]
2. Morelli, M.; Carta, A.R.; Jenner, P. Adenosine A_{2A} receptors and Parkinson’s disease. *Handb. Exp. Pharmacol.* **2009**, *193*, 589–615.
3. Rahman, A. The role of adenosine in Alzheimer’s disease. *Curr. Neuropharmacol.* **2009**, *7*, 207–216. [[CrossRef](#)] [[PubMed](#)]
4. Leone, R.D.; Lo, Y.-C.; Powell, J.D. A_{2A}R antagonists: Next generation checkpoint blockade for cancer immunotherapy. *Comput. Struct. Biotechnol. J.* **2015**, *13*, 265–272. [[CrossRef](#)] [[PubMed](#)]
5. Franco, R.; Oñatibia-Astibia, A.; Martínez-Pinilla, E. Health benefits of methylxanthines in cacao and chocolate. *Nutrients* **2013**, *5*, 4159–4173. [[CrossRef](#)] [[PubMed](#)]
6. Dungo, R.; Deeks, E.D. Istradefylline: First global approval. *Drugs* **2013**, *73*, 875–882. [[CrossRef](#)] [[PubMed](#)]
7. LeWitt, P.A.; Guttman, M.; Tetrud, J.W.; Tuite, P.J.; Mori, A.; Chaikin, P.; Sussman, N.M. Adenosine A_{2A} receptor antagonist istradefylline (KW-6002) reduces “off” time in Parkinsons disease: A double-blind, randomized, multicenter clinical trial (6002-US-005). *Ann. Neurol.* **2008**, *63*, 295–302. [[CrossRef](#)]
8. Sauer, R.; Maurinsh, J.; Reith, U.; Fülle, F.; Klotz, K.-N.; Müller, C.E. Water-soluble phosphate prodrugs of 1-propargyl-8-styrylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. *J. Med. Chem.* **2000**, *43*, 440–448. [[CrossRef](#)]

9. Hockemeyer, J.; Burbiel, J.C.; Müller, C.E. Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: potential drugs for Parkinson's Disease. *J. Org. Chem.* **2004**, *69*, 3308–3318. [[CrossRef](#)]
10. Müller, C.E.; Schober, U.; Hipp, J.; Geis, U.; Frobenius, W.; Pawlowski, M. Configurationally stable analogs of styrylxanthines as A_{2A} adenosine receptor antagonist. *Eur. J. Med. Chem.* **1997**, *32*, 709–719. [[CrossRef](#)]
11. Müller, C.E.; Hockemeyer, J.; Tzvetkov, N.; Burbiel, J.C. 8-Ethynylxanthine Derivatives as Selective A_{2A} Receptor Antagonists. WO Patent WO2008077557A1, 19 December 2007.
12. Lopes, B.A.; Miguez, E.; Kümmerle, E.A.; Rumjanek, M.V.; Fraga, A.C.; Barreiro, J.E. Characterization of amide bond conformers for a novel heterocyclic template of *N*-acylhydrazone derivatives. *Molecules* **2013**, *18*, 11683–11704. [[CrossRef](#)] [[PubMed](#)]
13. Quintanilla-Licea, R.; Colunga-Valladares, J.; Caballero-Quintero, A.; Rodríguez-Padilla, C.; Tamez-Guerra, R.; Gomez-Flores, R.; Waksman, N. NMR detection of isomers arising from restricted rotation of the C–N amide bond of *N*-formyl-*o*-toluidine and *N,N*-bis-formyl-*o*-toluidine. *Molecules* **2002**, *7*, 662–673. [[CrossRef](#)]
14. Mphahlele, J.M.; Maluleka, M.M.; Rhyman, L.; Ramasami, P.; Mampa, M.R. Spectroscopic, DFT, and XRD studies of hydrogen bonds in *N*-unsubstituted 2-aminobenzamides. *Molecules* **2017**, *22*, 83. [[CrossRef](#)] [[PubMed](#)]
15. Laursen, J.S.; Engel-Andreasen, J.; Fristrup, P.; Harris, P.; Olsen, C.A. Cis-trans amide bond rotamers in beta-peptoids and peptoids: Reevaluation of stereoelectronic effects in backbone and side chains. *J. Am. Chem. Soc.* **2013**, *135*, 2835–2844. [[CrossRef](#)] [[PubMed](#)]
16. Hu, X.; Zhang, W.; Carmichael, I.; Serianni, A.S. Amide cis–trans isomerization in aqueous solutions of methyl *N*-formyl-*d*-glucosaminides and methyl *N*-acetyl-*d*-glucosaminides: Chemical equilibria and exchange kinetics. *J. Am. Chem. Soc.* **2010**, *132*, 4641–4652. [[CrossRef](#)]
17. Marx, D.; Wingen, L.M.; Schnakenburg, G.; Müller, C.E.; Scholz, M.S. Fast, efficient, and versatile synthesis of 6-amino-5-carboxamidouracils as precursors for 8-substituted xanthines. *Front. Chem.* **2019**, *7*, 1–15. [[CrossRef](#)]
18. El-Sabbagh, O.I.; El-Sadek, M.E.; El-Kalyoubi, S.; Ismail, I. Synthesis, DNA binding and antiviral activity of new uracil, xanthine, and pteridine derivatives. *Arch. Pharm.* **2007**, *340*, 26–31. [[CrossRef](#)]
19. Hayallah, A.M.; Sandoval-Ramírez, J.; Reith, U.; Schober, U.; Preiss, B.; Schumacher, B.; Daly, J.W.; Müller, C.E. 1,8-Disubstituted xanthine derivatives: Synthesis of potent A_{2B}-selective adenosine receptor antagonists. *J. Med. Chem.* **2002**, *45*, 1500–1510. [[CrossRef](#)]
20. Yang, S.-M.; Yasgar, A.; Miller, B.; Lal-Nag, M.; Brimacombe, K.; Hu, X.; Sun, H.; Wang, A.; Xu, X.; Nguyen, K.; et al. Discovery of NCT-501, a potent and selective theophylline-based inhibitor of aldehyde dehydrogenase 1A1 (ALDH1A1). *J. Med. Chem.* **2015**, *58*, 5967–5978. [[CrossRef](#)]
21. Poulsen, A.; Ho, S.Y.; Wang, W.; Alam, J.; Jeyaraj, D.A.; Ang, S.H.; Tan, E.S.W.; Lin, G.R.; Cheong, V.W.W.; Ke, Z.; et al. Pharmacophore model for Wnt/porcupine inhibitors and its use in drug design. *J. Chem. Inf. Model.* **2015**, *55*, 1435–1448. [[CrossRef](#)] [[PubMed](#)]
22. Novak, P.; Skare, D.; Sekušak, S.; Vikić-Topić, D. Substituent, temperature and solvent effects on keto-enol equilibrium in symmetrical pentane-1,3,5-triones. Nuclear magnetic resonance and theoretical studies. *Croatica Chemica Acta* **2000**, *73*, 1153–1170.
23. Krishnan, V.V.; Thompson, W.B.; Goto, J.J.; Maitra, K.; Maitra, S. Modulations in restricted amide rotation by steric induced conformational trapping. *Chem. Phys. Lett.* **2012**, *523*, 124–127. [[CrossRef](#)] [[PubMed](#)]
24. Gorse, A.-D.; Pesquer, M. A theoretical study of aniline and some derivatives in their ground states. *J. Mol. Struct. Theochem.* **1993**, *281*, 21–32. [[CrossRef](#)]
25. Gasparro, F.P.; Kolodny, N.H. NMR determination of the rotational barrier in *N,N*-dimethylacetamide. A physical chemistry experiment. *J. Chem. Educ.* **1977**, *54*, 258. [[CrossRef](#)]
26. Mamat, C.; Pretze, M.; Gott, M.; Köckerling, M. Synthesis, dynamic NMR characterization and XRD studies of novel *N,N'*-substituted piperazines for bioorthogonal labeling. *Beilstein J. Org. Chem.* **2016**, *12*, 2478–2489. [[CrossRef](#)]
27. Modarresi-Alam, A.R.; Najafi, P.; Rostamizadeh, M.; Keykha, H.; Bijanzadeh, H.-R.; Kleinpeter, E. Dynamic ¹H NMR Study of the barrier to rotation about the C–N bond in primary carbamates and its solvent dependence. *J. Org. Chem.* **2007**, *72*, 2208–2211. [[CrossRef](#)]

28. Stärk, D.; Norrby, P.-O.; Jaroszewski, J.W. Conformational analysis of indole alkaloids corynantheine and dihydrocorynantheine by dynamic ^1H NMR spectroscopy and computational methods: steric effects of ethyl vs vinyl group. *J. Org. Chem.* **2001**, *66*, 2217–2221. [[CrossRef](#)]
29. Ahlrichs, R.; Bär, M.; Häser, M.; Horn, H.; Kölmel, C. Electronic structure calculations on workstation computers: The program system turbomole. *Chem. Phys. Lett.* **1989**, *162*, 165–169. [[CrossRef](#)]
30. Neese, F. *ORCA—An ab Initio, DFT and Semiempirical SCF-MO Package*; Max Planck Institute for Bioinorganic Chemistry: Mülheim an der Ruhr, Germany, 2012.
31. Grimme, S.; Brandenburg, J.G.; Bannwarth, C.; Hansen, A. Consistent structures and interactions by density functional theory with small atomic orbital basis sets. *J. Chem. Phys.* **2015**, *143*, 054107. [[CrossRef](#)]
32. Grimme, S.; Bannwarth, C.; Dohm, S.; Hansen, A.; Pisarek, J.; Pracht, P.; Seibert, J.; Neese, F. Fully automated quantum-chemistry-based computation of spin-spin-coupled nuclear magnetic resonance spectra. *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 14763–14769. [[CrossRef](#)] [[PubMed](#)]
33. Grimme, S.; Bannwarth, C.; Shushkov, P. A robust and accurate tight-binding quantum chemical method for structures, vibrational frequencies, and noncovalent interactions of large molecular systems parametrized for all spd-block elements ($Z = 1–86$). *J. Chem. Theory Comput.* **2017**, *13*, 1989–2009. [[CrossRef](#)] [[PubMed](#)]
34. Zimmerman, P. Reliable transition state searches integrated with the growing string method. *J. Chem. Theory Comput.* **2013**, *9*, 3043–3050. [[CrossRef](#)] [[PubMed](#)]
35. Grimme, S. *XTB, a Tight-Binding Quantum Chemistry Code for Large Molecules*, version 6.1; University of Bonn: Bonn, Germany, 2019.
36. Weigend, F.; Ahlrichs, R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297–3305. [[CrossRef](#)] [[PubMed](#)]
37. Kozuch, S.; Gruzman, D.; Martin, J.M.L. DSD-BLYP: A general purpose double hybrid density functional including spin component scaling and dispersion correction. *J. Phys. Chem. C* **2010**, *114*, 20801–20808. [[CrossRef](#)]
38. Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.* **2010**, *132*, 154104. [[CrossRef](#)]
39. Kozuch, S.; Martin, J.M.L. Spin-component-scaled double hybrids: An extensive search for the best fifth-rung functionals blending DFT and perturbation theory. *J. Comput. Chem.* **2013**, *34*, 2327–2344. [[CrossRef](#)]
40. Goerigk, L.; Hansen, A.; Bauer, C.; Ehrlich, S.; Najibi, A.; Grimme, S. A look at the density functional theory zoo with the advanced GMTKN55 database for general main group thermochemistry, kinetics and noncovalent interactions. *Phys. Chem. Chem. Phys.* **2017**, *19*, 32184–32215. [[CrossRef](#)]
41. Eichkorn, K.; Treutler, O.; Öhm, H.; Häser, M.; Ahlrichs, R. Auxiliary basis sets to approximate coulomb potentials. *Chem. Phys. Lett.* **1995**, *240*, 283–290. [[CrossRef](#)]
42. Grimme, S. Supramolecular binding thermodynamics by dispersion-corrected density functional theory. *Chem. - Eur. J.* **2012**, *18*, 9955–9964. [[CrossRef](#)]
43. Sure, R.; Grimme, S. Corrected small basis set Hartree-Fock method for large systems. *J. Comput. Chem.* **2013**, *34*, 1672–1685. [[CrossRef](#)] [[PubMed](#)]
44. Eckert, F.; Klamt, A. Fast solvent screening via quantum chemistry: COSMO-RS approach. *AIChE J.* **2004**, *48*, 369–385. [[CrossRef](#)]
45. Becke, A.D. Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys. Rev. A Gen. Phys.* **1988**, *38*, 3098–3100. [[CrossRef](#)] [[PubMed](#)]
46. Sinnecker, S.; Rajendran, A.; Klamt, A.; Diedenhofen, M.; Neese, F. Calculation of solvent shifts on electronic g-tensors with the conductor-like screening model (COSMO) and its self-consistent generalization to real solvents (direct COSMO-RS). *J. Phys. Chem. A* **2006**, *110*, 2235–2245. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of compound **8a** are available from the authors.



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Supplementary Materials

Structural and conformational studies on carboxamides of 5,6-diaminouracils – precursors of biologically active xanthine derivatives

Daniel Marx,^{1,2} Gregor Schnakenburg,³ Stefan Grimme,⁴ Christa E. Müller^{*1,2}

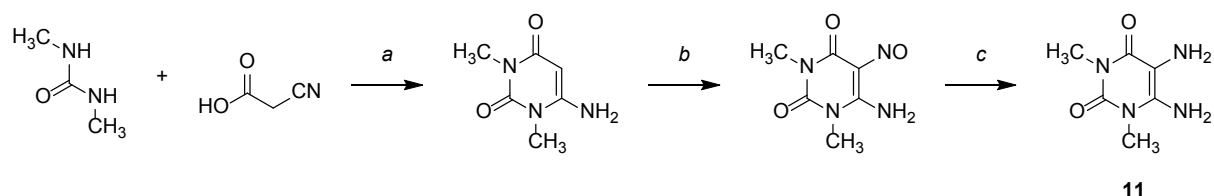
*Correspondence: Prof. Dr. Christa E. Müller, christa.mueller@uni-bonn.de, +49 (0) 228 / 73-2301

TABLE OF CONTENTS

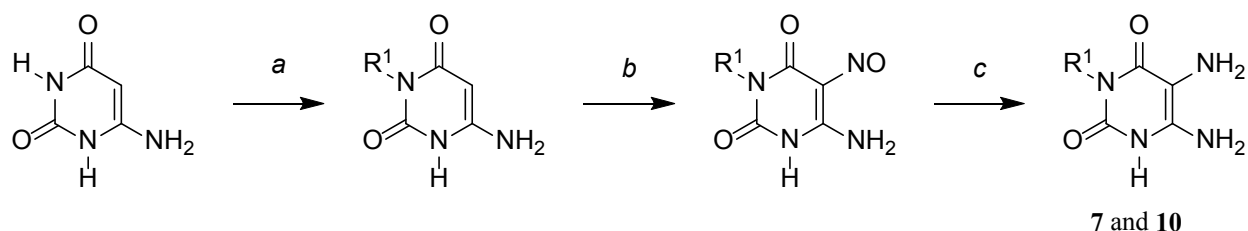
Synthesis of diaminouracil derivatives	2
Crystallographic data	3
¹ H- and ¹³ C-NMR spectra of compound 8, 12–17	8

Synthesis of diaminouracil derivatives

*N*1- and *N*3-substituted 5,6-diaminouracils were used as starting materials for the preparation of the corresponding 6-amino-5-carboxamidouracil derivatives and synthesized according to literature procedures [1-4].



Scheme S1. Synthesis of 5,6-diamino-1,3-dimethyluracil. Reagents and conditions: (a) Ac_2O , 60 °C, 3 h; (b) aq. AcOH , HNO_2 , 50-60 °C; (c) sodium dithionite, $\text{NH}_3/\text{H}_2\text{O}$, 60 °C.



7 $\text{R}^1 = \text{ethyl}$

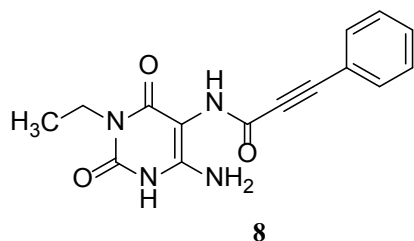
10 $\text{R}^1 = \text{propargyl}$

Scheme S2. Synthesis of *N*3-substituted 5,6-diaminouracil derivatives **7** and **10**. Reagents and conditions: (a) 2.1 equiv of hexamethyldisilazane (HMDS), reflux, 60–70 °C, 1.7 equiv of ethyl iodide for (**7**) or 3-bromopropyne for (**10**); (b) aq AcOH , HNO_2 , 50-60 °C; (c) sodium dithionite, $\text{NH}_3/\text{H}_2\text{O}$, 60 °C.

Analytical data were in accordance with published data. For details see [1, 2, 4].

Crystallographic data

Table S1. Crystal data and structure refinement for compound 8.



Identification code	GPHARM63, 8 // GXray5352
Crystal Habitus	clear colourless block
Device Type	STOE IPDS-2T
Empirical formula	C ₁₅ H ₁₄ N ₄ O ₃
Moiety formula	C ₁₅ H ₁₄ N ₄ O ₃
Formula weight	298.30
Temperature/K	123(2)
Crystal system	triclinic
Space group	P1
a/Å	4.6523(3)
b/Å	5.5387(4)
c/Å	13.7337(9)
α/°	82.818(5)
β/°	89.265(5)
γ/°	80.921(6)
Volume/Å ³	346.70(4)
Z	1
Q _{calc} /cm ³	1.429

μ/mm^{-1}	0.103
F(000)	156.0
Crystal size/ mm^3	$0.32 \times 0.3 \times 0.24$
Absorption correction	none
Radiation	MoK α ($\lambda = 0.71073$)
2Θ range for data collection/ $^\circ$	5.98 to 50.498 $^\circ$
Completeness to theta	0.999
Index ranges	$-5 \leq h \leq 5, -6 \leq k \leq 6, -16 \leq l \leq 16$
Reflections collected	8705
Independent reflections	2406 [$R_{\text{int}} = 0.0736, R_{\text{sigma}} = 0.0473$]p
Data/restraints/parameters	2406/3/201
Goodness-of-fit on F^2	1.070
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0281, wR_2 = 0.0730$
Final R indexes [all data]	$R_1 = 0.0297, wR_2 = 0.0735$
Largest diff. peak/hole / $e \text{ \AA}^{-3}$	0.16/-0.15
Flack parameter	0.5(6)

Table S2 Bond Lengths for compound 8.

Atom	Atom	Length/ \AA	Atom	Atom	Length/ \AA
O1	C3	1.214(3)	C1	C4	1.415(3)
O2	C4	1.249(3)	C5	C6	1.449(3)
O3	C5	1.231(3)	C6	C7	1.201(3)
N1	C2	1.367(3)	C7	C8	1.436(3)
N1	C3	1.375(3)	C8	C9	1.392(3)
N2	C3	1.384(3)	C8	C13	1.394(3)
N2	C4	1.401(3)	C9	C10	1.387(3)
N2	C14	1.475(3)	C10	C11	1.373(4)
N3	C1	1.419(3)	C11	C12	1.381(4)

N3	C5	1.347(3)	C12	C13	1.388(3)
N4	C2	1.339(3)	C14	C15	1.518(3)
C1	C2	1.375(3)			

Table S3 Bond Angles for compound 8.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C2	N1	C3	124.8(2)	N2	C4	C1	117.38(19)
C3	N2	C4	122.84(17)	O3	C5	N3	123.72(19)
C3	N2	C14	116.45(18)	O3	C5	C6	122.2(2)
C4	N2	C14	120.6(2)	N3	C5	C6	114.1(2)
C5	N3	C1	122.34(19)	C7	C6	C5	177.1(2)
C2	C1	N3	120.22(18)	C6	C7	C8	179.0(2)
C2	C1	C4	120.75(18)	C9	C8	C7	119.9(2)
C4	C1	N3	119.03(19)	C9	C8	C13	120.1(2)
N1	C2	C1	118.26(18)	C13	C8	C7	120.0(2)
N4	C2	N1	116.4(2)	C10	C9	C8	119.6(2)
N4	C2	C1	125.34(19)	C11	C10	C9	120.1(2)
O1	C3	N1	120.9(2)	C10	C11	C12	120.7(2)
O1	C3	N2	123.25(18)	C11	C12	C13	120.1(2)
N1	C3	N2	115.87(18)	C12	C13	C8	119.4(2)
O2	C4	N2	119.22(19)	N2	C14	C15	112.32(19)
O2	C4	C1	123.40(19)				

¹H- and ¹³C-NMR spectra of compounds **8**, **12-17**.

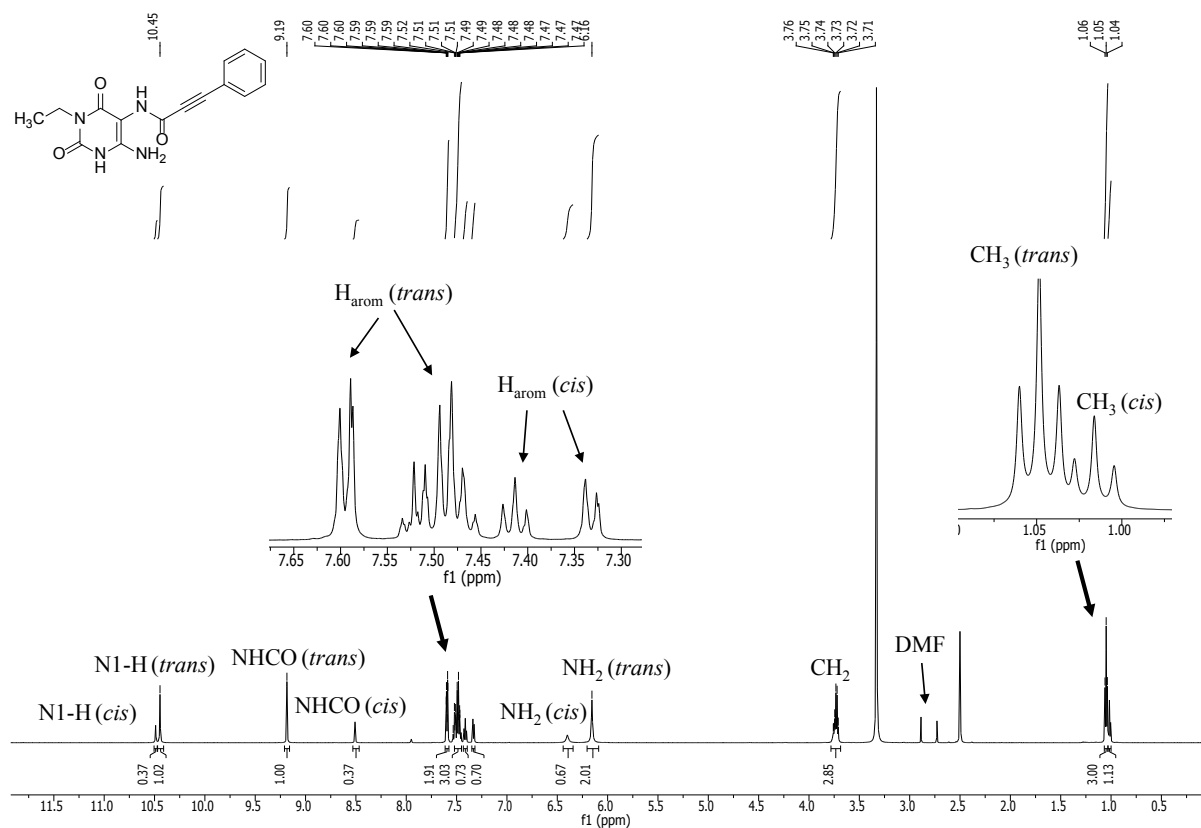


Figure S1. ¹H-NMR spectra of **8** in (CD₃)₂SO at room temperature.

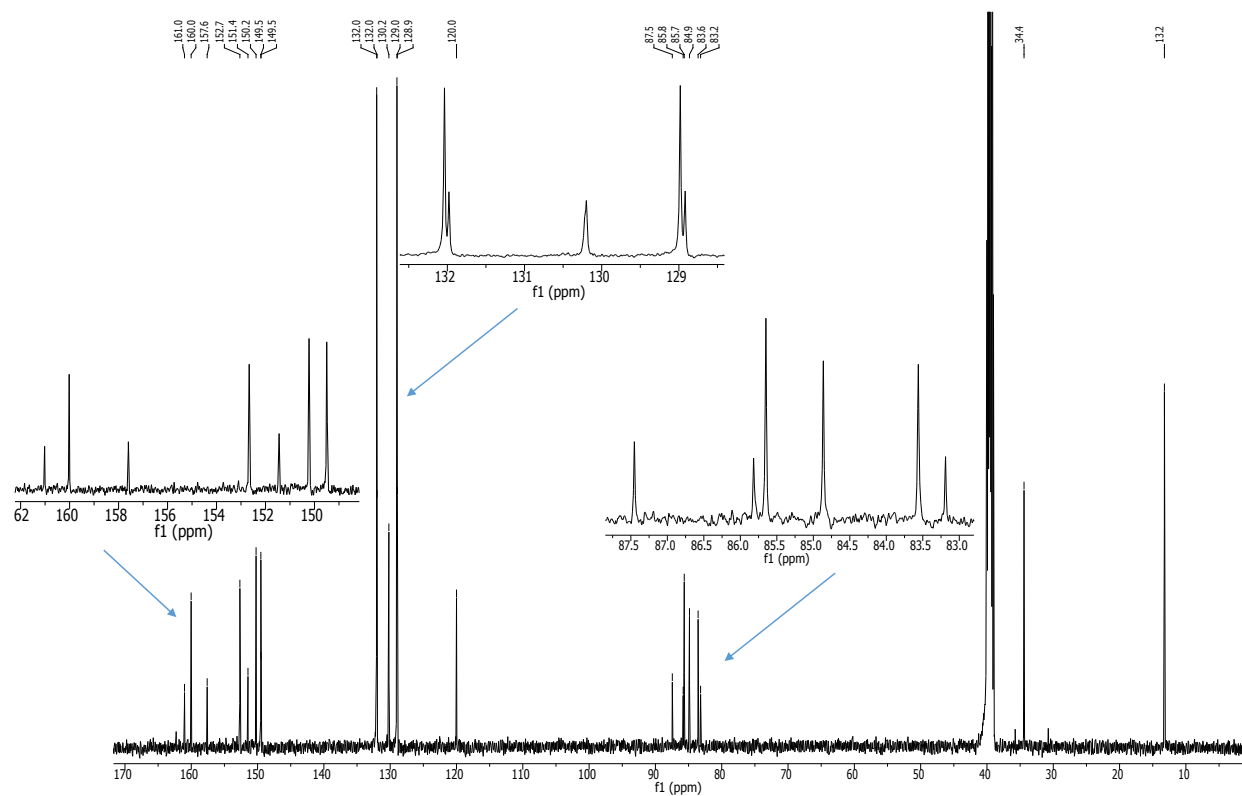


Figure S2. ¹³C-NMR spectra of **8** in (CD₃)₂SO at room temperature.

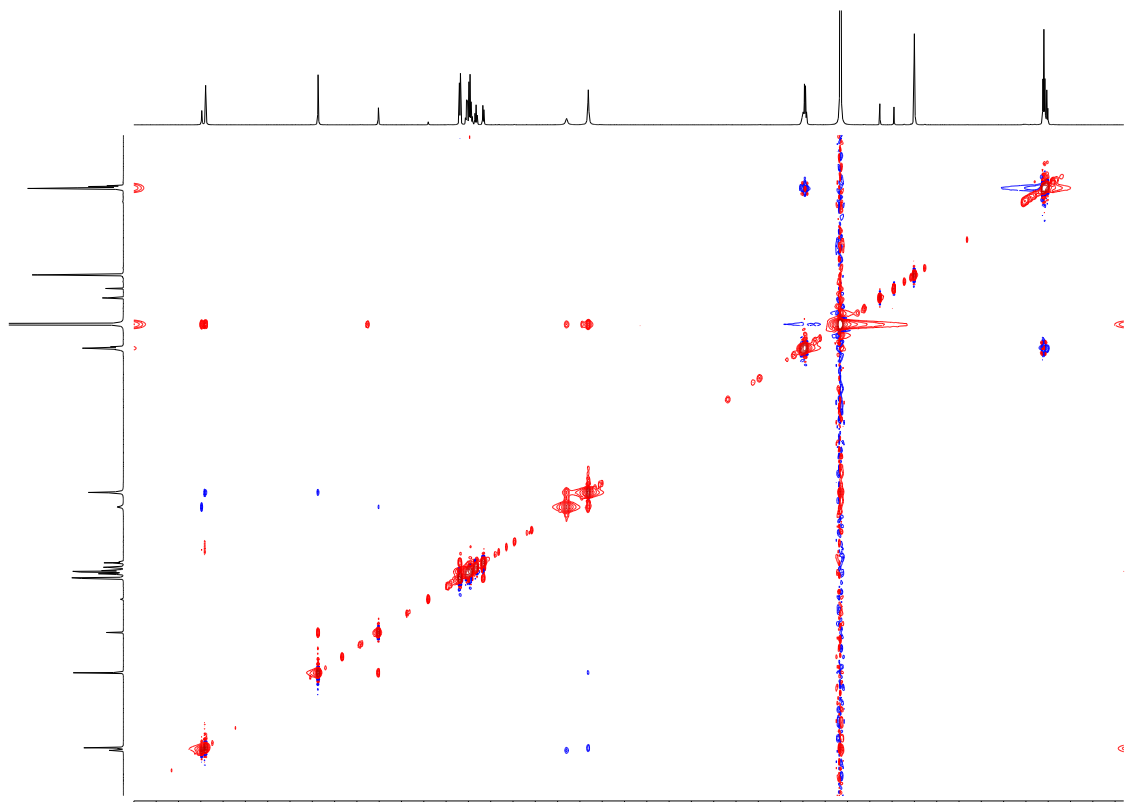


Figure S3. NOESY-NMR spectra of **8** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

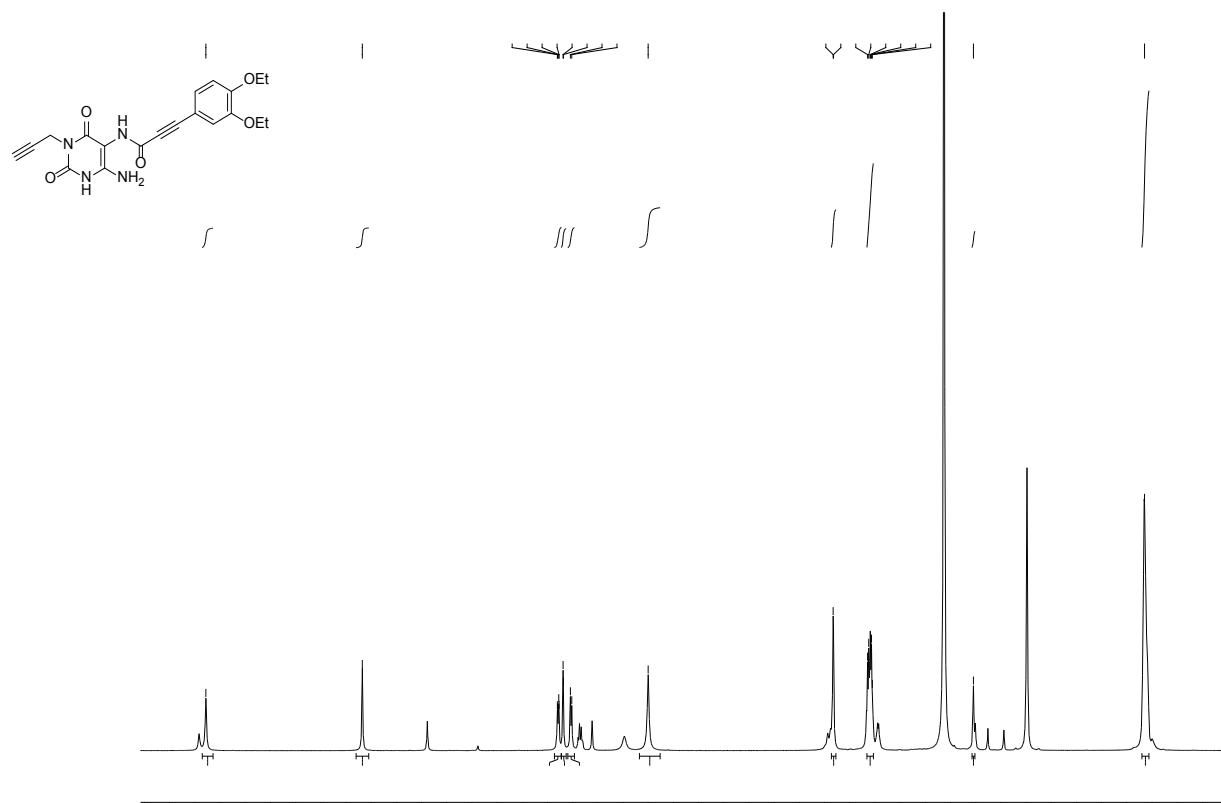


Figure S4. ^1H -NMR spectra of **12** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

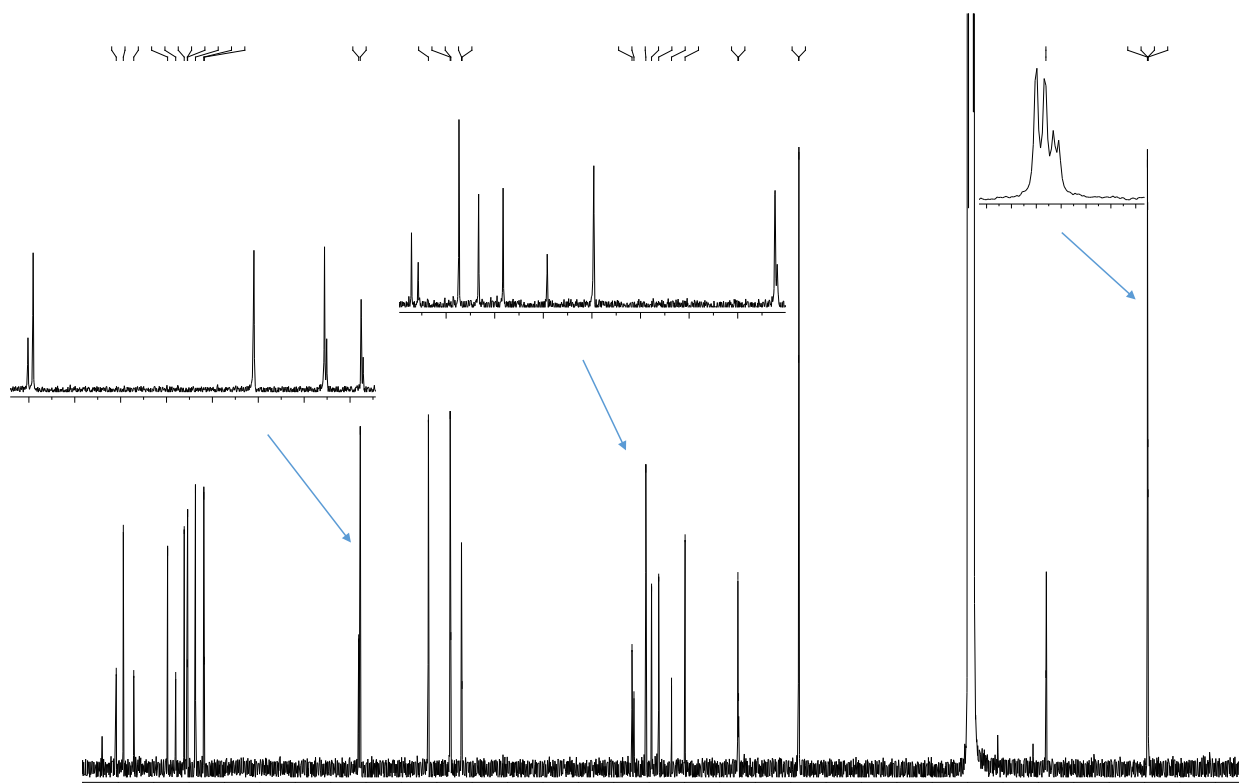


Figure S5. ^{13}C -NMR spectra of **12** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

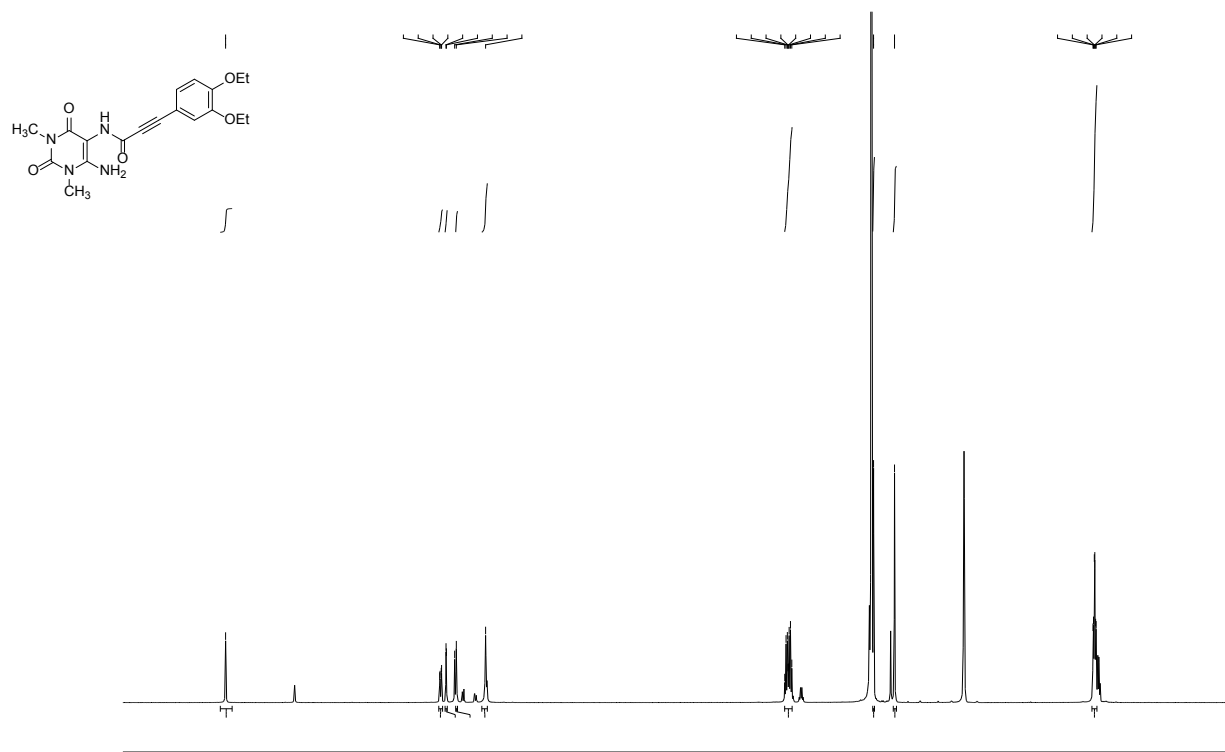


Figure S6. ^1H -NMR spectra of **13** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

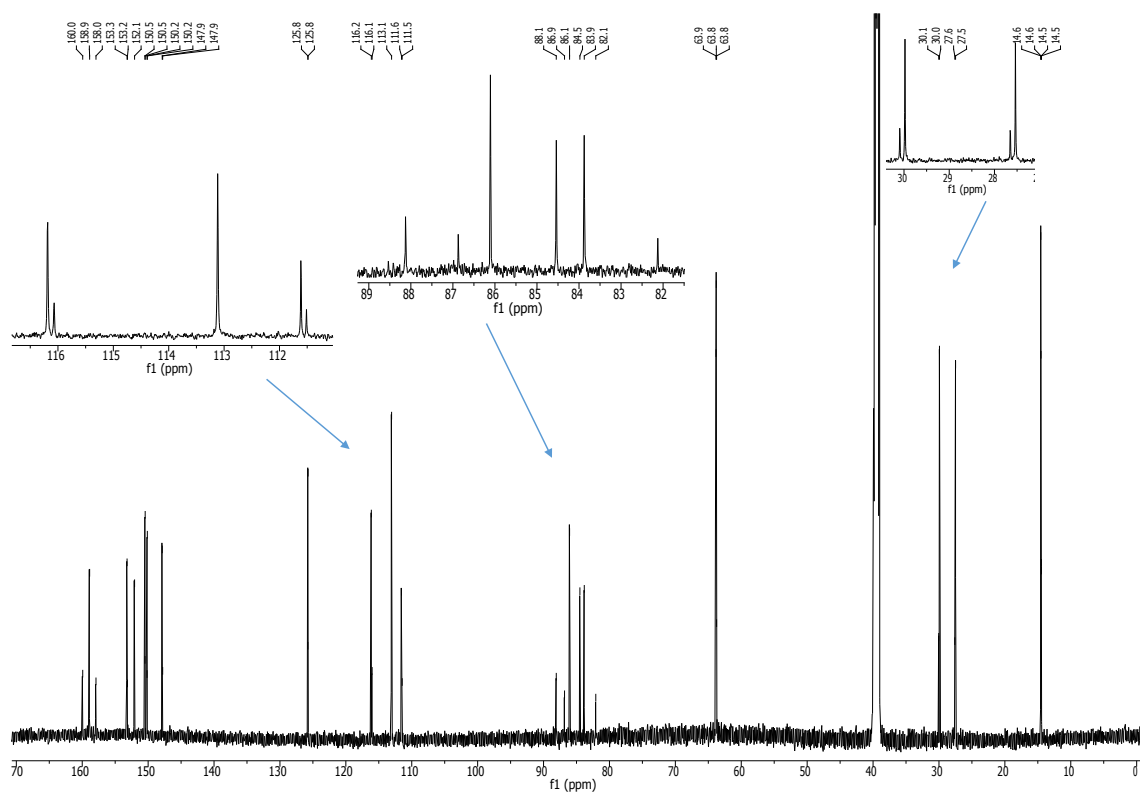


Figure S7. ^{13}C -NMR spectra of **13** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

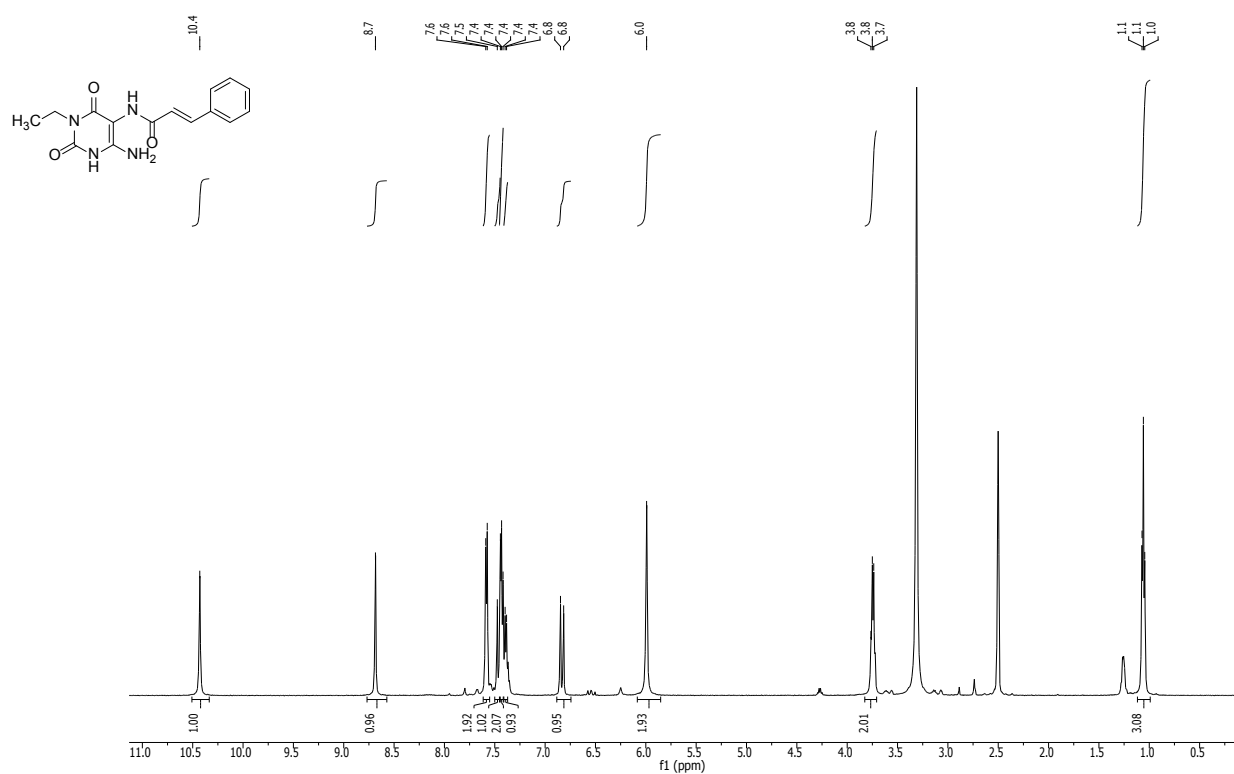


Figure S8. ^1H -NMR spectra of **14** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

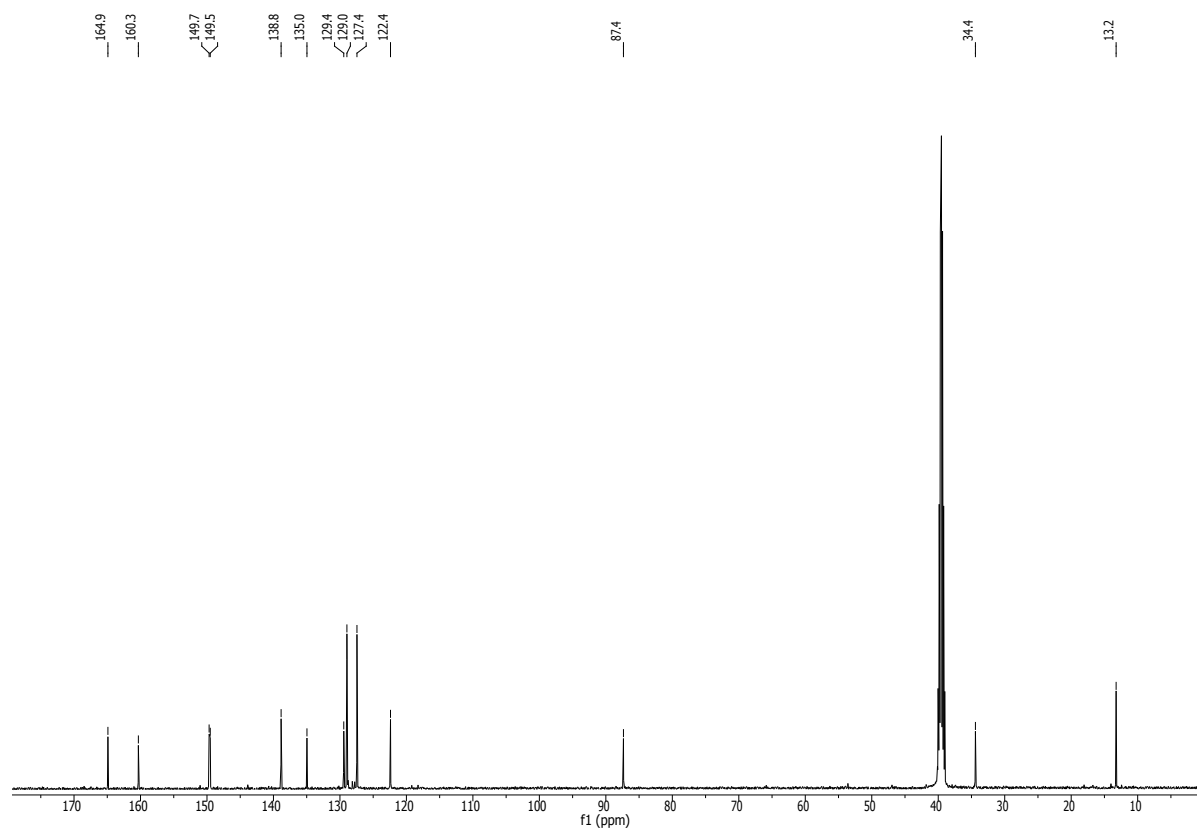


Figure S9. $^{13}\text{C-NMR}$ spectra of **14** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

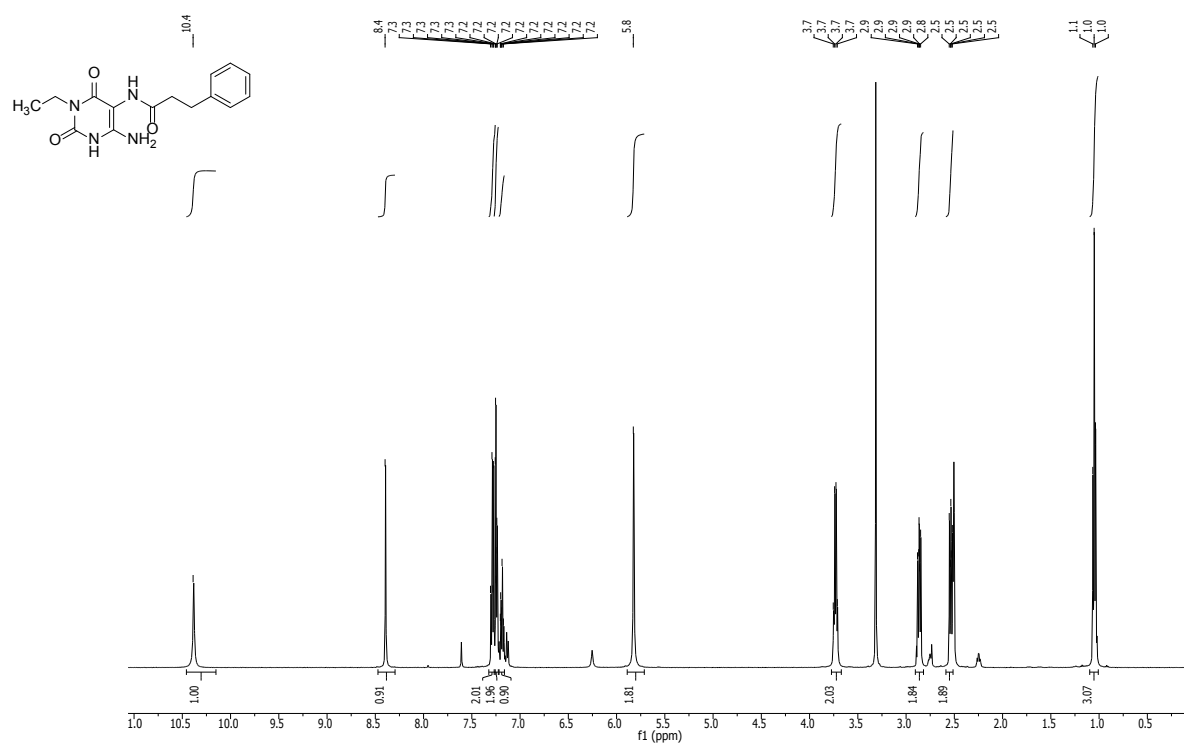


Figure S10. $^1\text{H-NMR}$ spectra of **15** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

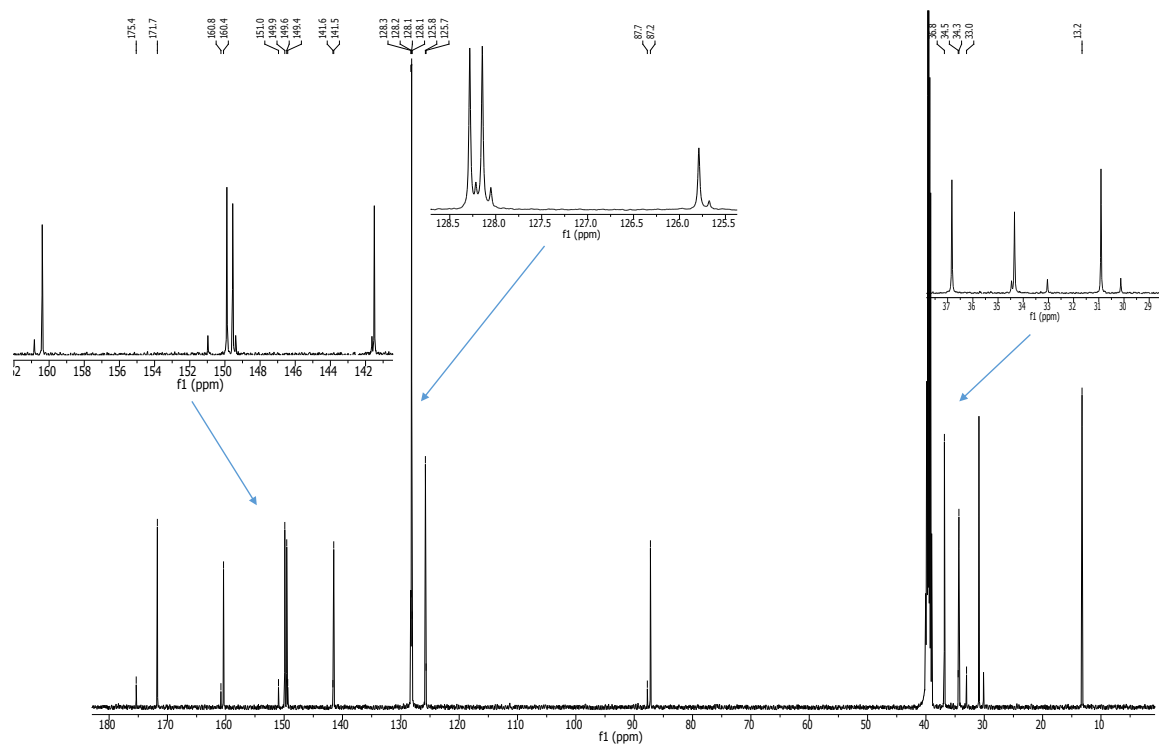


Figure S11. ^{13}C -NMR spectra of **15** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

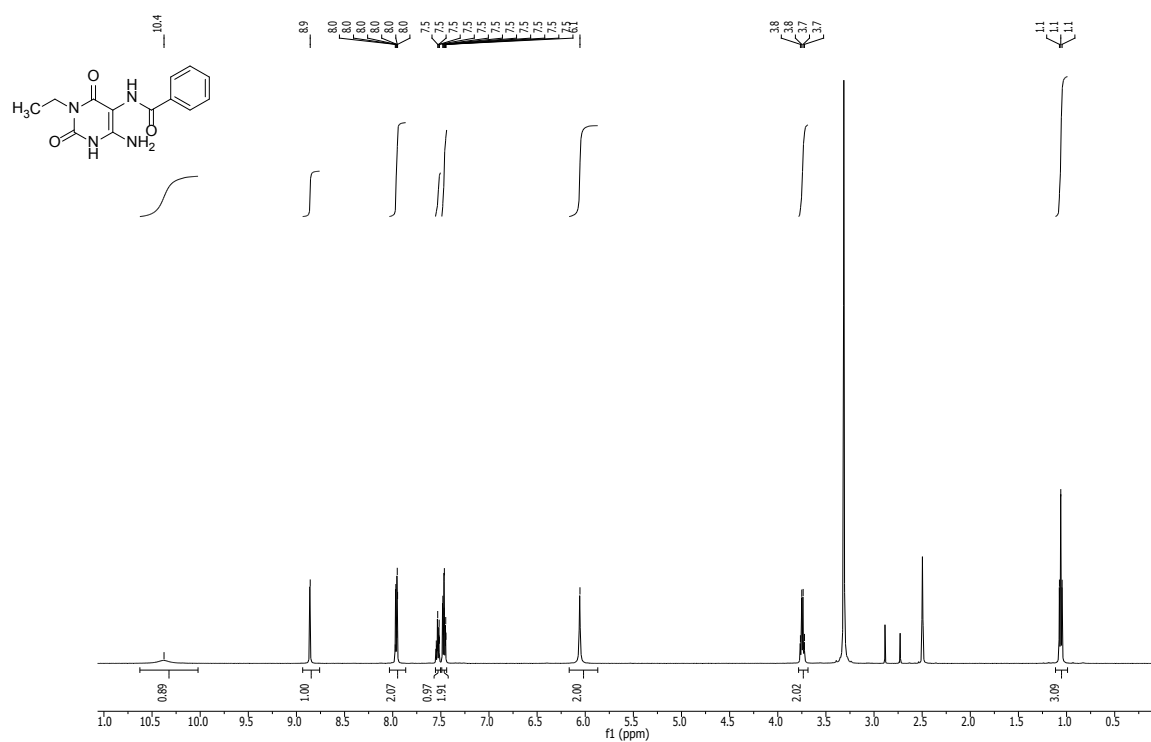


Figure S12. ^1H -NMR spectra of **16** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

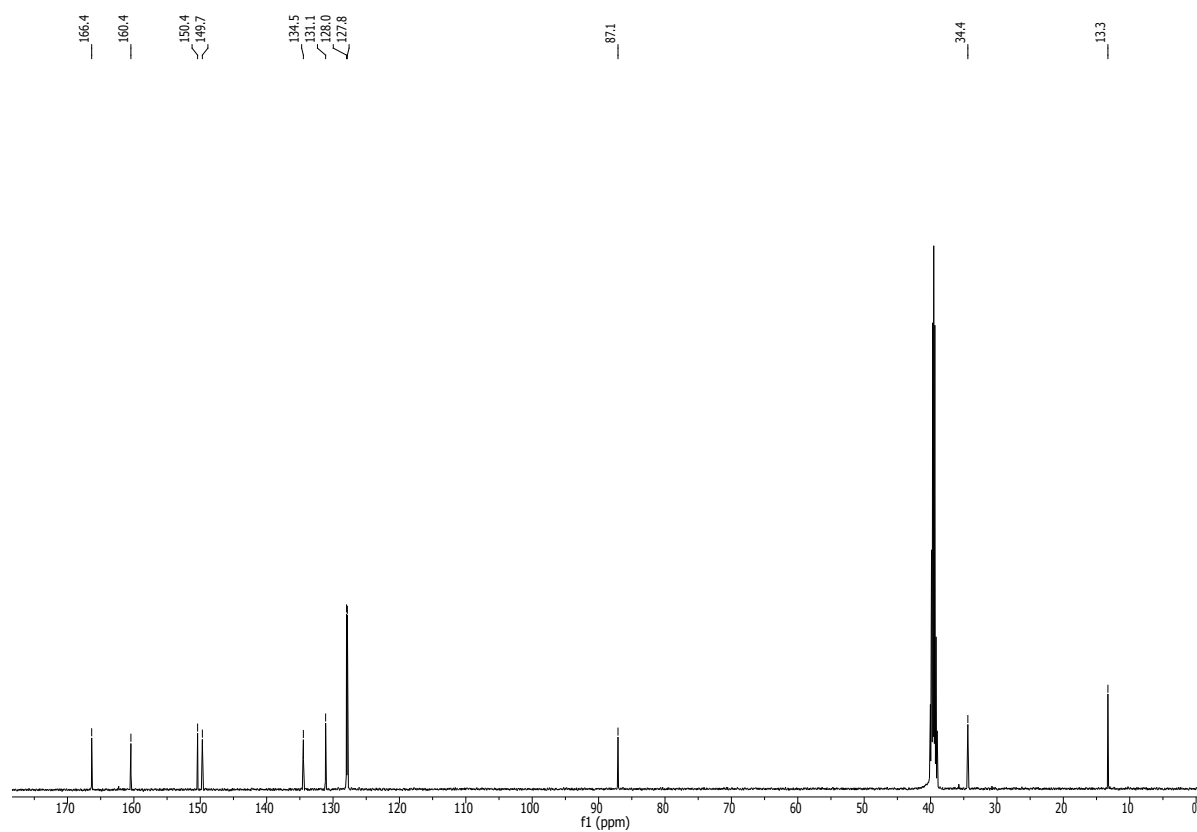


Figure S13. ^{13}C -NMR spectra of **16** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

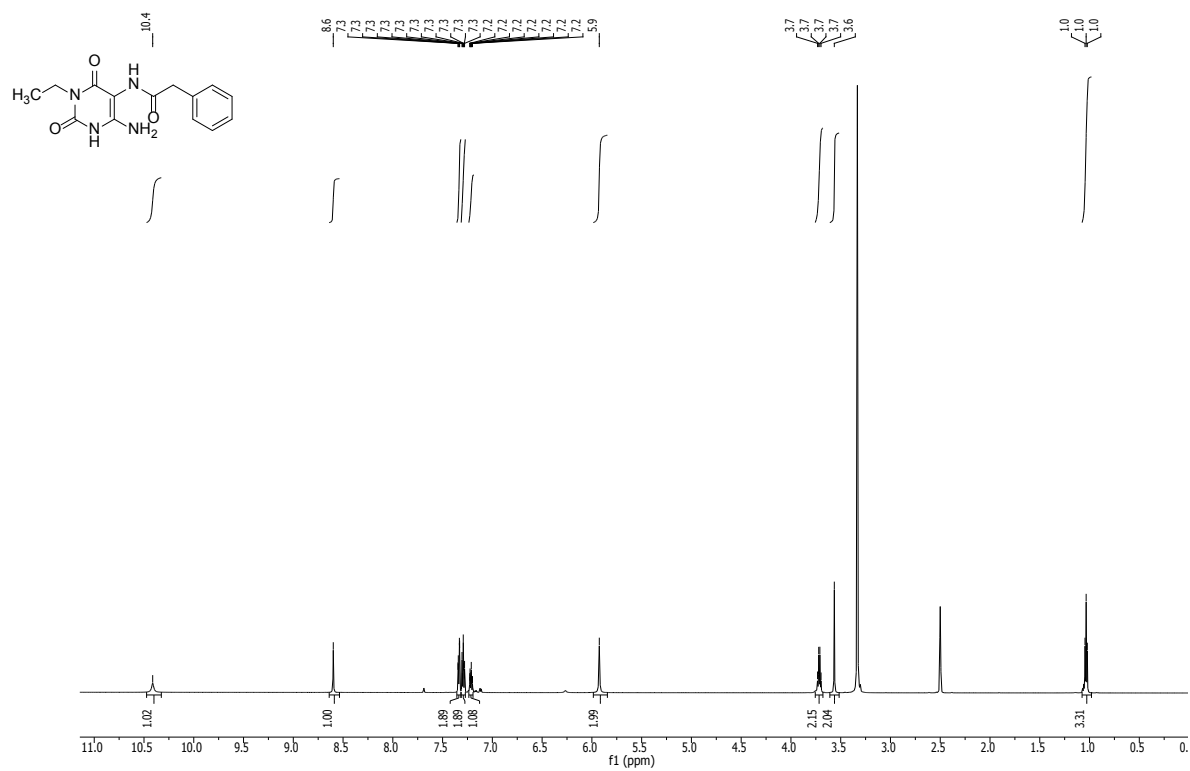


Figure S14. ^1H -NMR spectra of **17** in $(\text{CD}_3)_2\text{SO}$ at room temperature.

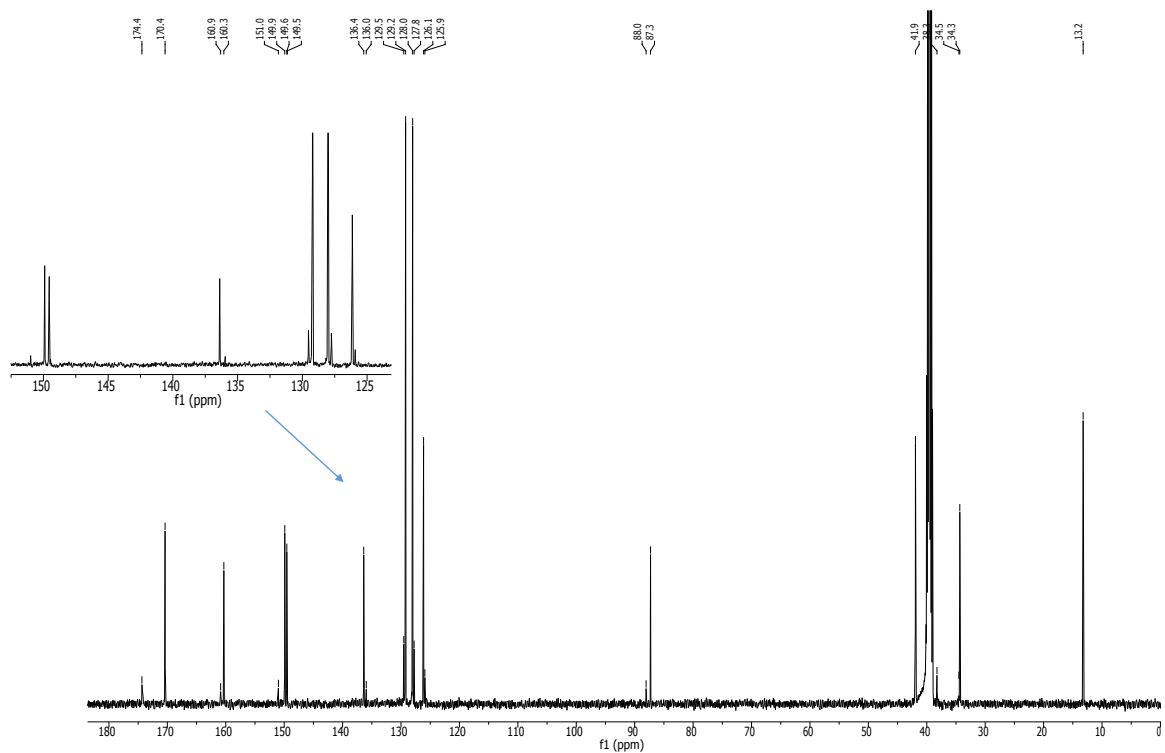


Figure S15. ^{13}C -NMR spectra of 17 in $(\text{CD}_3)_2\text{SO}$ at room temperature.

DHPLC analyses of **12**

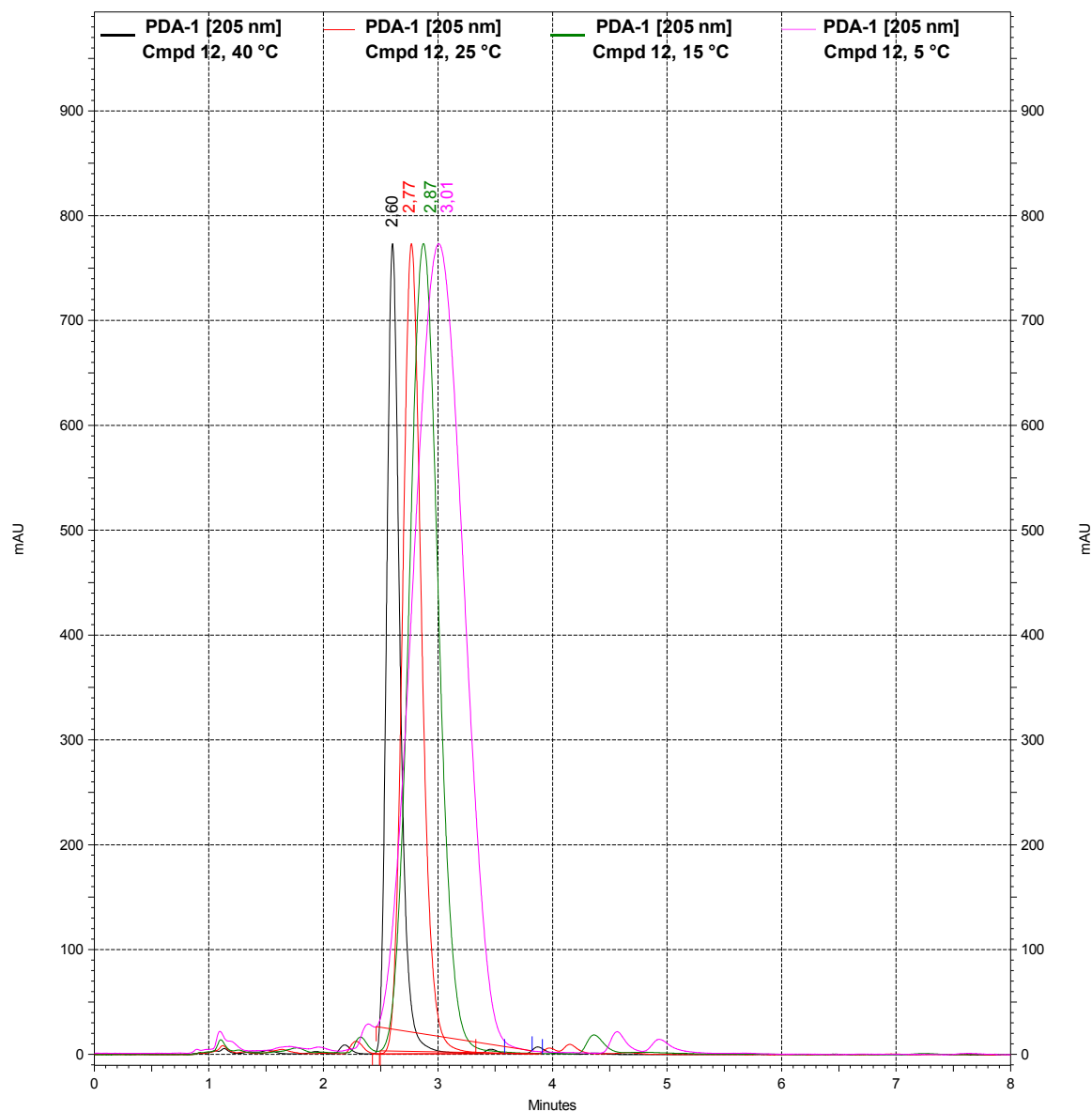


Figure S16: DHPLC analyses of **12** at 205 nm. Compound **12** (2.1 mg) was dissolved in 4.2 ml MeCN and 0.1 μ l injected for the DHPLC measurements. A mixture of 40% MeCN and 60% H₂O was used as eluent with a flow rate out of 0.2 ml/min. The pressure increased from 358 bar (25 °C) to 583 bar (5 °C). HPLC chromatogram at 40 °C is shown in black, at 25 °C in red, at 15 °C in green and at 5 °C in pink. The corresponding retention times shifted from 2.60 min at 40 °C to 3.01 min at 5 °C.

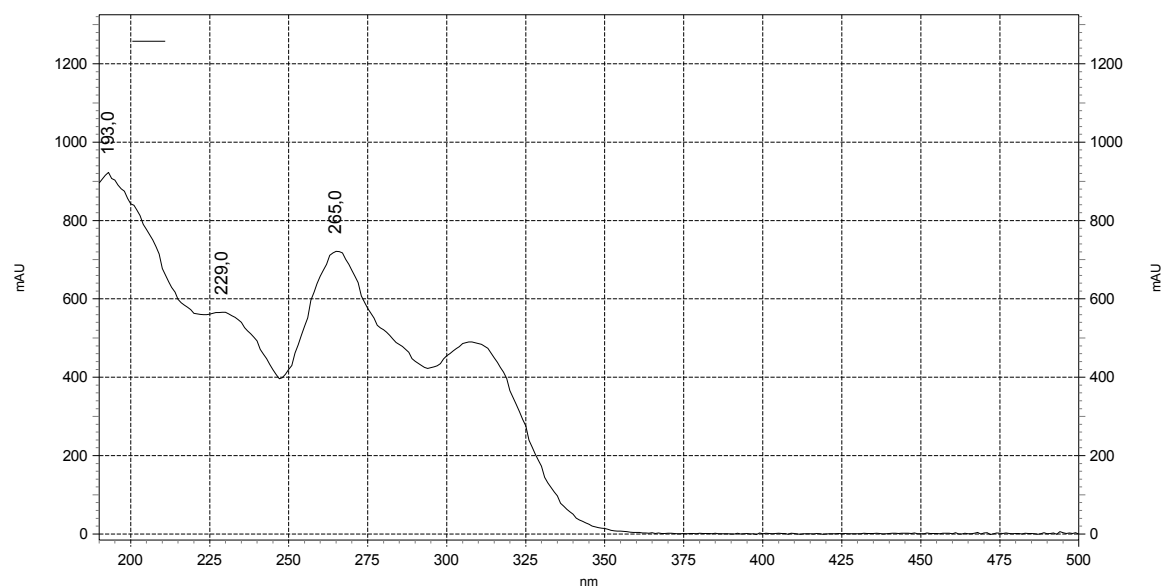


Figure S17: UV-spectra of compound 12

References

1. Hockemeyer, J.; Burbiel, J. C.; Müller, C. E. Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: potential drugs for Parkinson's Disease. *J. Org. Chem.* **2004**, *69*, 3308-3318.
2. Iii Charles E Maxwell, C. J. S. Method of preparing 4-aminouracils. *US2715625A* **1952**.
3. Marx, D.; Wingen, L. M.; Schnakenburg, G.; Müller, C. E.; Scholz, M. S. Fast, efficient, and versatile synthesis of 6-amino-5-carboxamidouracils as precursors for 8-substituted xanthines. *Frontiers in Chemistry* **2019**, *7*, 1-15.
4. Müller, C. E.; Shi, D.; Manning Jr, M.; Daly, J. W. Synthesis of paraxanthine analogs (1, 7-disubstituted xanthines) and other xanthines unsubstituted at the 3-position: structure-activity relationships at adenosine receptors. *J. Med. Chem.* **1993**, *36*, 3341-3349.

4 Medicinal Chemistry of Primate-Specific Mas-Related G Protein-Coupled Receptors (MRGPRX)

4.1 G protein-coupled receptors

The receptor family of G protein-coupled receptors (GPCRs) represent the largest class of transmembrane proteins. The first GPCR, bovine rhodopsin, was discovered in 1983 and since then GPCRs play an increasingly important role in drug research and development.¹ To date, more than 800 GPCRs are known, which have in common that they cross the cell membrane with 7 α helices and are therefore also called as 7 transmembrane receptors (7TM). Another important characteristic of GPCRs is that they are able to interact with heterotrimeric guanine nucleotide-binding proteins (G proteins) which are composed of three distinct subunits, α -, β - and γ -subunit.²⁻⁴ GPCRs are involved in almost all physiological and pathophysiological processes in the body, including hearing⁵, smelling⁶, tasting⁷, or even our pain sensation is controlled by GPCRs.⁸ These receptors are able to receive a signal from outside the cell in the form of light⁹, mechanical stress¹⁰, lipids¹¹, hormones or peptides and transmitted it into the cell interior in order to address various effector proteins (enzymes, ion channels) and trigger an intracellular signal cascade.¹² The activation of GPCRs can initiate G protein controlled reactions on the one hand, but also arrestin or GPCRkinases (GRKs) on the other hand. Many ligands stimulate the G protein and arrestin pathway simultaneously, but independent activation of these two signal transduction pathways is also possible. Ligands that activate only one of the two signal transduction pathways are called biased ligands.¹³

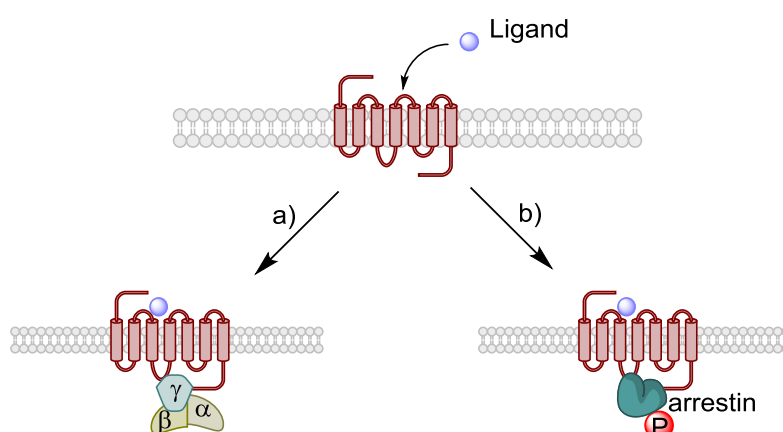


Figure 1: Illustration of GPCR activation. The activation of GPCR can trigger different signalling pathways, either via G proteins (a) or arrestin controlled (b) signal transduction pathways (according to Shimada *et al.*).¹³

The activation via the G protein signaling pathway proceeds as follows. Activation of the receptor by a corresponding signaling molecule leads to an exchange of GDP with GTP and the inactive $G\alpha\beta\gamma$ -GDP-complex dissociated into a $G\alpha$ -GTP and a $G\beta\gamma$ -subunit. The resulting $G\alpha$ -GTP and $G\beta\gamma$ -subunits subsequently bind to various enzymes such as adenylyl cyclase (AC), phospholipase C (PLC) or various ion channels, thus activate or deactivate them. This can promote the formation of secondary messengers such as cyclic adenosine monophosphate (cAMP), inositol-1,4,5-triphosphate (IP3) or diacylglycerol (DAG), which allows the regulation of ion concentrations within the cell. Depending on the $G\alpha$ subunit different effector systems are addressed.¹⁴

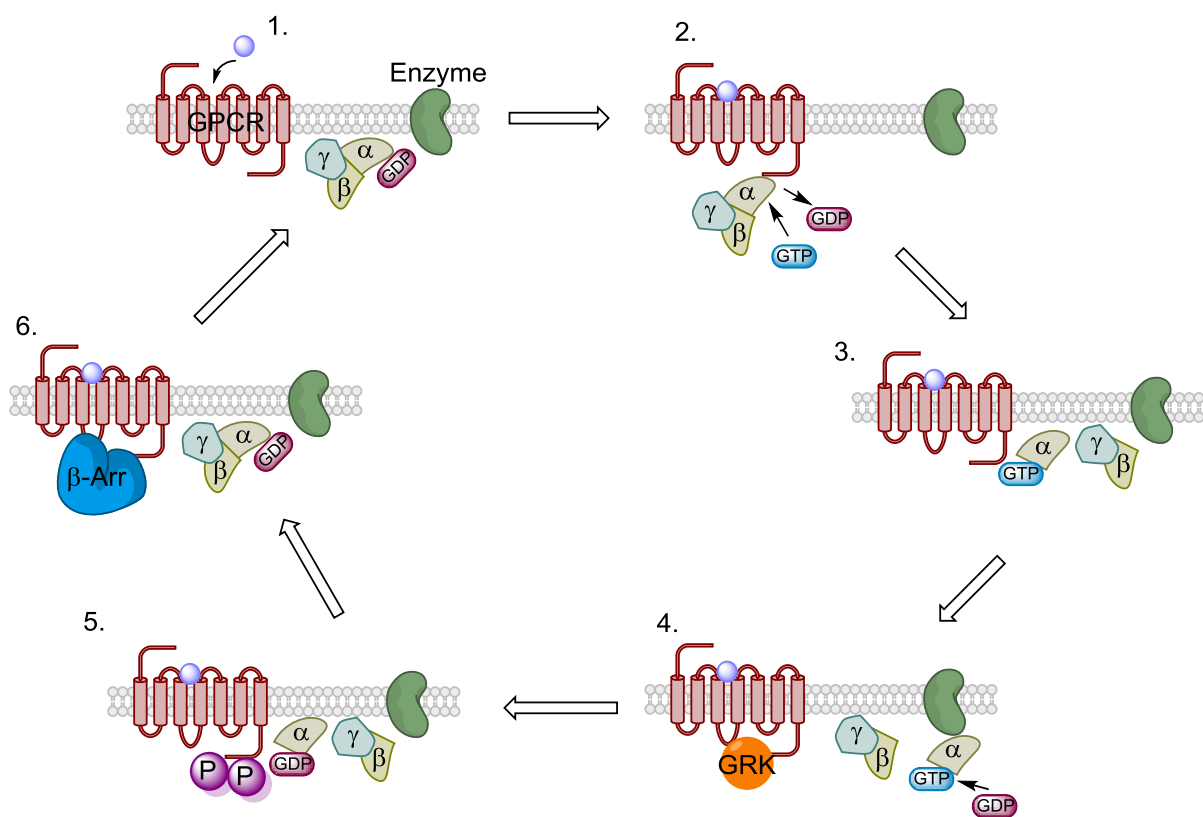


Figure 2: GPCR signaling pathway. Ligand binding changes the GPCR conformation and triggers the interaction with heterotrimeric G proteins and exchange of GDP with GTP binding (2). This leads to a separation of the $G\alpha\beta\gamma$ -GDP-complex (3) and subsequent binding to numerous enzymes (4). Phosphorylation of GPCR is triggered by a GRK and lead to β -arrestin binding (5+6). Removing of the ligand is reactivation the GPCR cycle.¹⁵

The $G\alpha_s$ subunit activates the (AC) and after its activation, the enzyme catalyzes the synthesis of cAMP from adenosine triphosphate (ATP). cAMP is a second messenger which transfers the extracellular signal by activating, for example, protein kinase A (PKA). PKA phosphorylates numerous kinds of proteins leading to different physiological effects. $G\alpha_o$ is important for olfactory receptors and activates also the AC which induces the depolarization of smelling cells.

Transmission to the *bulbus olfactorius*, a vertebrate structure of the forebrain, leads to odor perception. $G\alpha_i$ are inhibitor proteins so that conversion of ATP to cAMP will be blocked. Furthermore, the $G\beta\gamma$ -subunit can activate the isoenzymes β_2 and β_3 of the PLC. These soluble enzymes are attached to the cell membrane where they catalyze the reaction of phosphatidylinositol-4,5-bisphosphate (PIP_2) to the second messenger DAG and IP_3 . Receptors of endoplasmic reticulum (ER) are activated by these second messengers which leads to influx of Ca^{2+} into the cytosol out of the ER. $G\alpha_q$ and $G\alpha_{11}$ activate the isoenzymes β_1 and β_4 which also result in mobilization of Ca^{2+} out of the ER. Calmodulin binds Ca^{2+} and starts cellular reactions, for example muscle contraction. $G\alpha_{12}$ and $G\alpha_{13}$ activate the small GTP-binding protein Rho. Rho is important for phosphorylation of myosin consequently also for muscle work. The last α -subunit $G\alpha_t$ is necessary for the eyes photosensitivity by stimulating phosphorylation of phosphodiesterase 6 (PDE 6).⁴

Table 1: Most important $G\alpha$ protein families and their effector systems^{4, 14}

<i>G protein family</i>	<i>Effector System</i>	<i>GPCR examples</i>
$G\alpha_s$	<ul style="list-style-type: none"> ↑ Adenylate cyclase ↓ Ca^{2+} channels ↓ Na^+ channels 	A_{2A} - and A_{2B} -adenosine receptors, dopamine receptor D_1 and D_5
$G\alpha_{i/o}$	<ul style="list-style-type: none"> ↓ Adenylate cyclase ↓ Ca^{2+} channels ↑ K^+ channels ↑ Phospholipase A_2 and C ↑ cGMP-specific phosphodiesterase 	$P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, D_2 , D_3 receptor
$G\alpha_{q/11}$	<ul style="list-style-type: none"> ↑ Phospholipase C (PLC) → cleaves membrane-bound phosphatidylinositol 4,5-bisphosphate into inositol trisphosphate (IP_3) and diacylglycerol (DAG) 	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, A_{2B} adenosine receptor
$G\alpha_{12/13}$	<ul style="list-style-type: none"> ↑ Rho guanine-nucleotide-exchange factors (GEFs) → activates Rho 	GPR35

↑ stimulating, ↓ inhibiting

Due to their diversity, the GRAFS classification system was designed and all GPCRs were assigned to one of five subgroups named *Glutamate* (G, 15 members), *Rhodopsin* (R, 701), *Adhesion* (A, 24), *Frizzled/Taste2* (F, 24) and *Secretin* (S, 15) based on phylogenetic analysis.¹⁶⁻¹⁸ The rhodopsin family is by far the largest of the five GPCR families and can be further divided into four subgroups α - δ .¹⁹⁻²⁰ Most of the receptors belonging to this group have characteristic transmembrane motifs such as the NSxxNPxxY motif in TMVII or the DRY motif between TMIII and IL2. The α group of the rhodopsin family can be further divided into five subgroups, prostaglandin receptors, amine receptors, opsin receptors, melatonin receptors, and MECA receptors. The β -group contains 36 receptors. All known ligands of these receptors are peptides. The γ -group of rhodopsin GPCRs can also be divided into the three subgroups named SOG receptors, MCH receptors, and the chemochine receptors. The δ -group of the rhodopsin family can as well be divided into four subgroups, the MAS-related G protein-coupled receptors, glycoprotein receptors, purine receptors, and the olfactory receptors.¹⁸

GPCRs are among the most important targets in drug development and in 2017, 134 GPCRs were targets for FDA or EU approved drugs. Almost 35% (approx. 700) of all approved drugs exert their effect via GPCRs.²¹ However, the potential of this class of substances is far from exhausted. Currently there are still more than 130 orphan GPCRs whose natural ligand is unknown and therefore the (patho)physiological role of the receptor is not yet understood.²² These orphan receptors represent a high potential for future drug research.

4.2 Primate specific Mas-related G protein-coupled receptor

In 2001, Dong *et al.* discovered a set of orphan receptors with over 50 rodent and human GPCRs, called as Mas-related gene receptors (MRGPRs).²³ Later in 2002, Lembo *et al.* discovered the same GPCR family during a search in the RNA isolated from a primary culture of rat dorsal root ganglia (DRG) and named as sensory neuron-specific receptors (SNSRs).²⁴ In general, the receptor family can be classified into nine distinct subfamilies (MRGPRA–H and -X) and belongs to the δ -branch of class A GPCR family. The International Union of Basic and Clinical Pharmacology (IUPHAR) classify all members as orphan receptors and most of these receptors are expressed in the sensory neurons of the small-diameter DRG. The primary sensory neurons in the DRG play a role in nociception and itching. Due to their expression in the DRG, MRGPRs are therefore considered to be involved in somatosensory functions including pain or itch.²³⁻²⁶ Among the nine subfamilies of MRGPRs, four members (MRGPRX1–4) are primate-specific. Cloning of MRGPRX members from other primates like crab-eating macaque (*Macaca fascicularis*) and rhesus monkey was possible, which enables development of animal

models for this subfamily.²⁷ The MRGPRX sub-types have sequence identities from 45.0% to 83.2% and sequence similarities between 58.4% and 88.5% (Table 1). Although the human MRGPRX2 and the mouse MRGPRB2 receptor have only a 50% sequence identity, several publications suggest that MRGPRB2 could be the orthologous receptor to MRGPRX2.²⁸⁻³⁰ In order to investigate this further, the new investigated MRGPRX2 antagonists presented in this thesis were also tested at the mouse MRGPRB2 (see unpublished results).

Table 2: Sequence identities (blue) and similarities (orange) between the MRGPRX sub-types.²⁹ The numbers describe the identical amino acids (blue) and similar amino acids (orange) between two receptors. The identity is the percentage of identical matches between the two sequences over the reported aligned region. Similarity indicate functional, structural and/or evolutionary relationships between two biological sequences.

	MRGPRX1	MRGPRX2	MRGPRX3	MRGPRX4	MRGPRB2
MRGPRX1	-	213/331 (64.4%)	268/322 (83.2%)	254/322 (78.9%)	164/341 (48.1%)
MRGPRX2	240/331 (72.5%)	-	207/337 (61.4%)	206/331 (62.2%)	171/342 (50.0%)
MRGPRX3	285/322 (88.5%)	235/337 (69.7%)	-	264/322 (82.0%)	160/344 (46.5%)
MRGPRX4	274/322 (85.1%)	238/331 (71.9%)	285/322 (88.5%)	-	159/353 (45.0%)
MRGPRB2	218/341 (63.9%)	212/342 (62.0%)	209/344 (60.8%)	206/353 (58.4%)	-

overdose deaths were opioid-related.³¹ The discovery of opioid alternatives as painkillers is of great interest. Current nonopioid analgesic drugs like, e.g., nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen are not as effective as opioids for the treatment of acute and especially chronic pain.³⁶

4.2.1 MRGPRX1

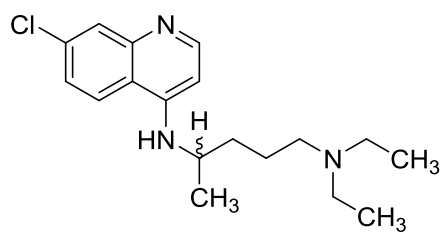
The MRGPRX1 represents a promising non-opioid dependent alternative for treating pain. The first agonist found for MRGPRX1 was the proenkephalin A degradation product bovine adrenal medulla peptide 22 (BAM22).²⁴ Proenkephalin A is an opioid peptide precursor that can be degraded to several natural opioid ligands, including [met]enkephalin, [leu]enkephalin and prodynorphin the endogenous ligands of the μ , δ and κ opioid receptors. The other degradation products of proenkephalin A resulting from proteolytic cleavage include peptide F, peptide E, BAM22, BAM20 and BAM12. BAM22 also binds with high affinity to all three known opioid receptors due to the Met-enkephalin motif YGGFM. Thus, a connection between BAM22 and pain transmission in humans is suspected.^{24, 37} It could be shown that BAM22 can activate the MRGPRX1 receptor and its closest rodent orthologues, mouse MRGPRC11 and rat MRGPRC, independently of its YGGFM motif and a significantly increase of its proportion is observed in the spinal cord after tissue inflammation and nerve injury. Thus, it is assumed that there is another receptor system besides the opioid system which might play a role in pain perception and transmission in humans. Since MRGPRX are only found in humans and therefore the investigation of the physiological properties of these receptors is difficult, the research group around Li *et al.* expressed the receptor in a humanized mouse model.³⁷ It was shown in the transgenic MrgprX1 mouse model that BAM8-22, a modification of BAM22 lacking to typical opioid motif, thus unable to activate opioid receptor but acting as a fully MRGPRX1 agonist, could alleviate persistent pain, including persistent pain after nerve injury. The results suggested that pain inhibition by BAM8-22 was MRGPRX1-dependent and probably due to a reduction in calcium-dependent neurotransmitter release from primary sensory neurons.³⁷ To learn more about the physiological properties of the receptor Chen *et al.* expressed the MRGPRX1 in rat superior cervical ganglion (SCG), dorsal root ganglion (DRG), and hippocampal neurons using nuclear injection or recombinant adenoviruses. They were able to show that MRGPRX1 heterologously expressed in rat neurons can be activated by BAM8-22 and acts via a G_q - and $G_{i/o}$ -coupled signaling pathway and modulates neuronal Ca^{2+} - and K^+ -channels.³⁸

Besides the expression of MRGPRX1 in small-diameter DRG neurons, the receptor could also be detected at mRNA level in connective tissue mast cells (CTMC), although to a lesser extent

than MRGPRX2,³⁹ and in leukaemia-derived human mast cell line (LAD)-2.³⁹ MRGPRX1 induces the release of the chemokine receptor 2 (CCR2) agonist chemokine ligand 2 (CCL2) in LAD-2 mast cells (MC) after activation by BAM8-22. Activation of the CCR2 receptors is expected to be responsible for the sensation of pain and therefore the inhibition of these receptors is a promising approach for the development of new analgesics. The MRGPRX1 receptor could play a crucial role in this process.⁴⁰

The expression in MC is not only an interesting approach for the development of new pain medication, but also for the treatment of pruritus. Basically, two forms of pruritus can be distinguished: histamine-dependent and histamine-independent pruritus, the latter being extremely difficult to treat so far. It has been shown in human volunteers that BAM8-22 causes non histaminergic itching. Since BAM8-22 is an MRGPRX1 agonist, this receptor could be a novel target for the treatment of pruritus.⁴¹ Furthermore, a correlation between the MRGPRX1 activation triggered by BAM8-22 and cholestatic pruritus was described in an obstructive cholestasis model named bile duct ligation (BDL) mice.⁴² Several ligands have already been synthesized for MRGPRX1, which facilitates the investigation of the physiological properties at this receptor.

Wroblowski *et al.* synthesized pyridazinone derivatives as potent agonists for the human MRGPRX1.⁴³ Also, a docking study was reported for the most potent pyridazinone derived agonists based on the homology model of the human MRGPRX1 using bovine rhodopsin crystal structure (PDB ID: 1f88) as a template that showed an overall sequence identity of 18.6%.⁴³ Liu *et al.* was able to prove that chloroquine activates the human MRGPRX1, thus might function as itch receptor. Chloroquine-induced pruritus is one of the most common side effects of this widely used anti-malarial drug. Malaria is caused by protozoa of the genus *plasmodium* and can be spread to humans by the bite of female mosquitoes. Chloroquine caps hemozoin molecules to prevent further bio crystallization of heme. A chloroquine-heme complex that is highly toxic for the malaria cell is formed.²⁵

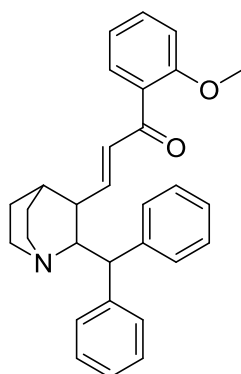


Chloroquine

Figure 4: Structure of chloroquine, one of the most important antimalarial drugs and MRGPRX1 agonist.

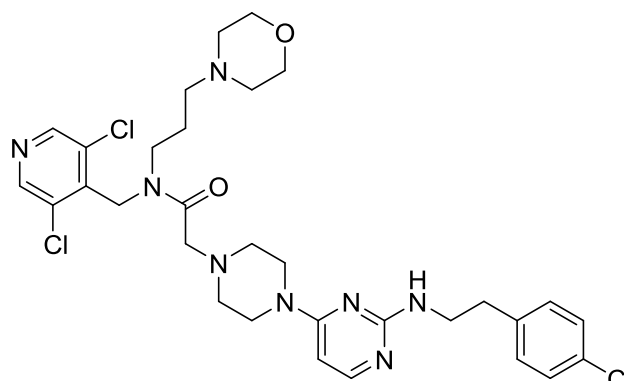
In 2006, Kunapuli *et al.* identified 2,3-disubstituted quinuclidine derivatives as MRGPRX1 antagonists that have a putative role in pruritus (Figure 5A).^{25, 44} Later in 2011, Bayrakdarian *et al.* reported 2,4-diaminopyrimidine derivatives as another scaffold with antagonistic potency for MRGPRX1 (Figure 5B).⁴⁵ However, the exact physiological functions of the receptor are still not completely understood.

A)



2,3-Disubstituted quinuclidine derivative
 $IC_{50} = 50 \text{ nM}$ in BLA assay

B)



2,4,-Diaminopyrimidine derivatives
 $IC_{50} = 14 \text{ nM}$

Figure 5: Reported antagonists for the human MRGPRX1.⁴⁴⁻⁴⁵ (BLA Assay = Cell-based beta-lactamase (BLA) reporter gene assay)

In addition, new highly potent benzamidine- and 1-aminoisoquinoline-based MRGPRX1 agonists have recently been developed by Prchalová *et al.* The agonists will be further tested in humanized mouse models for their physiological properties in connection with chronic pain.⁴⁶

4.2.2 MRGPRX2 and its role in human mast cells

Due to a proliferation assay Burstein *et al.* suggested a G_i - and G_q -coupled pathway for

MRGPRX2 both in human and rhesus monkey.²⁶ MRGPRX2, like the other receptor subtypes, was initially detected in DRG.²³ Furthermore, it was shown that the receptor is expressed in human skin mast cells (MCs), cord blood-derived MCs, CD34⁺ cell-derived MCs, and in human mast cell line LAD2 (Laboratory of Allergic Diseases 2) but not in lung MCs (HLMCs).⁴⁷ In addition, an expression of the receptor in peripheral blood basophils and eosinophils but not neutrophils has recently been demonstrated by Wedi *et al.*⁴⁸ In basophils the upregulation of the receptor of allergic subjects upon a tricolor granulocyte activation test using grass pollen has been shown.⁴⁸ MRGPRX2 mRNA could be further detected in the skin, fatty tissue, bladder and colon.⁴⁹

Due to an increased expression of the receptor in human MCs, targeting of MRGPRX2 is mainly associated with allergic diseases.⁵⁰ MCs are present in connective tissues throughout the body, and activation and degranulation of MCs leads to non-IgE-mediated (so-called pseudoallergic or anaphylactoid) hypersensitivity reactions. MC's are involved in physiological processes such as vasodilation, vascular homeostasis, innate and adaptive immune response, angiogenesis and toxin detoxification, as well as pathophysiological processes such as allergy, asthma, anaphylaxis, gastrointestinal disorders, many types of malignancies and cardiovascular diseases.⁵¹

Mast cells are located in close proximity to nociceptors and pruriceptors on sensory nerve fibers in the periphery so that they can communicate with each other. In the brain, mast cells interact either directly with nociceptive neurons or indirectly with micro- and astroglia in pathophysiological diseases.⁵²⁻⁵⁵ Due to these cross-links, mast cells contribute to neuronal sensitization⁵⁶, increased vascular permeability⁵⁷, chronic pain⁵⁸, itching and neuroinflammation.⁵⁸⁻⁵⁹ In patients with chronic urticaria, in the lungs of asthma patients and in human glandular cells of patients with allergic rhinitis an upregulation of the receptor could be observed.⁶⁰⁻⁶² Although the receptor subtypes of the MRGPRX family have so far only been found in primates, there are several scientific papers suggesting that MRGPRB2 is the murine orthologue of MRGPRX2.⁶³⁻⁶⁴

A large number of compounds has been reported to activate the MRGPRX2. A distinction can be made between peptides and small molecule MRGX2 receptor agonists.

4.2.2.1 Peptide derived MRGPX2 agonists

Robas *et al.* identified CST-14 (Figure 3) as the first putative ligand for MRGPRX2 that showed an EC₅₀ value of 25 nM in calcium assay in HEK cells. Cortistatin is a cyclic peptide that is able to activate five somatostatin receptors (SSTR1-5) with a potency comparable to that of somatostatin. It has been shown to play a role in the induction of slow-wave sleep, inhibition of motor activity and inhibition of cell proliferation. Cortistatin-14 is very similar to somatostatin-14, but despite this high similarity they are products of different genes. Many of the effects attributed to cortistatin are due to the activation of somatostatin receptors. Nevertheless, there are other modes of action that indicate a specific type of receptor. Moderate expression levels of MrgX2 in subgroups of neurons in the CA2, CA3 and CA4 regions of the hippocampus might indicate an effect mediated by cortistatin.⁶⁵

Another neuropeptide activating MRGPRX2 is substance P (SP). SP is considered an inflammatory neuropeptide and a powerful endogenous pruritic agent whose action is mediated by the neurokinin-1 receptor (NK-1R). Antagonists developed for this receptor to inhibit the nociceptive and proinflammatory properties of SP have been effective in animal models for various diseases, but surprisingly not in humans. SP is believed to activate MRGPRX2 in humans and not NK-1R to induce pruritus.¹⁵

Further ligands that were able to activate MRGPRX2 are proadrenomedullin N-terminal 20 peptide (PAMP-20) and PAMP-9-20/PAMP-12.⁶⁶ The proadrenomedullin N-terminal 20-peptide (PAMP-20) is produced from the adrenomedullin (AM) precursor and induced a strong hypotensive effect when injected intravenously into anesthetized rats. Furthermore, PAMP-20 inhibits carbachol-induced catecholamine synthesis and secretion in cultured bovine adrenal medulla cells, which may lead to vasodilating activity caused by PAMP-20. This suggests that PAMP-20 may play a key role in cardiovascular control.⁶⁷ Therefore, MRGX2 receptor could regulate catecholamine secretion from adrenal glands.⁶⁶

The next class of compounds that are able to activate MRGPRX2 are antimicrobial host defense peptides (HDPs).⁶⁸ The main function of HDPs is to promote innate immunity against various pathogens and further to maintain the homeostasis of the immune system. Both human β -defensins, an HDP released from epithelium, and cathelicidin LL-37 from neutrophils can induce chemotaxis and initiate mast cell degranulation through the activation of MRGPRX2. It was found that the expression of HDPs in the gingiva and saliva of patients with periodontitis is upregulated compared to that of healthy subjects, suggesting host defense by MRGPRX2-mediated mast cell activation.⁶⁹

In addition, it could be shown for various alkaloids and peptidergic or non peptidergic drugs such as morphine⁷⁰, complanadine A^{5, 71}, TAN-67⁷², ciprofloxacin³⁰ as well as the Bradykinin B₂ Receptor Antagonist icatibant that they activate MRGPRX2 to a greater or lesser extent. Mast cell degranulation was also caused by compound 48/80 in RBL-2H3 and LAD2 mast cell lines expressing MRGPRX1 and MRGPRX2. Furthermore, this compound utilizes Ca²⁺ mobilization in HEK293 cells expressing MRGPRX2.⁷³

Small chemokine (C-X-C motif) ligand 14 (CXCL14) is able to activate the MRGX2 receptor in a dose-dependent manner using cellosaurus CHEM1 cell line recombinantly expressing MRGPRX2.⁷⁴

4.2.2.2 Small-molecule MRGPRX2 modulators

Lansu *et. al.* identified the opioid-related MRGPRX2 agonists including the drugs morphine, hydrocodone and dextromethorphan and subsequently generated a homology model of the human MRGPRX2 based on the X-ray structure of the human κ -opioid receptor in complex with a selective antagonist, JD1c (PDB 4DJH) as a template and searched for novel agonists using a structure-based virtual screening approach.⁷⁵

Recently, the first two small molecule competitive antagonists for MRGPRX2 have been described, on the one hand 1-(phenazin-5(10*H*)-yl)ethan-1-one and on the other hand 3-(pyridin-2-ylmethyl)-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one with IC₅₀ values of 1.6 and 2.5 μ M respectively, in Ca²⁺-mobilization assay against 1 μ M of CST-14. Both compounds were able to block icatibant-induced hCMC degranulation and completely inhibited the degranulation of human cord blood-derived MCs (hCMCs), which suggests, that MRGPRX2 antagonists are suitable therapeutics for IgE-independent inflammatory diseases and drug-induced pseudoallergies.⁷⁶

In a compound screening, Alnouri and Müller found three hits as MRGPRX2 antagonists. One of these compounds, **CB8** (Figure 6), was taken as a lead compound for the development of more potent derivatives in collaboration with Professor Herdewijn and his coworkers.⁷⁷

intermediate filaments. Keratinocytes play further an important role in wound healing³⁷, in protecting the skin from UV radiation⁸² and against dehydration.³⁸ Wound healing is a complicated process which include several successive mechanisms such as haemostasis, inflammation, proliferation and maturation.⁸³ The first step after injury is the coagulation and haemostasis in order to prevent exanguination.⁸⁴⁻⁸⁵ In the inflammation phase first neutrophils, then macrophages and afterwards lymphocytes begin phagocytosis to destroy and remove bacteria, foreign particles and damaged tissue, as infected wounds do not heal.^{2, 86} The proliferation phase starts about three days after the injury and includes several mechanisms such as fibroblast migration,¹⁷ collagen synthesis,⁸⁴ angiogenesis,²⁰ epithelialization and granulation tissue formation.¹⁶ In the final phase of remodeling synthesis of collagen and of the extracellular matrix takes place.^{18, 83, 87} Keratinocytes produce antimicrobial peptides also known as host defense peptides (HDPs). On the one hand, HDPs are effective against microorganisms such as bacteria, fungi or viruses, but are also involved in immunomodulatory functions including chemotaxis, cytokine/chemokine production, cell differentiation and apoptosis as well as in the acceleration of angiogenesis. The HDP angiogenic peptide AG-30 regulated the expression of angiogenesis-related cytokines and growth factors in human aortic endothelial cells.⁸⁸ AG-30 showed similar angiogenic properties to those of LL37 or PR39. In order to further increase the angiogenic and antimicrobial properties of AG-30 Nakagami *et al.* modified the peptide sequence of AG-30 by the substitution of five amino acids yielding AG30/5C (Figure 2). Since both epidermal cell growth and angiogenesis are responsible for accelerating the time course of wound healing, AG30/5C is proposed as a possible drug to improve wound healing.⁸⁹

AG30 M L S L I F L H R L K S M R K R L D R K L R L W H R K N Y P
AG30/5C M L K L I F L H R L K R M R K R L K R K L R L W H R K R Y K

Figure 4: Amino acid sequences of AG30 and AG30/5C.⁸⁹ The letters stand for the different amino acids.

In 2016 Kiatsurayanon *et al.* could show that the activation of keratinocytes triggered by AG-30/5C is controlled by MRGPRX3 and MRGPRX4. There is evidence these receptors can mediate not only AG-30/5C-induced cytokine/chemokine production, but also keratinocyte migration and proliferation, because the ability of AG-30/5C to promote both keratinocyte migration and proliferation is significantly suppressed in keratinocytes transfected with MRGPRX3 or -X4 siRNAs. TLR ligands such as PGN, flagellin, LPS, and polyinosinic:polycytidylic acid (poly I:C) increased MRGPRX3 mRNA expression 3- to 36-fold and both hBD-2 and LL-37 are able to stimulate keratinocytes via MRGPRX3 and -X4. Furthermore, a connection between MRGPRX3 and -X4 with the MAPK and NF-κB pathways

is assumed, since both receptors in keratinocytes reduce AG30/5C-induced MAPK and I κ B phosphorylation.^{68, 90} In order to describe more precise physiological and pathophysiological effects of MRGPRX3, agonists and antagonists for this receptor are urgently needed.

4.2.5 MRGPRX4

MRGPRX4, the fourth member of the MRGPRX4 family, primarily stimulates G $_q$ -regulated pathways.²⁶ The expression of the MRGPRX4 receptor could be upregulated by poly I:C. Poly I:C is a toll-like receptor 3 (TLR3) agonist which accelerated wound closure in patients.³⁹ TLRs support the innate immune system in the coordination of inflammatory signal transduction and the functions of these receptors are discussed and explored in wound healing⁴¹, chronic pain⁹¹ and itch⁹². Both AG-30/5C and other host defense peptides (HDPs) such as hBD-2 and LL-37 are able to activate keratinocytes via MRGPRX3 and -X4, which suggests that these receptors play a crucial role in HDP-induced regulation of skin immunity. Furthermore, the cytokine/chemokine production and keratinocyte migration and proliferation initiated by AG-30/5C is mediated by MRGPRX3 and -X4.⁶⁸ Recently, Roy *et al.* confirmed that AG-30/5C acts as an MRGPRX2 biased agonist in mast cells and as an MRGPRX4 biased agonist in keratinocytes. In this publication it was proved, that the diabetes type 2 drug nateglinide, the first described MRGPRX4 agonist, activates both the β -arrestin and the Ca²⁺-mediated signaling pathway of MRGPRX4.^{90, 93} A frequently observed side effect of nateglinide is pruritus, which may be a first indication that MRGPRX4 could be an itch receptor.⁹⁴ Itching is also a frequently observed phenomenon in wound healing.⁴⁹ Therefore, it is reasonable to assume that if MRGPRX4 is involved in wound healing, itching could also be mediated by this receptor. This question was investigated by the research group of Yu *et al.* and Meixiong *et al.*^{47-48, 95} First, bilirubin was found as a possible ligand for MRGPRX4. Bilirubin is a yellowish bile acid, which is formed when the heme portion of the red blood pigment is degraded and is responsible for the yellowing of jaundice. Basically, a distinction can be made between two types of itch. On the one hand histaminergic or acute itch and nonhistaminergic or chronic itch. In nonhistaminergic itch a communication between keratinocytes, the immune system and the nonhistaminergic sensory nerves is responsible for the occurrence of itching. In non-histaminergic itching, as the name suggests, histamine is not involved, so that antihistamine therapies are often unsuccessful and therefore this type of itching in particular requires new therapies.⁶⁷ Nonhistaminergic itch is frequently observed in jaundice which could be triggered by bilirubin. However, the observed EC₅₀ value of bilirubin at MRGPRX4 was only 61.9 μ M.⁴⁸ Later, the scientists were able to identify other bile acids that additionally activated MRGPRX4

and induce itching in a humanized mouse model as well as in human subjects.^{47, 95} These bile acid derivatives such as DCA, CDCA and also bilirubin show increased plasma levels in case of cholestatic itch, which can precipitate in the skin and then presumably activate the MRGPRX4 receptor and thus trigger itching. EC₅₀ values for DCA and CDCA are much higher than those of bilirubin and are 2,7 μM and 2,6 μM, respectively.^{47, 94} A first homology model of this receptor was published in a doctoral thesis by Lansu using the κ-opioid receptor (PDB code 4DJH) as a template. Several phenylalanine derivatives as well as different glinides were further described as MRGPRX4 agonists and their SAR evaluated by various docking studies in the published MRGPRX4 homology model. Mutagenesis studies revealed a potential interaction of the agonists with R86 and R95.⁹⁶

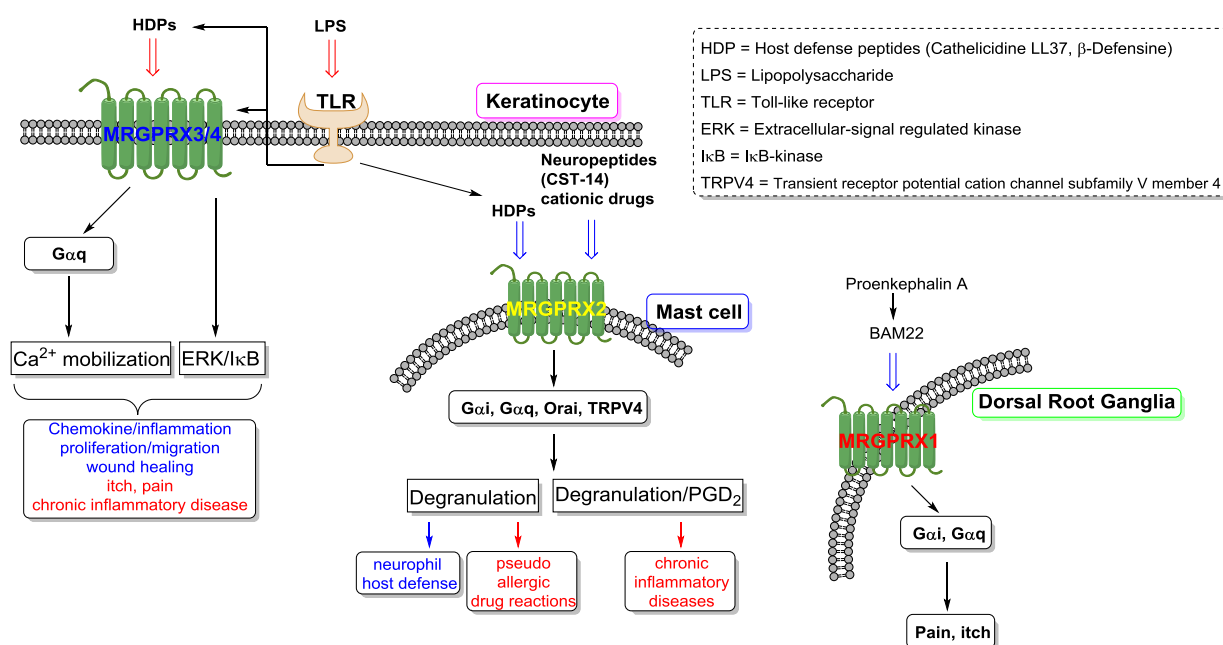


Figure 5: Summary of the proposed (patho)physiological roles of MRGPRX subtypes.⁹⁷

In summary, the MRGPRX family represents interesting opportunities for research into potential pain therapeutics, drugs against histamine-independent itching and for the development of anti-allergic drugs. Significant experiments have already been conducted for MRGPRX1, which propose this receptor as a non-opioid-dependent pain target. Due to the expression of MRGPRX2 preferably in mast cells, the activation of this receptor represents an interesting new treatment option especially in the field of allergies. So far, little is known about MRGPRX3, but the receptor can at present be associated with eye diseases and wound healing. MRGPRX4 is also associated with wound healing due to its expression in keratinocytes. A number of publications also show that this receptor can be associated with non-histamine-dependent itching.

Advances have been made in medicinal chemistry of MRGPRX over the last years, but the knowledge about these primate-specific receptors is still sparse. Highly potent and selective agonists and antagonists are urgently needed for the investigation of the physiological and pathophysiological roles of these receptors. Therefore, the aim of the present work was to design, synthesize and analyze the structure-activity relationship of (i) MRGPRGX2 antagonists, and (ii) MRGPRX4 agonists as well as antagonists.

4.3 References

- (1) George, B.; Janis, J. E.; Attinger, C. E. The basic science of wound healing. *Plast. Reconstr. Surg.* **2006**, *117* (7), 12S–34S.
- (2) Robson, M. C.; Steed, D. L.; Franz, M. G. Wound healing: biologic features and approaches to maximize healing trajectories. *Curr. Probl. Surg.* **2001**, *2* (38), 72–140.
- (3) Giulian, D.; Chen, J.; Ingeman, J.; George, J.; Noponen, M. The role of mononuclear phagocytes in wound healing after traumatic injury to adult mammalian brain. *J. Neurosci.* **1989**, *9* (12), 4416–4429.
- (4) Hepler, J. R.; Gilman, A. G. G proteins. *Trends. Biochem. Sci.* **1992**, *17* (10), 383–387.
- (5) Sun, J.-P.; Li, R.; Ren, H.-Z.; Xu, A.-T.; Yu, X.; Xu, Z.-G. The very large G protein coupled receptor (Vlgr1) in hair cells. *J. Mol. Neurosci.* **2013**, *50* (1), 204–214.
- (6) Spehr, M.; Munger, S. D. Olfactory receptors: G protein-coupled receptors and beyond. *J. Neurochem.* **2009**, *109* (6), 1570–1583.
- (7) Di Pizio, A.; Behrens, M.; Krautwurst, D. Beyond the flavour: the potential druggability of chemosensory G protein-coupled receptors. *Int. J. Mol. Sci.* **2019**, *20* (6), 1402.
- (8) Li, H.; Wang, R.; Lu, Y.; Xu, X.; Ni, J. Targeting G protein-coupled receptor for pain management. *Brain Circ.* **2017**, *3* (2), 109–113.
- (9) Masseck, O. A.; Rubelowski, J. M.; Spoida, K.; Herlitze, S. Light and drug activated G protein-coupled receptors to control intracellular signalling. *Exp. Physiol.* **2011**, *96* (1), 51–56.
- (10) Iliff, A. J.; Xu, X. S. A mechanosensitive GPCR that detects the bloody force. *Cell* **2018**, *173* (3), 542–544.
- (11) Yin, H.; Chu, A.; Li, W.; Wang, B.; Shelton, F.; Otero, F.; Nguyen, D. G.; Caldwell, J. S.; Chen, Y. A. Lipid G protein-coupled receptor ligand identification using β -arrestin PathHunter™ assay. *J. Biol. Chem.* **2009**, *284* (18), 12328–12338.
- (12) Culhane, K. J.; Liu, Y.; Cai, Y.; Yan, E. C. Transmembrane signal transduction by peptide hormones via family BG protein-coupled receptors. *Front. Pharmacol.* **2015**, *6*, 264.
- (13) Shimada, I.; Ueda, T.; Kofuku, Y.; Eddy, M. T.; Wüthrich, K. GPCR drug discovery: integrating solution NMR data with crystal and cryo-EM structures. *Nat. Rev. Drug Discov.* **2019**, *18* (1), 59–82.
- (14) Young, A.; McNaught, C.-E. The physiology of wound healing. *Surgery* **2011**, *29* (10), 475–479.

- (15) Hanlon, C. D.; Andrew, D. J. Outside-in signaling – a brief review of GPCR signaling with a focus on the Drosophila GPCR family. *J. Cell. Sci.* **2015**, *128* (19), 3533–3542.
- (16) Witte, M. B.; Barbul, A. General principles of wound healing. *Surg. Clin. North Am.* **1997**, *77* (3), 509–528.
- (17) Schreier, T.; Degen, E.; Baschong, W. Fibroblast migration and proliferation during in vitro wound healing. *Res. Exp. Med.* **1993**, *193* (1), 195–205.
- (18) O'Kane, S. Wound remodelling and scarring. *J. Wound Care* **2002**, *11* (8), 296–299.
- (19) Kojima, K.; Okamoto, Y.; Kojima, K.; Miytake, K.; Fujise, H.; Shigemasa, Y.; Minami, S. Effects of chitin and chitosan on collagen synthesis in wound healing. *J. Vet. Med. Sci.* **2004**, *66* (12), 1595–1598.
- (20) Eming, S. A.; Brachvogel, B.; Odorisio, T.; Koch, M. Regulation of angiogenesis: wound healing as a model. *Prog. Histochem. Cytochem.* **2007**, *42* (3), 115–170.
- (21) Beall, R. F.; Hwang, T. J.; Kesselheim, A. S. Pre-market development times for biologic versus small-molecule drugs. *Nat. Biotechnol.* **2019**, *37* (7), 708–711.
- (22) G de la Torre, B.; Albericio, F. The pharmaceutical industry in 2018. An analysis of FDA drug approvals from the perspective of molecules. *Molecules* **2019**, *24* (4), 809.
- (23) Dong, X.; Han, S.-k.; Zylka, M. J.; Simon, M. I.; Anderson, D. J. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell* **2001**, *106* (5), 619–632.
- (24) Lembo, P. M. C.; Grazzini, E.; Groblewski, T.; O'Donnell, D.; Roy, M.-O.; Zhang, J.; Hoffert, C.; Cao, J.; Schmidt, R.; Pelletier, M.; Labarre, M.; Gosselin, M.; Fortin, Y.; Banville, D.; Shen, S. H.; Ström, P.; Payza, K.; Dray, A.; Walker, P.; Ahmad, S. Proenkephalin A gene products activate a new family of sensory neuron-specific GPCRs. *Nat. Neurosci.* **2002**, *5*, 201–209.
- (25) Liu, Q.; Tang, Z.; Surdenikova, L.; Kim, S.; Patel, K. N.; Kim, A.; Ru, F.; Guan, Y.; Weng, H.-J.; Geng, Y. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell* **2009**, *139* (7), 1353–1365.
- (26) Burstein, E. S.; Ott, T. R.; Feddock, M.; Ma, J.-N.; Fuhs, S.; Wong, S.; Schiffer, H. H.; Brann, M. R.; Nash, N. R. Characterization of the Mas-related gene family: structural and functional conservation of human and rhesus MrgX receptors. *Br. J. Pharmacol.* **2006**, *147* (1), 73–82.
- (27) Zhang, L.; Taylor, N.; Xie, Y.; Ford, R.; Johnson, J.; Paulsen, J. E.; Bates, B. Cloning and expression of MRG receptors in macaque, mouse, and human. *Mol. Brain* **2005**, *133* (2), 187–197.

- (28) Subramanian, H.; Gupta, K.; Ali, H. Roles of Mas-related G protein-coupled receptor X2 on mast cell-mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. *J. Allergy Clin. Immunol.* **2016**, *138* (3), 700–710.
- (29) Kanz, C.; Aldebert, P.; Althorpe, N.; Baker, W.; Baldwin, A.; Bates, K.; Browne, P.; van den Broek, A.; Castro, M.; Cochrane, G. The EMBL nucleotide sequence database. *Nucleic Acids Res.* **2005**, *33* (suppl_1), D29–D33.
- (30) Porebski, G.; Kwiecien, K.; Pawica, M.; Kwitniewski, M. Mas-Related G protein-coupled receptor-X2 (MRGPRX2) in drug hypersensitivity reactions. *Front. Immunol.* **2018**, *9*, 3027-3027.
- (31) Vadivelu, N.; Kai, A. M.; Kodumudi, V.; Sramcik, J.; Kaye, A. D. The opioid crisis: a comprehensive overview. *Curr. Pain Headache Rep.* **2018**, *22* (3), 16.
- (32) Nicol, A. L.; Hurley, R. W.; Benzon, H. T. Alternatives to opioids in the pharmacologic management of chronic pain syndromes: a narrative review of randomized, controlled, and blinded clinical trials. *Anesth Analg* **2017**, *125* (5), 1682–1703.
- (33) Smith, H. S. Opioid metabolism. *Mayo Clin. Proc.* **2009**, *84* (7), 613–624.
- (34) Ricardo Buenaventura, M.; Rajive Adlaka, M.; Nalini Sehgal, M. Opioid complications and side effects. *Pain physician* **2008**, *11*, S105–S120.
- (35) Jones, M. R.; Viswanath, O.; Peck, J.; Kaye, A. D.; Gill, J. S.; Simopoulos, T. T. A Brief history of the opioid epidemic and strategies for pain medicine. *Pain Ther.* **2018**, *7* (1), 13–21.
- (36) Kaye, A. D.; Cornett, E. M.; Hart, B.; Patil, S.; Pham, A.; Spalitta, M.; Mancuso, K. F. Novel Pharmacological Nonopioid Therapies in Chronic Pain. *Curr. Pain Headache Rep.* **2018**, *22* (4), 31.
- (37) Pastar, I.; Stojadinovic, O.; Tomic-Canic, M. Role of keratinocytes in healing of chronic wounds. *Surg. Technol. Int.* **2008**, *17*, 105–112.
- (38) Siefken, W.; Carstensen, S.; Springmann, G.; Wittern, K.-P.; Wenck, H.; Stäb, F.; Sauermann, G.; Schreiner, V.; Doering, T.; Janeke, G. Role of taurine accumulation in keratinocyte hydration. *J. Invest. Dermatol.* **2003**, *121* (2), 354–361.
- (39) Lin, Q.; Wang, L.; Lin, Y.; Liu, X.; Ren, X.; Wen, S.; Du, X.; Lu, T.; Su, S. Y.; Yang, X. Toll-like receptor 3 ligand polyinosinic: polycytidylic acid promotes wound healing in human and murine skin. *J. Invest. Dermatol.* **2012**, *132* (8), 2085–2092.
- (40) Lacagnina, M. J.; Watkins, L. R.; Grace, P. M. Toll-like receptors and their role in persistent pain. *Pharmacol Ther.* **2018**, *184*, 145–158.
- (41) Chen, L.; DiPietro, L. A. Toll-like receptor function in acute wounds. *Adv. Wound Care* **2017**, *6* (10), 344–355.

(42) Taves, S.; Ji, R.-R., Itch control by Toll-like receptors. In *Handb. Exp. Pharmacol.*, Springer: 2015; pp 135–150.

(43) Wroblowski, B.; Wigglesworth, M. J.; Szekeres, P. G.; Smith, G. D.; Rahman, S. S.; Nicholson, N. H.; Muir, A. I.; Hall, A.; Heer, J. P.; Garland, S. L. The discovery of a selective, small molecule agonist for the MAS-related gene X1 receptor. *J. Med. Chem.* **2009**, *52* (3), 818–825.

(44) Kunapuli, P.; Lee, S.; Zheng, W.; Alberts, M.; Kornienko, O.; Mull, R.; Kreamer, A.; Hwang, J.-I.; Simon, M. I.; Strulovici, B. Identification of small molecule antagonists of the human mas-related gene-X1 receptor. *Anal. Biochem.* **2006**, *351* (1), 50–61.

(45) Bayrakdarian, M.; Butterworth, J.; Hu, Y.-J.; Santhakumar, V.; Tomaszewski, M. J. Development of 2, 4-diaminopyrimidine derivatives as novel SNSR4 antagonists. *Bioorg. Med. Chem. Lett.* **2011**, *21* (7), 2102–2105.

(46) Roy, S.; Ganguly, A.; Haque, M.; Ali, H. Angiogenic Host Defense Peptide AG-30/5C and Bradykinin B2 Receptor Antagonist Icatibant Are G Protein Biased Agonists for MRGPRX2 in Mast Cells. *The Journal of Immunology* **2019**, *202* (4), 1229–1238.

(47) Yu, H.; Zhao, T.; Liu, S.; Wu, Q.; Johnson, O.; Wu, Z.; Zhuang, Z.; Shi, Y.; Peng, L.; He, R. MRGPRX4 is a bile acid receptor for human cholestatic itch. *eLife* **2019**, *8*.

(48) Meixiong, J.; Vasavda, C.; Green, D.; Zheng, Q.; Qi, L.; Kwatra, S. G.; Hamilton, J. P.; Snyder, S. H.; Dong, X. Identification of a bilirubin receptor that may mediate a component of cholestatic itch. *eLife* **2019**, *8*, e44116.

(49) Lerner, E. Why Do Wounds Itch? *Wounds* **2018**, *30* (1), 1–3.

(50) Motakis, E.; Guhl, S.; Ishizu, Y.; Itoh, M.; Kawaji, H.; de Hoon, M.; Lassmann, T.; Carninci, P.; Hayashizaki, Y.; Zuberbier, T. Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* **2014**, *123* (17), e58–e67.

(51) Meixiong, J.; Vasavda, C.; Snyder, S. H.; Dong, X. MRGPRX4 is a G protein-coupled receptor activated by bile acids that may contribute to cholestatic pruritus. *Proc. Natl. Acad. Sci* **2019**, *116* (21), 10525–10530.

(52) Hendriksen, E.; van Bergeijk, D.; Oosting, R. S.; Redegeld, F. A. Mast cells in neuroinflammation and brain disorders. *Neurosci. Biobehav. Rev.* **2017**, *79*, 119–133.

(53) Silver, R.; Curley, J. P. Mast cells on the mind: new insights and opportunities. *Trends Neurosci.* **2013**, *36* (9), 513–521.

(54) McKay, D. M.; Bienenstock, J. The interaction between mast cells and nerves in the gastrointestinal tract. *Immunol. Today* **1994**, *15* (11), 533–538.

(55) Gupta, K.; Harvima, I. T. Mast cell-neural interactions contribute to pain and itch. *Immunol. Rev.* **2018**, *282* (1), 168–187.

(56) Zhang, X.-C.; Strassman, A. M.; Burstein, R.; Levy, D. Sensitization and activation of intracranial meningeal nociceptors by mast cell mediators. *J. Pharmacol. Exp. Ther.* **2007**, *322* (2), 806–812.

(57) Boesiger, J.; Tsai, M.; Maurer, M.; Yamaguchi, M.; Brown, L. F.; Claffey, K. P.; Dvorak, H. F.; Galli, S. J. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of Fcε receptor I expression. *J. Exp. Med.* **1998**, *188* (6), 1135–1145.

(58) Graziottin, A.; Skaper, S. D.; Fusco, M. Mast cells in chronic inflammation, pelvic pain and depression in women. *Gynecol. Endocrinol.* **2014**, *30* (7), 472–477.

(59) Cohen, E. P.; Russell, T. J.; Garancis, J. C. Mast cells and calcium in severe uremic itching. *Am. J. Med. Sci.* **1992**, *303* (6), 360–365.

(60) Fujisawa, D.; Kashiwakura, J.-i.; Kita, H.; Kikukawa, Y.; Fujitani, Y.; Sasaki-Sakamoto, T.; Kuroda, K.; Nunomura, S.; Hayama, K.; Terui, T. Expression of mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J. Allergy Clin. Immunol.* **2014**, *134* (3), 622–633. e9.

(61) Manorak, W.; Idahosa, C.; Gupta, K.; Roy, S.; Panettieri, R.; Ali, H. Upregulation of Mas-related G Protein coupled receptor X2 in asthmatic lung mast cells and its activation by the novel neuropeptide hemokinin-1. *Respiratory research* **2018**, *19* (1), 1.

(62) Le, D. D.; Schmit, D.; Heck, S.; Omlor, A. J.; Sester, M.; Herr, C.; Schick, B.; Daubeuf, F.; Fähndrich, S.; Bals, R. Increase of mast cell-nerve association and neuropeptide receptor expression on mast cells in perennial allergic rhinitis. *Neuroimmunomodulation* **2016**, *23* (5-6), 261–270.

(63) Takamori, A.; Izawa, K.; Kaitani, A.; Ando, T.; Okamoto, Y.; Maehara, A.; Tanabe, A.; Nagamine, M.; Yamada, H.; Uchida, S. Identification of inhibitory mechanisms in pseudo-allergy involving Mrgprb2/MRGPRX2-mediated mast cell activation. *J. Allergy. Clin. Immunol.* **2019**, *143* (3), 1231–1235.

(64) Green, D. P.; Limjunyawong, N.; Gour, N.; Pundir, P.; Dong, X. A mast-cell-specific receptor mediates neurogenic inflammation and pain. *Neuron* **2019**, *101* (3), 412–420.

(65) Robas, N.; Mead, E.; Fidock, M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J. Biol. Chem.* **2003**, *278* (45), 44400–44404.

(66) Kamohara, M.; Matsuo, A.; Takasaki, J.; Kohda, M.; Matsumoto, M.; Matsumoto, S.-i.; Soga, T.; Hiyama, H.; Kobori, M.; Katou, M. Identification of MrgX2 as a human G-protein-coupled receptor for proadrenomedullin N-terminal peptides. *Biochem. Biophys. Res. Commun.* **2005**, *330* (4), 1146–1152.

(67) Yosipovitch, G.; Rosen, J. D.; Hashimoto, T. Itch: from mechanism to (novel) therapeutic approaches. *J. Allergy Clin. Immunol.* **2018**, *142* (5), 1375–1390.

(68) Kiatsurayanon, C.; Niyonsaba, F.; Chieosilapatham, P.; Okumura, K.; Ikeda, S.; Ogawa, H. Angiogenic peptide (AG)-30/5C activates human keratinocytes to produce cytokines/chemokines and to migrate and proliferate via MrgX receptors. *J. Dermatol. Sci.* **2016**, *83* (3), 190–199.

(69) Gupta, K.; Idahosa, C.; Roy, S.; Lee, D.; Subramanian, H.; Dhingra, A.; Boesze-Battaglia, K.; Korostoff, J.; Ali, H. Differential regulation of Mas-related G protein-coupled receptor X2-mediated mast cell degranulation by antimicrobial host defense peptides and porphyromonas gingivalis lipopolysaccharide. *Infect. Immun.* **2017**, *85* (10), e00246-17.

(70) Akuzawa, N.; Obinata, H.; Izumi, T.; Takeda, S. Morphine is an exogenous ligand for MrgX2, a G protein-coupled receptor for cortistatin. *J. Cell. Animal Biol.* **2007**, *2* (1), 004–009.

(71) Johnson, T.; Siegel, D. Complanadine A, a selective agonist for the Mas-related G protein-coupled receptor X2. *Bioorg. Med. Chem. Lett.* **2014**, *24* (15), 3512–3515.

(72) Southern, C.; Cook, J. M.; Neetoo-Isseljee, Z.; Taylor, D. L.; Kettleborough, C. A.; Merritt, A.; Bassoni, D. L.; Raab, W. J.; Quinn, E.; Wehrman, T. S. Screening β -arrestin recruitment for the identification of natural ligands for orphan G protein-coupled receptors. *J. Biomol. Screen.* **2013**, *18* (5), 599–609.

(73) Kashem, S. W.; Subramanian, H.; Collington, S. J.; Magotti, P.; Lambris, J. D.; Ali, H. G protein-coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. *Eur. J. Pharmacol.* **2011**, *668* (1-2), 299–304.

(74) Golz, S. Mrg receptor modulators. *EP3011340B1* **2017**.

(75) Lansu, K.; Karpiak, J.; Liu, J.; Huang, X.-P.; McCorvy, J. D.; Kroeze, W. K.; Che, T.; Nagase, H.; Carroll, F. I.; Jin, J.; Shoichet, B. K.; Roth, B. L. In silico design of novel probes for the atypical opioid receptor MRGPRX2. *Nat. Chem. Biol.* **2017**, *13* (5), 529–536.

(76) Ogasawara, H.; Furuno, M.; Edamura, K.; Noguchi, M. Novel MRGPRX2 antagonists inhibit IgE independent activation of human umbilical cord blood derived mast cells. *J. Leukocyte. Biol.* **2019**, *106* (5), 1069–1077.

(77) Müller, C. E. MrgX2 Antagonists. *UBN0004(US)* **2019**.

(78) Kaisho, Y.; Watanabe, T.; Nakata, M.; Yano, T.; Yasuhara, Y.; Shimakawa, K.; Mori, I.; Sakura, Y.; Terao, Y.; Matsui, H. Transgenic rats overexpressing the human MrgX3 gene show cataracts and an abnormal skin phenotype. *Biochem. Biophys. Res. Commun.* **2005**, *330* (3), 653–657.

(79) Yoshihara, M.; Ohmiya, H.; Hara, S.; Kawasaki, S.; Hayashizaki, Y.; Itoh, M.; Kawaji, H.; Tsujikawa, M.; Nishida, K.; Consortium, F. Discovery of molecular markers to discriminate corneal endothelial cells in the human body. *PLoS One* **2015**, *10* (3), e0117581.

(80) Hsu, Y.-H.; Liu, Y.; Hannan, M. T.; Maixner, W.; Smith, S. B.; Diatchenko, L.; Golightly, Y. M.; Menz, H. B.; Kraus, V. B.; Doherty, M. Genome-wide association meta-analyses to identify common genetic variants associated with hallux valgus in Caucasian and African Americans. *J. Med. Genet.* **2015**, *52* (11), 762–769.

(81) Solleti, S. K.; Bhattacharya, S.; Ahmad, A.; Wang, Q.; Mereness, J.; Rangasamy, T.; Mariani, T. J. MicroRNA expression profiling defines the impact of electronic cigarettes on human airway epithelial cells. *Sci. Rep.* **2017**, *7* (1), 1–10.

(82) Ishida, T.; Sakaguchi, I. Protection of human keratinocytes from UVB-induced inflammation using root extract of lithospermum erythrorhizon. *Biol. Pharm. Bull.* **2007**, *30* (5), 928-934.

(83) Velnar, T.; Bailey, T.; Smrkolj, V. The wound healing process: an overview of the cellular and molecular mechanisms. *J. Int. Med. Res.* **2009**, *37* (5), 1528-1542.

(84) Pool, J. Normal hemostatic mechanisms: a review. *Am. J. Med. Technol.* **1977**, *43* (8), 776–780.

(85) George Broughton, I.; Janis, J. E.; Attinger, C. E. The basic science of wound healing. *Plast. Reconstr. Surg.* **2006**, *117* (7S), 12S–34S.

(86) Giulian, D.; Chen, J.; Ingeman, J.; George, J.; Nojonen, M. The role of mononuclear phagocytes in wound healing after traumatic injury to adult mammalian brain. *J. Neurosci. Res.* **1989**, *9* (12), 4416-4429.

(87) Young, A.; McNaught, C.-E. The physiology of wound healing. *Ann. Emerg. Med.* **2011**, *29* (10), 475-479.

(88) Nishikawa, T.; Nakagami, H.; Maeda, A.; Morishita, R.; Miyazaki, N.; Ogawa, T.; Tabata, Y.; Kikuchi, Y.; Hayashi, H.; Tatsu, Y. Development of a novel antimicrobial peptide, AG-30, with angiogenic properties. *J. Cell Mol. Med.* **2009**, *13* (3), 535-546.

(89) Nakagami, H.; Nishikawa, T.; Tamura, N.; Maeda, A.; Hibino, H.; Mochizuki, M.; Shimosato, T.; Moriya, T.; Morishita, R.; Tamai, K.; Tomono, K.; Kaneda, Y. Modification of a novel angiogenic peptide, AG30, for the development of novel therapeutic agents. *J. Cell Mol. Med.* **2012**, *16* (7), 1629–1639.

(90) Roy, S.; Ganguly, A.; Haque, M.; Ali, H. Angiogenic host defense peptide AG-30/5C and bradykinin B2 receptor antagonist icatibant are G protein biased agonists for MRGPRX2 in mast cells. *J. Immunol.* **2019**, *202* (4), 1229–1238.

(91) Lacagnina, M. J.; Watkins, L. R.; Grace, P. M. Toll-like receptors and their role in persistent pain. *Pharmacol. Ther.* **2018**, *184*, 145-158.

(92) Taves, S.; Ji, R.-R. Itch control by Toll-like receptors. *Handb. Exp. Pharmacol.* **2015**, *226*, 135–150.

(93) Kroeze, W. K.; Sassano, M. F.; Huang, X.-P.; Lansu, K.; McCorvy, J. D.; Giguère, P. M.; Sciaky, N.; Roth, B. L. PRESTO-Tango: as an open-source resource for interrogation of the druggable human GPCR-ome. *Nat. Struct. Mol. Biol.* **2015**, *22* (5), 362–369.

(94) Yu, H.; Zhao, T.; Liu, S.; Wu, Q.; Johnson, O.; Wu, Z.; Zhuang, Z.; Shi, Y.; Peng, L.; He, R.; Yang, Y.; Sun, J.; Wang, X.; Xu, H.; Zeng, Z.; Zou, P.; Lei, X.; Luo, W.; Li, Y. MRGPRX4 is a bile acid receptor for human cholestatic itch. *eLife* **2019**, *8*, 1–27.

(95) Meixiong, J.; Vasavda, C.; Snyder, S. H.; Dong, X. MRGPRX4 is a G protein-coupled receptor activated by bile acids that may contribute to cholestatic pruritus. *PNAS* **2019**, *116* (21), 10525–10530.

(96) Lansu, K. Ligand discovery and functional characterization of MRGPRX family orphan GPCRs. University of North Carolina at Chapel Hill, 2017.

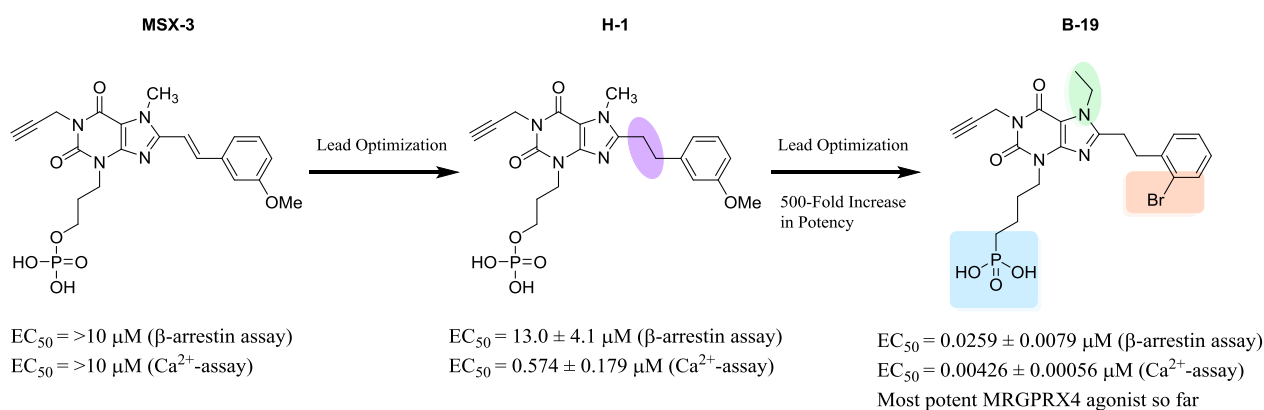
(97) Ali, H., Emerging roles for MAS-related G protein-coupled receptor X2 in host defense peptide, opioid, and neuropeptide-mediated inflammatory reactions. In *Adv. Immunol.*, Elsevier: 2017; Vol. 136, pp 123–162.

5. Patent I: MRGPRX4 Agonists and Antagonists

Marx, D.; Müller, C.; Alnouri, W.; Riedel, Y.; Namasivayam, V.; Pillaiyar, T.; Thimm, D.; Hockemeyer, J.

In a compound screening campaign of the internal, proprietary compound library, MSX-3 was discovered to show an agonistic effect on the rare natural variant L83S of the MRGPRX4 receptor, which represents an orphan G protein-coupled receptor which is only expressed in primates. In order to increase its activity and to additionally identify agonists for the wild-type (WT) MRGPRX4 as well, a variety of chemical modifications was performed. Since the phosphate group is expected to be enzymatically unstable, it was replaced by a phosphonate group as well as other bioisosteric functions. To further increase the stability of MSX-3 derivatives, the photosensitive double bond linker between C8 of the xanthine core and the aromatic residue attached to it via a linker was to be replaced by a more stable linker. Since no synthetic procedure was known in literature for this kind of target compounds, a broad array of chemical methods had to be studied in order to finally obtain the desired target compounds in 6 to 9 steps synthetic procedures.

Through these modifications we were able to increase the stability of the agonists on the one hand, and to increase the agonistic activity at the natural variant of the MRGPRX4 receptor on the other hand. We were able to obtain the first potent xanthine derivative with agonistic activity on the WT MRGPRX4 (**H-1**). Through the synthesis of more than 50 xanthine derivatives by substitution of the different side groups, the most active MRGPRX4 receptor agonist described so far could be synthesized showing an EC_{50} value of 26 nM in a β -arrestin assay and of 4 nM in a G protein-coupled Ca^{2+} mobilization assay (**B-19**). The final compounds were characterized by 1H -, ^{13}C - and ^{31}P -NMR and by high-resolution mass spectrometry (HRMS), and analyzed by HPLC-UV proving their high purity of more than 95%, which is required for subsequent biological studies.



For selectivity studies, selected MRGPRX4 agonists were tested for their potential agonistic and antagonistic activity on the other MRGPRX subtypes (MRGPRX1 to -X3). The new developed compounds show only agonistic activity at the MRGPRX4 receptor and were inactive at the other MRGPRX subtypes due to their different binding pockets. Homology Modeling and docking studies of selected MRGPRX4 agonists was performed in order to explain their structure-activity relationships and the most important amino acids involved in ligand binding.

In addition to an adequate activity at the target protein and an acceptable safety profile, drug candidates must demonstrate a balanced ratio of gastrointestinal absorption, distribution, clearance and elimination (ADME), the so-called Drug Metabolism and Pharmacokinetic (DMPK) properties. Selected MRGPRX4 agonists were tested in *in vitro* ADME studies performed by Pharmacelsus and showed excellent water solubility and good metabolic stability in liver microsomes for example for compound B-16 $t_{1/2} = 53.7$ min. Furthermore **B-16** showed a clearance of 25.8 $\mu\text{l}/\text{min}/\text{mg}$ protein and a plasma protein binding of 91.20 % \pm 0.03 %.

Since a weak, non-selective MRGPRX4 agonist has been described to activate human keratinocytes to produce cytokines and chemokines and to induce their migration and proliferation via MRGPRX4, the newly synthesized MRGPRX4 agonists were protected in the following patent application and will further investigated for their wound healing properties. Some of the synthesized derivatives showed (weak) partial agonistic activity and may be further optimized as antagonists, which are of high interest as novel drugs for the treatment of pain and inflammation. Furthermore, the newly synthesized compounds can be used as tool compound to find out more about the pharmacology of the so far sparsely investigated MRGPRX4 receptor.

MRGPRX4 Agonists and Antagonists

The invention relates to MRGPRX4 receptor agonists and antagonists useful for treating, alleviating and/or preventing diseases and disorders related to MRGPRX4 receptor function as well as pharmaceutical compositions comprising such compounds and methods for preparing such compounds. The invention is further directed to the use of these compounds, alone or in combination with other therapeutic agents, for alleviating, preventing and/or treating diseases and disorders, especially the use as wound healing medicaments or medicaments for different types of pain including itching.

MRGPRX4 (in the literature also referred to as “MAS-related G protein-coupled receptor X4”, MRGX4), is a member of the MRGX receptor family (in the literature also referred to as “MAS-related G protein-coupled receptor X”, MRGX, MRGPRX, and the like).

MAS-related gene receptors (MRG receptors, MRGPR) form a large family of G protein-coupled receptors. The MRGPRX (MRGX) subfamily of MRGPRs is expressed in small-diameter sensory neurons of dorsal root ganglia, in keratinocytes and few other tissues. The MRGPRX family consists of 4 subtypes (MRGPRX1-X4) which are expressed in primates including humans, but not found, e.g., in rodents. Non-primate orthologs of MRGPRX4 have not been identified to date. Proliferative responses mediated by human MRGPRX4 are pertussis toxin- (PTX-) insensitive MRGPRX4 and primarily mediated by the stimulation of Gq-regulated pathways (E. S. Burnstein et al., *Br J Pharmacol.* 2006 Jan;147(1):73-82. doi: 10.1038/sj.bjp.0706448). Besides, GPCRs induce β -arrestin recruitment followed by internalization of the receptor, and in fact, β -arrestin recruitment was shown to be induced by MRGPRX4 activation.

It is known that angiogenic peptide (AG)-30/5C activates human keratinocytes to produce cytokines/chemokines and to migrate and proliferate via MRGX receptors. There is evidence that AG-30/5C may be a useful therapeutic agent for wound healing by activating human keratinocytes (Ch. Kiatsurayanon et al., *J Dermatol Sci.* 2016 Sep;83(3):190-9. doi: 10.1016/j.jdermsci.2016.05.006).

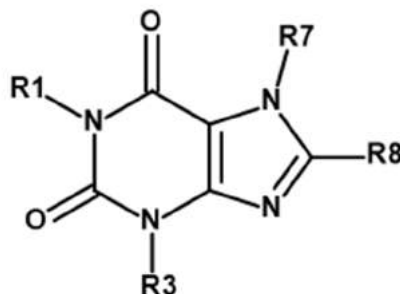
The human MRGPRX4 receptor represents a fundamentally new drug target, and the development of potent MRGPRX4 receptor agonists, partial agonists, and antagonists/inverse agonists to be used for the treatment of MRGPRX4-associated conditions, disorders or diseases requires the design of novel drugs targeting the MRGPRX4 receptor.

It is an object of the invention to provide compounds that have advantages compared to the compounds of the prior art. The compounds should act as potent and selective agonists or antagonists of the MRGPRX4 receptor, in particular of the human wildtype MRGPRX4 receptor, and thus may be useful as drugs or for the prevention or treatment of MRGPRX4-associated conditions, disorders or diseases. Moreover, it was an object of the invention to provide methods for preparing said compounds. It was furthermore an object of the invention to provide compounds and pharmaceutical formulations for the treatment, alleviation and/or prevention of MRGPRX4-associated conditions, disorders or diseases. It was a further object of the invention to provide the use of these compounds for alleviating, preventing and/or treating conditions, diseases and disorders connected to MRGPRX4 function, particularly for, but not limited to the use for treating open or closed wounds, e.g. for wound healing, and for the treatment of different types of pain and itch.

This object has been solved by the subject-matter of the patent claims.

The invention is directed to MRGPRX4 receptor agonists or antagonists, respectively, that are useful for preventing or treating MRGPRX4-associated conditions, disorders or diseases.

A first aspect of the invention relates to a compound according to general Formula 1



wherein

- R1** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;
- R3** represents -C₁₋₁₀-alkyl-P(=O)(OC₁₋₁₀-alkyl)₂, -C₁₋₁₀-alkyl-P(=O)(OH)(OC₁₋₁₀-alkyl), -C₁₋₁₀-alkyl-S(=O)₂(OH), -C₁₋₁₀-alkyl-S(=O)₂(NH₂), -C₁₋₁₀-alkyl-C(=O)(OH), or -C₁₋₁₀-alkyl-P(=O)(OH)₂;
- R7** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl; and
- R8** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-O-aryl, -C₃₋₆-cycloalkyl-aryl, -C₃₋₆-cycloalkyl-heteroaryl, -C₁₋₁₀-alkyl-heteroaryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

wherein in each case "C₁₋₁₀-alkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

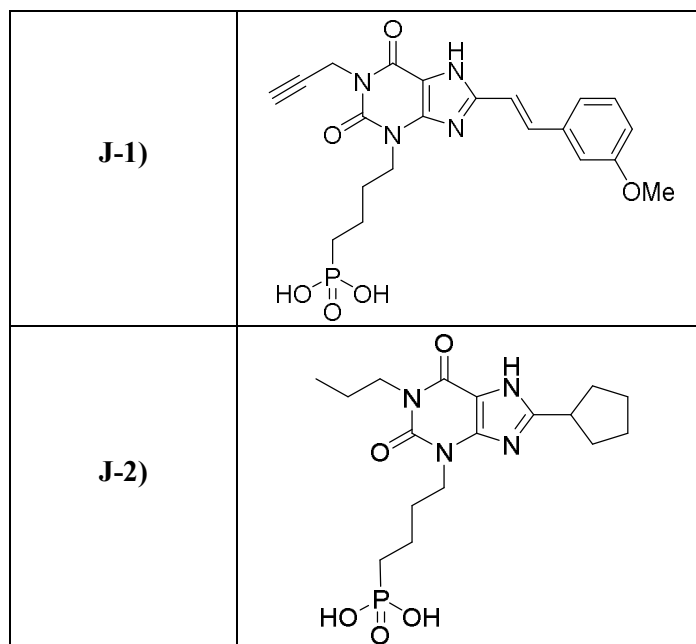
wherein in each case "C₃₋₁₀-cycloalkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

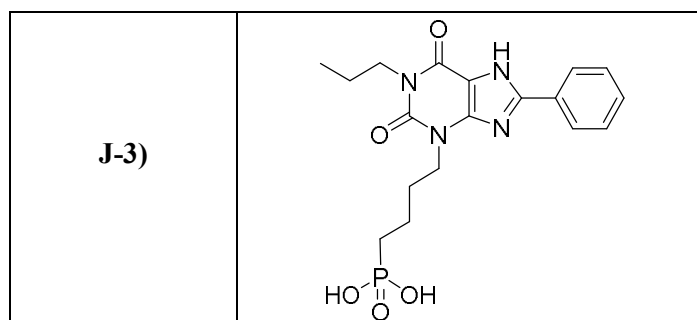
wherein in each case "aryl" is a 6-14-membered aryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from -C≡CH, -CF₃, -CCl₃, -CBr₃, -Cl₃, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "heteroaryl" is a 5-14-membered heteroaryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from -C≡CH, -CF₃, -CCl₃, -CBr₃, -Cl₃, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

or a physiologically acceptable salt thereof;

with the proviso that the compound is not a compound selected from the group consisting of compounds J-1 to J-3:





The compounds according to the invention are derivatives of xanthine (2,6-dihydroxypurine). The numbering of substituents **R1**, **R3**, **R7** and **R8** corresponds to the numbering of ring atoms of the xanthine scaffold.

The compounds according to the invention may reflect two substitution patterns, for the purpose of the specification indicated as (i) and (ii), respectively.

According to substitution pattern (i),

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl;

R3 represents -C₁₋₁₀-alkyl-P(=O)(OH)₂;

R7 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl; and

R8 represents -C₃₋₆-cycloalkyl-aryl, -C₃₋₆-cycloalkyl-heteroaryl, -C₁₋₁₀-alkyl-aryl or -C₁₋₁₀-alkyl-heteroaryl (wherein in case of -C₂-alkyl-aryl, the alkyl-moiety is saturated).

According to substitution pattern (ii),

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

R3 represents -C₁₋₁₀-alkyl-P(=O)(OC₁₋₁₀-alkyl)₂; -C₁₋₁₀-alkyl-P(=O)(OH)(OC₁₋₁₀-alkyl); -C₁₋₁₀-alkyl-S(=O)₂(OH); -C₁₋₁₀-alkyl-S(=O)₂(NH₂); or -C₁₋₁₀-alkyl-C(=O)(OH);

R7 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl; and

R8 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl.

Within the above definitions of the compounds according to substitution patterns (i) and (ii), in each case "C₁₋₁₀-alkyl" independently may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I.

Unless expressly stated otherwise, "alkyl" preferably refers to an aliphatic hydrocarbon including straight chain, or branched chain groups. Preferably, the alkyl group has 1 to 10 carbon atoms (C₁-C₁₀ alkyl), more preferably 1 to 6 carbon atoms (C₁-C₆ alkyl) and most preferably 1 to 4 carbon atoms (C₁-C₄ alkyl), e. g., methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and the like. The aliphatic hydrocarbon may be saturated or unsaturated. When it is unsaturated, it may contain one or more unsaturations, i.e., -C=C-double and/or -C≡C-triple bonds. If there is more than one unsaturation, the unsaturations may be conjugated or isolated. Thus, for the purpose of the specification the term "alkyl" encompasses saturated hydrocarbons as well as alkenyl, alkynyl and alkenynyl residues. "Alkenyl" preferably refers to an alkyl group, as defined above, consisting of at least two carbon atoms and at least one carbon-carbon double bond e.g., ethenyl, propenyl, butenyl or pentenyl and their structural isomeric forms such as 1- or 2-propenyl, 1-, 2-, or 3-butenyl and the like. "Alkynyl" preferably refers to an alkyl group, as defined above, consisting of at least two carbon atoms and at least one carbon-carbon triple bond e. g., acetylene, ethynyl, propynyl, butynyl, or pentynyl and their structural isomeric forms as described above. Alkyl may be substituted or unsubstituted. When substituted, the substituent group(s) is one or more, for example one or two groups, individually selected from the group consisting of -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I.

Within the above definitions of the compounds according to substitution patterns (i) and (ii), in each case "C₃₋₁₀-cycloalkyl" independently may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I.

Unless expressly stated otherwise, "cycloalkyl" preferably refers to cyclic hydrocarbon residue that contains no heteroatoms as ring members and that is not aromatic. "Cyclo-alkyl" may encompass a single cycle or more than one cycle. Preferably, cycloalkyl has 3 to 8 carbon atoms (-C₃-C₈ cycloalkyl). Cycloalkyl may be saturated, e.g., cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, adamantane; or unsaturated (e.g., cycloalkenyl, cycloalkynyl), e.g., cyclobutenyl, cyclopentenyl, cyclohexenyl, cyclo-hexadiene, cycloheptatriene and the like. Cycloalkyl may be substituted or unsubstituted. When substituted, the substituent group(s) is one or more, for example one or two groups, individually

selected from $-C\equiv CH$, $-C(=O)OH$, $-C(=O)O-C_{1-6}$ -alkyl, $-OH$, $-O-C_{1-10}$ -alkyl, $-NH_2$, $-NH-C_{1-10}$ -alkyl, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

Within the above definitions of the compounds according to substitution patterns (i) and (ii), in each case "aryl" independently is a 6-14-membered aryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}$ -alkyl, $-OH$, $-O-C_{1-10}$ -alkyl, $-NH_2$, $-NH-C_{1-10}$ -alkyl, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

Unless expressly stated otherwise, "aryl" preferably refers to an aromatic all-carbon monocyclic or fused-ring polycyclic group (i.e., rings which share adjacent pairs of carbon atoms) of 6 to 14 ring atoms and having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is one or more, for example one, two, or three substituents, independently selected from the group consisting of $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}$ -alkyl, $-OH$, $-O-C_{1-10}$ -alkyl, $-NH_2$, $-NH-C_{1-10}$ -alkyl, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

Within the above definitions of the compounds according to substitution patterns (i) and (ii), in each case "heteroaryl" independently is a 5-14-membered heteroaryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}$ -alkyl, $-OH$, $-O-C_{1-10}$ -alkyl, $-NH_2$, $-NH-C_{1-10}$ -alkyl, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

Unless expressly stated otherwise, "heteroaryl" preferably refers to a monocyclic or fused aromatic ring (i.e., rings which share an adjacent pair of atoms) of 5 to 10 ring atoms in which one, two, three or four ring atoms are selected from the group consisting of N, O and S and the rest being carbon. Examples, without limitation, of heteroaryl groups are pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, quinoliziny, quinazoliny, pthalaziny, quinoxaliny, cinnolinyl, naphthyridinyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5, 6, 7, 8-tetra-hydroisoquinolyl, purinyl, pteridinyl, pyridinyl, pyrimidinyl, carbazolyl, xanthenyl or benzoquinolyl. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is one or more, for example one or two substituents, independently selected from the group consisting of $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}$ -alkyl, $-OH$, $-O-C_{1-10}$ -alkyl, $-NH_2$, $-NH-C_{1-10}$ -alkyl, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

If not expressly stated otherwise, any residue, group or moiety defined herein that can be substituted is preferably substituted with one or more substituents independently selected from the group consisting of $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}\text{-alkyl}$, $-OH$, $-O-C_{1-10}\text{-alkyl}$, $-NH_2$, $-NH-C_{1-10}\text{-alkyl}$, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

The invention also relates to the stereoisomers of the compounds according to general formula 1 e.g. the enantiomers or diastereomers in racemic, enriched or substantially pure form.

In preferred embodiments of the compounds according to substitution patterns (i) and (ii), **R1** represents $-C_{1-10}\text{-alkyl}$, optionally substituted with $-C\equiv CH$; or $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$;
preferably $-C_{1-6}\text{-alkyl}$, optionally substituted with $-C\equiv CH$; or $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$;
more preferably $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH_2C\equiv CH$, or $-CH_2\text{-cyclobutyl}$.

In preferred embodiments of the compounds according to substitution patterns (i) and (ii), **R3** represents $-C_{3-5}\text{-alkyl-P}(=O)(OH)_2$; $-C_{3-5}\text{-alkyl-P}(=O)(OC_{1-6}\text{-alkyl})_2$; $-C_{3-5}\text{-alkyl-P}(=O)(OH)(OC_{1-6}\text{-alkyl})$; $-C_{3-5}\text{-alkyl-S}(=O)_2(OH)$; $-C_{3-5}\text{-alkyl-S}(=O)_2(NH_2)$; or $-C_{3-5}\text{-alkyl-C}(=O)(OH)$;
preferably $-C_{3-5}\text{-alkyl-P}(=O)(OH)_2$; more preferably $-C_4\text{-alkyl-P}(=O)(OH)_2$.

In preferred embodiments of the compounds according to substitution patterns (i) and (ii), **R7** represents $-H$; $-C_{1-10}\text{-alkyl}$, optionally substituted with $-OH$; $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$; or $-C_{1-10}\text{-alkyl-aryl}$;
preferably $-H$; $-C_{1-6}\text{-alkyl}$, optionally substituted with $-OH$; $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$; or $-C_{1-6}\text{-alkyl-aryl}$;
more preferably $-H$, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2-OH$, $-CH_2CH_2CH_3$, $-CH_2\text{-cyclopropyl}$, or $-CH_2\text{-phenyl}$.

In preferred embodiments of the compounds according to substitution patterns (i) and (ii), **R8** represents $-C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, $-aryl$, $-C_{1-10}\text{-alkyl-aryl}$, $-C_{3-6}\text{-cycloalkyl-aryl}$, or $-C_{1-10}\text{-alkyl-O-aryl}$;

preferably $-phenyl$, optionally substituted with $-F$, $-Br$, $-Cl$, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2\text{-phenyl}$, optionally substituted with $-F$, $-Br$, $-Cl$, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2CH_2\text{-phenyl}$, optionally substituted with one or two substituents independently of one another selected from $-F$, $-Br$, $-Cl$, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH=CH\text{-phenyl}$, optionally substituted with $-F$, $-Br$, $-Cl$, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2\text{-O-phenyl}$, optionally substituted with $-F$, $-Br$, $-Cl$, $-CH_3$, $-CF_3$, $-OCH_3$; $-cyclopropyl\text{-phenyl}$, optionally substituted with $-F$, $-Br$, $-Cl$, $-CH_3$, $-CF_3$, $-OCH_3$; or $-C_{3-6}\text{-cycloalkyl}$;

more preferably $-phenyl$, optionally substituted with $-OCH_3$; $-CH_2\text{-phenyl}$, optionally substituted with $-Cl$; $-CH_2CH_2\text{-phenyl}$, optionally substituted with one or two substituents independently of one another

selected from -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH=CH-phenyl, optionally substituted with -OCH₃; -CH₂-O-phenyl; -cyclopropyl-phenyl; or -cyclopentyl.

In preferred embodiments of the compounds according to substitution pattern (i)

R1 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, or -C₁₋₆-alkyl-heteroaryl; or

R3 represents -C₁₋₆-alkyl-P(=O)(OH)₂;

R7 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, or -C₁₋₆-alkyl-heteroaryl; and

R8 represents -C₃₋₆-cycloalkyl-phenyl or -C₁₋₆-alkyl-phenyl (wherein in case of -C₂-alkyl-phenyl, the alkyl-moiety is saturated).

In preferred embodiments of the compounds according to substitution pattern (ii),

R1 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, -C₁₋₆-alkyl-heteroaryl, -C₃₋₆-cycloalkyl, -C₁₋₆-O-aryl, or -C₁₋₆-O-heteroaryl;

R3 represents -C₁₋₆-alkyl-P(=O)(OC₁₋₆-alkyl)₂; -C₁₋₆-alkyl-P(=O)(OH)(OC₁₋₆-alkyl); -C₁₋₆-alkyl-S(=O)₂(OH); -C₁₋₆-alkyl-S(=O)₂(NH₂); or -C₁₋₆-alkyl-C(=O)(OH);

R7 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, -C₁₋₆-alkyl-heteroaryl, -C₃₋₆-cycloalkyl, -C₁₋₆-O-aryl, or -C₁₋₆-O-heteroaryl; and

R8 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, -C₁₋₆-alkyl-heteroaryl, -C₃₋₆-cycloalkyl, -C₁₋₆-O-aryl, or -C₁₋₆-O-heteroaryl.

In more preferred embodiments of the compounds according to substitution pattern (i),

R1 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, or -C₁₋₆-alkyl-heteroaryl;

R3 represents -C₄-alkyl-P(=O)(OH)₂;

R7 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, or -C₁₋₆-alkyl-heteroaryl; and

R8 represents -C₃-cycloalkyl-aryl or -C₁₋₆-alkyl-phenyl (wherein in case of -C₂-alkyl-phenyl, the alkyl-moiety is saturated).

In more preferred embodiments of the compounds according to substitution pattern (ii),

R1 represents -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, -C₁₋₆-alkyl-heteroaryl, -C₃₋₆-cycloalkyl, -C₁₋₆-O-aryl, or -C₁₋₆-O-heteroaryl;

- R3** represents $-C_4\text{-alkyl-P(=O)(OC}_{1-6}\text{-alkyl)}_2$; $-C_4\text{-alkyl-P(=O)(OH)(OC}_{1-6}\text{-alkyl)}$; $-C_4\text{-alkyl-S(=O)}_2\text{(OH)}$; $-C_4\text{-alkyl-S(=O)}_2\text{(NH}_2\text{)}$; or $-C_4\text{-alkyl-C(=O)(OH)}$;
- R7** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, $-C_{1-6}\text{-alkyl-heteroaryl}$, $-C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-O-aryl}$, or $-C_{1-6}\text{-O-heteroaryl}$; and
- R8** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, $-C_{1-6}\text{-alkyl-heteroaryl}$, $-C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-O-aryl}$, or $-C_{1-6}\text{-O-heteroaryl}$.

In still more preferred embodiments of the compounds according to substitution pattern (i),

- R1** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, or $-C_{1-6}\text{-alkyl-heteroaryl}$;
- R3** represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-P(=O)(OH)}_2$;
- R7** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, or $-C_{1-6}\text{-alkyl-heteroaryl}$; and
- R8** represents $-\text{cyclopropyl-aryl}$, $-\text{CH}_2\text{-phenyl}$ or $-\text{CH}_2\text{CH}_2\text{-phenyl}$.

In still more preferred embodiments of the compounds according to substitution pattern (ii),

- R1** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, $-C_{1-6}\text{-alkyl-heteroaryl}$, $-C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-O-aryl}$, or $-C_{1-6}\text{-O-heteroaryl}$;
- R3** represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-P(=O)(OC}_{1-6}\text{-alkyl)}_2$; $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-P(=O)(OH)(OC}_{1-6}\text{-alkyl)}$; $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-S(=O)}_2\text{(OH)}$; $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-S(=O)}_2\text{(NH}_2\text{)}$; or $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C(=O)(OH)}$;
- R7** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, $-C_{1-6}\text{-alkyl-heteroaryl}$, $-C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-O-aryl}$, or $-C_{1-6}\text{-O-heteroaryl}$; and
- R8** represents $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, $-C_{1-6}\text{-alkyl-heteroaryl}$, $-C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-O-aryl}$, or $-C_{1-6}\text{-O-heteroaryl}$.

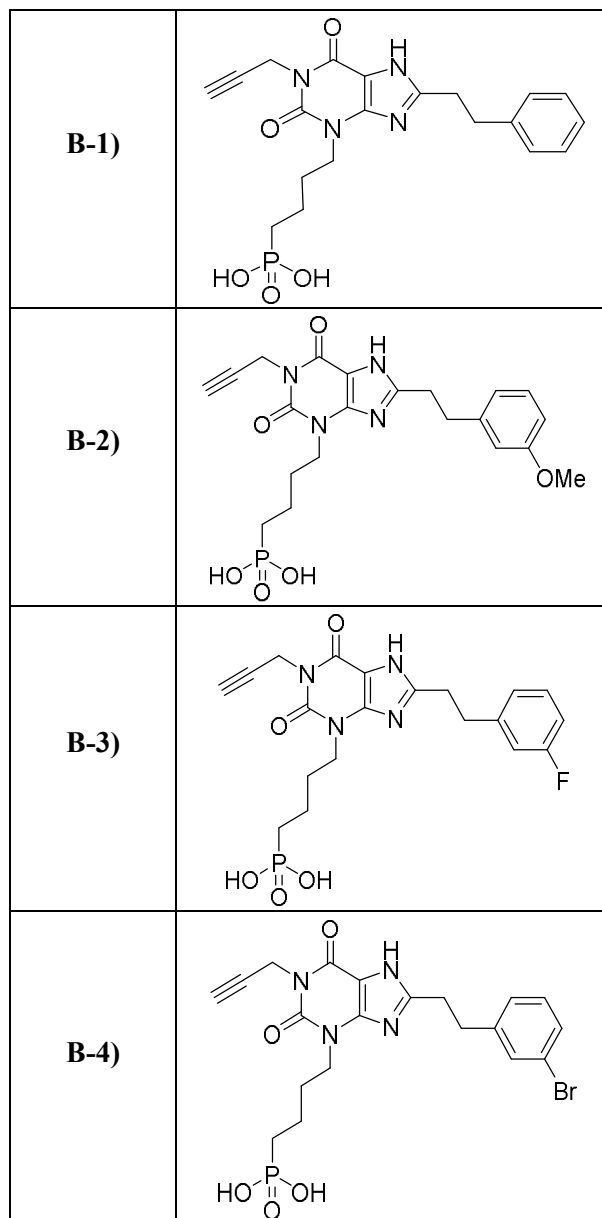
Within any of the above definitions of the preferred, more preferred and still more preferred embodiments of the compounds according to substitution patterns (i) and (ii), in each case " $C_{1-6}\text{-alkyl}$ " may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or mono-substituted with a substituent selected from $-\text{C}\equiv\text{CH}$, $-\text{C(=O)OH}$, $-\text{C(=O)O-C}_{1-10}\text{-alkyl}$, $-\text{OH}$, $-\text{O-C}_{1-10}\text{-alkyl}$, $-\text{NH}_2$, $-\text{NH-C}_{1-10}\text{-alkyl}$, $-\text{N(C}_{1-10}\text{-alkyl)}_2$, $-\text{N}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, and $-\text{I}$.

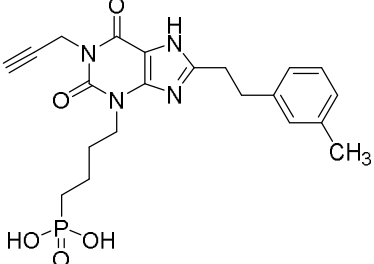
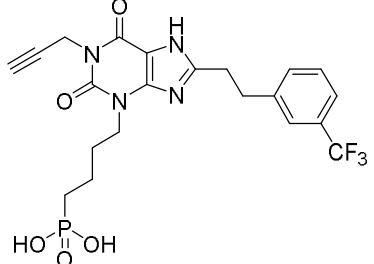
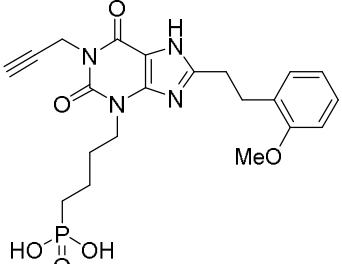
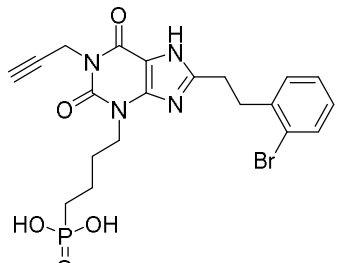
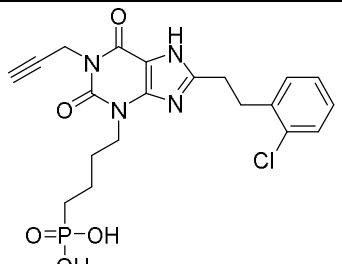
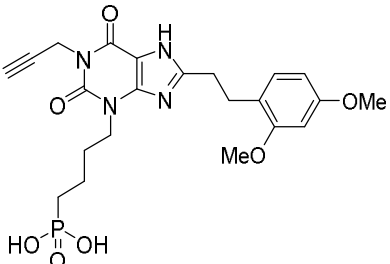
Within any of the above definitions of the preferred, more preferred and still more preferred embodiments of the compounds according to substitution patterns (i) and (ii), in each case " $C_{3-6}\text{-cycloalkyl}$ " may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or

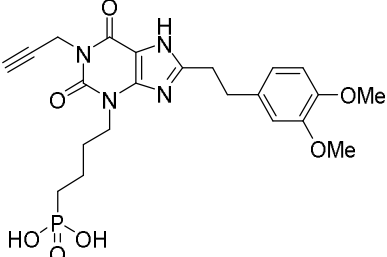
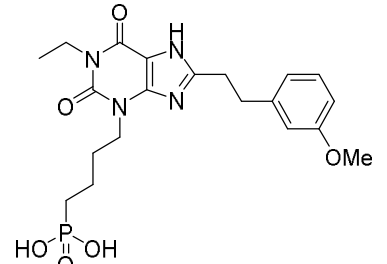
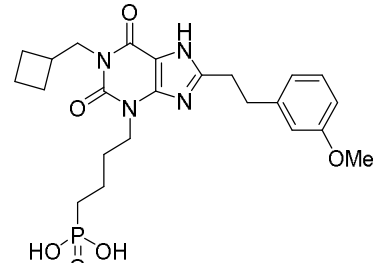
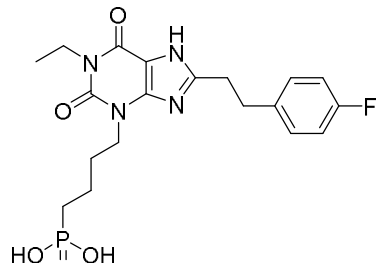
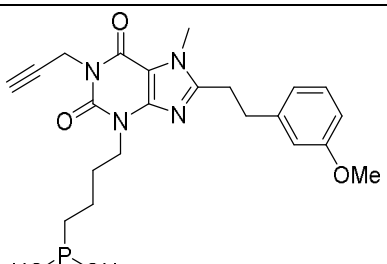
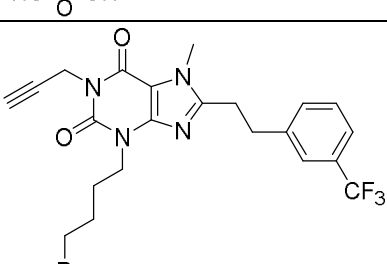
monosubstituted with a substituent selected from $-C\equiv CH$, $-C(=O)OH$, $-C(=O)O-C_{1-6}$ -alkyl, $-OH$, $-O-C_{1-10}$ -alkyl, $-NH_2$, $-NH-C_{1-10}$ -alkyl, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$.

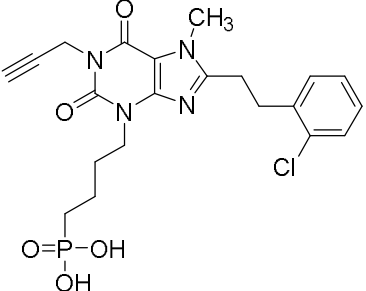
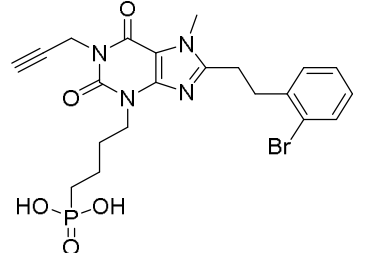
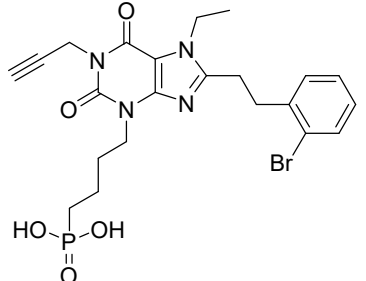
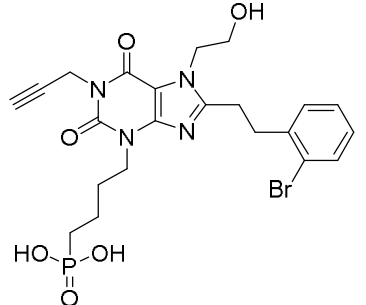
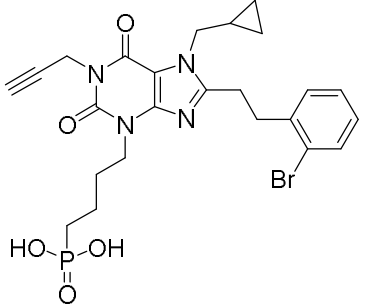
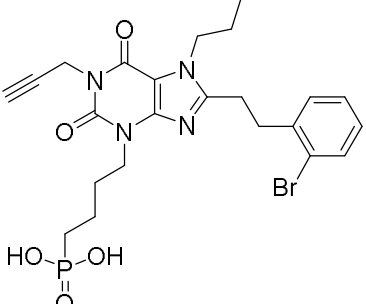
Within any of the above definitions of the preferred, more preferred and still more preferred embodiments of the compounds according to substitution patterns (i) and (ii), in each case "aryl" and "heteroaryl" are as defined above.

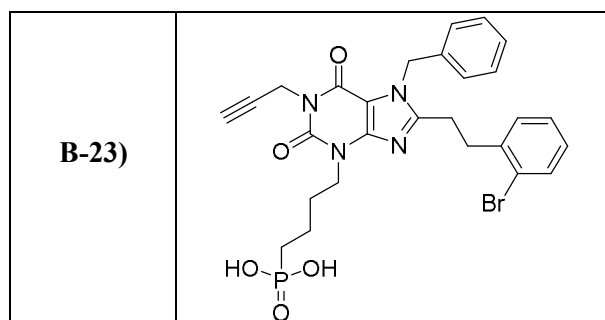
Particularly preferred compounds according to substitution pattern (i) are compounds B-1 to B-23:



B-5)	 <chem>C#CCN1C(=O)N(C2=NC(=NC(=N2)CCc3ccc(C)cc3)C(=O)N1CCCCP(=O)(O)O</chem>
B-6)	 <chem>C#CCN1C(=O)N(C2=NC(=NC(=N2)CCc3ccc(C(F)(F)F)cc3)C(=O)N1CCCCP(=O)(O)O</chem>
B-7)	 <chem>C#CCN1C(=O)N(C2=NC(=NC(=N2)CCc3cccc(OC)c3)C(=O)N1CCCCP(=O)(O)O</chem>
B-8)	 <chem>C#CCN1C(=O)N(C2=NC(=NC(=N2)CCc3cccc(Br)c3)C(=O)N1CCCCP(=O)(O)O</chem>
B-9)	 <chem>C#CCN1C(=O)N(C2=NC(=NC(=N2)CCc3ccccc3Cl)C(=O)N1CCCCP(=O)(O)O</chem>
B-10)	 <chem>C#CCN1C(=O)N(C2=NC(=NC(=N2)CCc3cc(OC)cc(OC)c3)C(=O)N1CCCCP(=O)(O)O</chem>

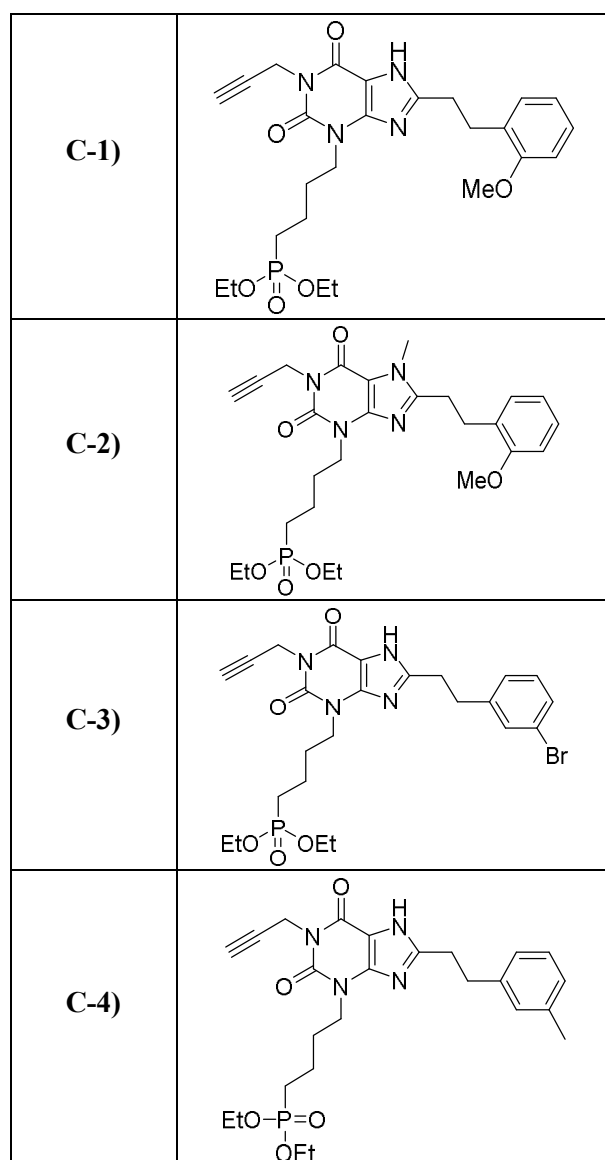
B-11)	 <chem>C#CCN1C(=O)N(C2=NC=NC=C2N1C(=O)N)CCCCOP(=O)(O)O</chem>
B-12)	 <chem>CCN1C(=O)N(C2=NC=NC=C2N1C(=O)N)CCCCOP(=O)(O)O</chem>
B-13)	 <chem>C1CCN1C(=O)N(C2=NC=NC=C2N1C(=O)N)CCCCOP(=O)(O)O</chem>
B-14)	 <chem>CCN1C(=O)N(C2=NC=NC=C2N1C(=O)N)CCCCOP(=O)(O)O</chem>
B-15)	 <chem>C#CCN1C(=O)N(C2=NC=NC=C2N1C(=O)N)CCCCOP(=O)(O)O</chem>
B-16)	 <chem>C#CCN1C(=O)N(C2=NC=NC=C2N1C(=O)N)CCCCOP(=O)(O)O</chem>

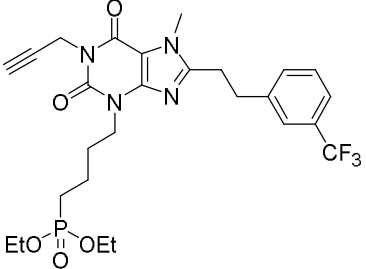
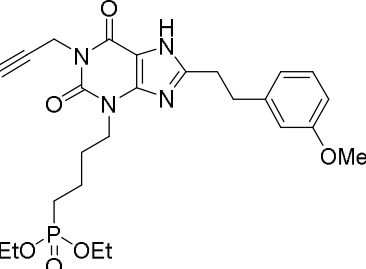
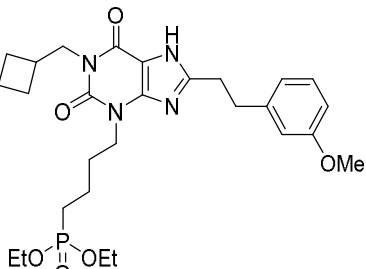
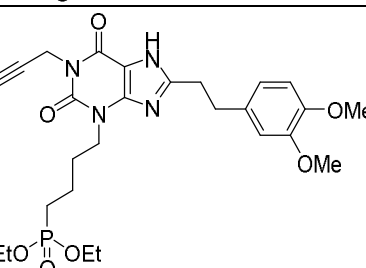
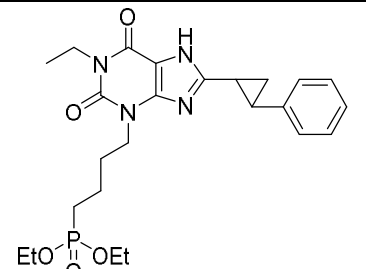
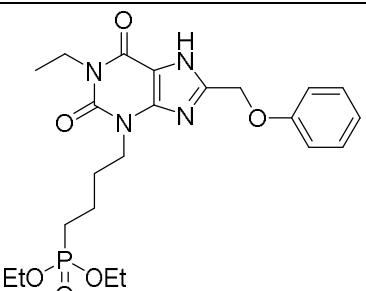
B-17)	 <chem>CN1C=NC2C(=O)N(C#CC)C(=O)N2C1CCCCOP(=O)(O)O</chem>
B-18)	 <chem>CN1C=NC2C(=O)N(C#CC)C(=O)N2C1CCCCOP(=O)(O)O</chem>
B-19)	 <chem>CCN1C=NC2C(=O)N(C#CC)C(=O)N2C1CCCCOP(=O)(O)O</chem>
B-20)	 <chem>OCN1C=NC2C(=O)N(C#CC)C(=O)N2C1CCCCOP(=O)(O)O</chem>
B-21)	 <chem>C1CC1CN1C=NC2C(=O)N(C#CC)C(=O)N2C1CCCCOP(=O)(O)O</chem>
B-22)	 <chem>CCCN1C=NC2C(=O)N(C#CC)C(=O)N2C1CCCCOP(=O)(O)O</chem>



and the physiologically acceptable salts thereof.

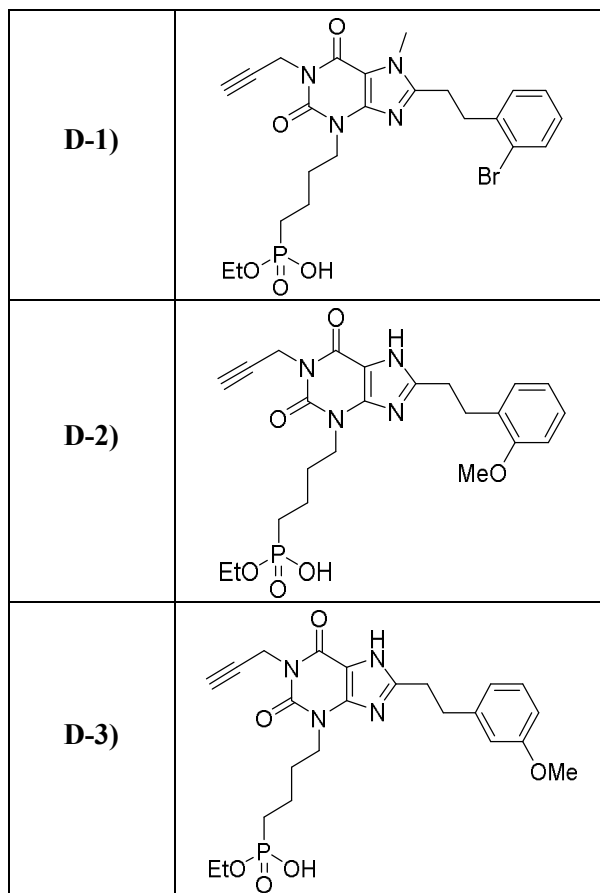
Particularly preferred compounds according to substitution pattern (ii) where **R3** represents $-C_{1-10}$ -alkyl- $P(=O)(OC_{1-10}\text{-alkyl})_2$ are compounds C-1 to C-10:



C-5)	
C-6)	
C-7)	
C-8)	
C-9)	
C-10)	

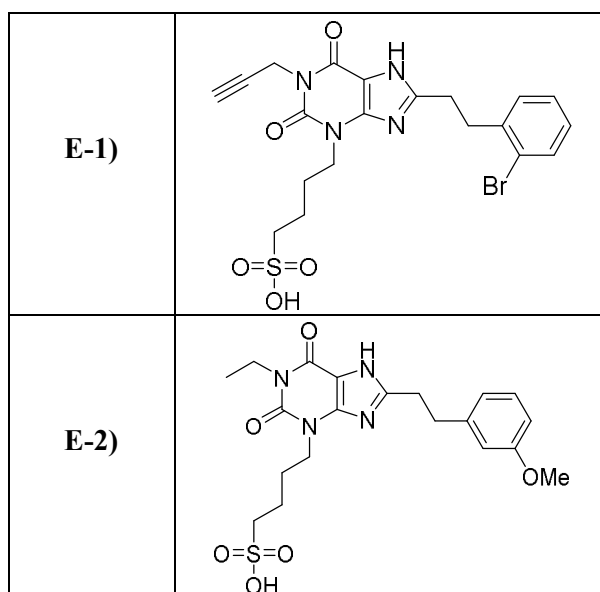
and the physiologically acceptable salts thereof.

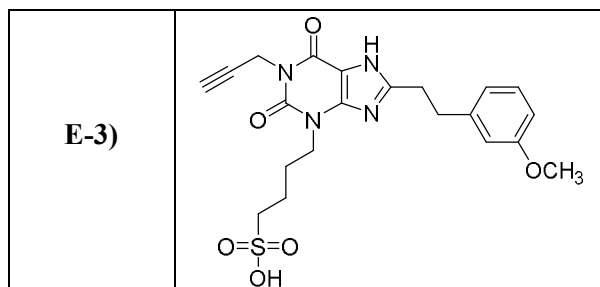
Particularly preferred compounds according to substitution pattern (ii) where **R3** represents $-C_{1-10}$ -alkyl- $P(=O)(OH)(OC_{1-10}\text{-alkyl})$ are compounds D-1 to D-3:



and the physiologically acceptable salts thereof.

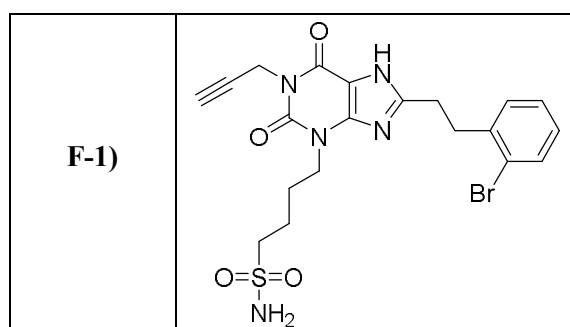
Particularly preferred compounds according to substitution pattern (ii) where **R3** represents $-C_{1-10}$ -alkyl- $S(=O)_2(OH)$ are compounds E-1 to E-3:





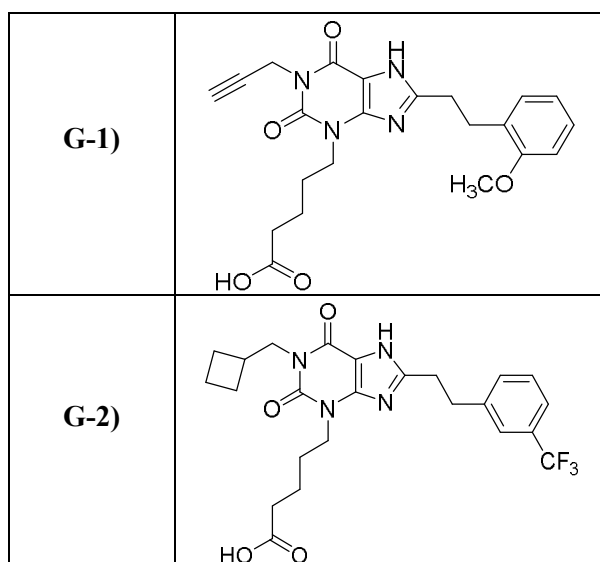
and the physiologically acceptable salts thereof.

A particularly preferred compound according to substitution pattern (ii) where **R3** represents $-C_{1-10}$ -alkyl-S(=O)₂(NH₂) is compound F-1:



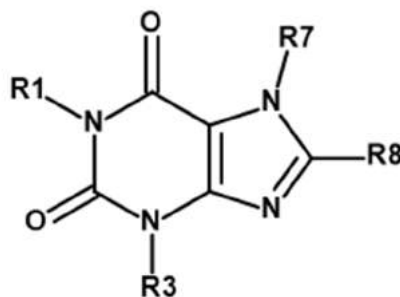
and the physiologically acceptable salts thereof.

Particularly preferred compounds according to substitution pattern (ii) where **R3** represents $-C_{1-10}$ -alkyl-C(=O)(OH) are compounds G-1 and G-2:



and the physiologically acceptable salts thereof.

Another aspect of the invention relates to a compound of general formula 1



wherein

R1 represents $-C_{1-10}$ -alkyl, optionally substituted with $-C\equiv CH$; or $-C_{1-10}$ -alkyl- C_{3-10} -cycloalkyl; preferably $-C_{1-6}$ -alkyl, optionally substituted with $-C\equiv CH$; or $-C_{1-6}$ -alkyl- C_{3-6} -cycloalkyl; more preferably $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH_2C\equiv CH$, or $-CH_2$ -cyclobutyl;

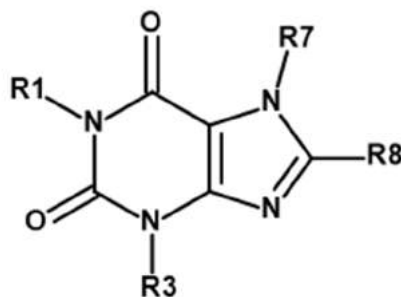
R3 represents $-C_{3-5}$ -alkyl-OH, $-C_{3-5}$ -alkyl-O-C(=O) C_{1-6} -alkyl, $-C_{3-5}$ -alkyl-P(=O)(OH)₂; $-C_{3-5}$ -alkyl-P(=O)(OC C_{1-6} -alkyl)₂; $-C_{3-5}$ -alkyl-P(=O)(OH)(OC C_{1-6} -alkyl); $-C_{3-5}$ -alkyl-S(=O)₂(OH); $-C_{3-5}$ -alkyl-S(=O)₂(NH₂); or $-C_{3-5}$ -alkyl-C(=O)(OH); preferably $-C_{3-5}$ -alkyl-P(=O)(OH)₂; more preferably $-C_4$ -alkyl-P(=O)(OH)₂;

R7 represents -H; $-C_{1-10}$ -alkyl, optionally substituted with -OH; $-C_{1-10}$ -alkyl- C_{3-10} -cycloalkyl; or $-C_{1-10}$ -alkyl-aryl; preferably -H; $-C_{1-6}$ -alkyl, optionally substituted with -OH; $-C_{1-6}$ -alkyl- C_{3-6} -cycloalkyl; or $-C_{1-6}$ -alkyl-aryl; more preferably -H, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2$ -OH, $-CH_2CH_2CH_3$, $-CH_2$ -cyclopropyl, or $-CH_2$ -phenyl;

R8 represents $-C_{3-10}$ -cycloalkyl, $-C_{1-10}$ -alkyl- C_{3-10} -cycloalkyl, -aryl, $-C_{1-10}$ -alkyl-aryl, $-C_{3-6}$ -cycloalkyl-aryl, or $-C_{1-10}$ -alkyl-O-aryl; preferably -phenyl, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2$ -phenyl, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2CH_2$ -phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH=CH$ -phenyl, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2$ -O-phenyl, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; cyclopropyl-phenyl, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; or $-C_{3-6}$ -cycloalkyl; more preferably -phenyl, optionally substituted with $-OCH_3$; $-CH_2$ -phenyl, optionally substituted with -Cl; $-CH_2CH_2$ -phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH=CH$ -phenyl, optionally substituted with $-OCH_3$; $-CH_2$ -O-phenyl; cyclopropyl-phenyl; or -cyclopentyl;

and the physiologically acceptable salts thereof.

Another aspect of the invention relates to a compound of general formula 1



wherein

R1 represents $-C_{1-10}$ -alkyl, optionally substituted with $-C\equiv CH$; or $-C_{1-10}$ -alkyl- C_{3-10} -cycloalkyl; preferably $-C_{1-6}$ -alkyl, optionally substituted with $-C\equiv CH$; or $-C_{1-6}$ -alkyl- C_{3-6} -cycloalkyl; more preferably $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH_2C\equiv CH$, or $-CH_2$ -cyclobutyl;

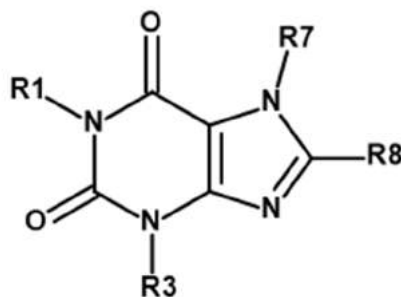
R3 represents $-C_{3-5}$ -alkyl-OH, $-C_{3-5}$ -alkyl-O-C(=O) C_{1-6} -alkyl, $-C_{3-5}$ -alkyl-P(=O)(OH) $_2$; $-C_{3-5}$ -alkyl-P(=O)(OC $_{1-6}$ -alkyl) $_2$; $-C_{3-5}$ -alkyl-P(=O)(OH)(OC $_{1-6}$ -alkyl); $-C_{3-5}$ -alkyl-S(=O) $_2$ (OH); $-C_{3-5}$ -alkyl-S(=O) $_2$ (NH $_2$); or $-C_{3-5}$ -alkyl-C(=O)(OH); preferably $-C_{3-5}$ -alkyl-P(=O)(OH) $_2$; more preferably $-C_4$ -alkyl-P(=O)(OH) $_2$.

R7 represents $-C_{2-10}$ -alkyl, optionally substituted with -OH; $-C_{2-10}$ -alkyl- C_{3-10} -cycloalkyl; or $-C_{2-10}$ -alkyl-aryl; preferably $-C_{2-6}$ -alkyl, optionally substituted with -OH; $-C_{2-6}$ -alkyl- C_{3-6} -cycloalkyl; or $-C_{2-6}$ -alkyl-aryl; more preferably $-CH_2CH_3$, $-CH_2CH_2$ -OH, or $-CH_2CH_2CH_3$;

R8 represents $-C_{3-10}$ -cycloalkyl, $-C_{1-10}$ -alkyl- C_{3-10} -cycloalkyl, -aryl, $-C_{1-10}$ -alkyl-aryl, $-C_{3-6}$ -cycloalkyl-aryl, or $-C_{1-10}$ -alkyl-O-aryl; preferably -phenyl, optionally substituted with -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; $-CH_2$ -phenyl, optionally substituted with -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; $-CH_2CH_2$ -phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; $-CH=CH$ -phenyl, optionally substituted with -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; $-CH_2$ -O-phenyl, optionally substituted with -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; -cyclopropyl-phenyl, optionally substituted with -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; or $-C_{3-6}$ -cycloalkyl; more preferably -phenyl, optionally substituted with -OCH $_3$; $-CH_2$ -phenyl, optionally substituted with -Cl; $-CH_2CH_2$ -phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, -CH $_3$, -CF $_3$, -OCH $_3$; $-CH=CH$ -phenyl, optionally substituted with -OCH $_3$; $-CH_2$ -O-phenyl; -cyclopropyl-phenyl; or -cyclopentyl;

and the physiologically acceptable salts thereof.

Another aspect of the invention relates to a compound of general formula 1



wherein

R1 represents $-\text{CH}_2\text{C}\equiv\text{CH}$;

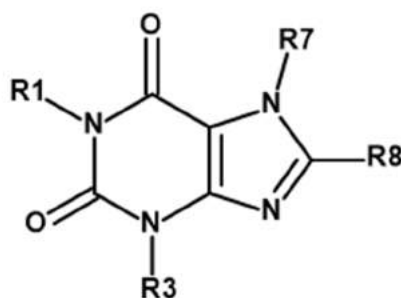
R3 represents $-\text{C}_{3-5}\text{-alkyl-OH}$, $-\text{C}_{3-5}\text{-alkyl-O-C(=O)C}_{1-6}\text{-alkyl}$, $-\text{C}_{3-5}\text{-alkyl-P(=O)(OH)}_2$; $-\text{C}_{3-5}\text{-alkyl-P(=O)(OC}_{1-6}\text{-alkyl)}_2$; $-\text{C}_{3-5}\text{-alkyl-P(=O)(OH)(OC}_{1-6}\text{-alkyl)}$; $-\text{C}_{3-5}\text{-alkyl-S(=O)}_2\text{(OH)}$; $-\text{C}_{3-5}\text{-alkyl-S(=O)}_2\text{(NH}_2\text{)}$; or $-\text{C}_{3-5}\text{-alkyl-C(=O)(OH)}$; preferably $-\text{C}_{3-5}\text{-alkyl-P(=O)(OH)}_2$; more preferably $-\text{C}_4\text{-alkyl-P(=O)(OH)}_2$;

R7 represents $-\text{H}$; $-\text{C}_{1-10}\text{-alkyl}$, optionally substituted with $-\text{OH}$; $-\text{C}_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$; or $-\text{C}_{1-10}\text{-alkyl-aryl}$; preferably $-\text{H}$; $-\text{C}_{1-6}\text{-alkyl}$, optionally substituted with $-\text{OH}$; $-\text{C}_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$; or $-\text{C}_{1-6}\text{-alkyl-aryl}$; more preferably $-\text{H}$, $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{-OH}$, $-\text{CH}_2\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{-cyclopropyl}$, or $-\text{CH}_2\text{-phenyl}$;

R8 represents $-\text{C}_{2-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, $-\text{C}_{2-10}\text{-alkyl-aryl}$, $-\text{C}_{3-6}\text{-cycloalkyl-aryl}$, or $-\text{C}_{1-10}\text{-alkyl-O-aryl}$; preferably $-\text{CH}_2\text{CH}_2\text{-phenyl}$, optionally substituted with one or two substituents independently of one another selected from $-\text{F}$, $-\text{Br}$, $-\text{Cl}$, $-\text{CH}_3$, $-\text{CF}_3$, $-\text{OCH}_3$; $-\text{CH}_2\text{-O-phenyl}$, optionally substituted with $-\text{F}$, $-\text{Br}$, $-\text{Cl}$, $-\text{CH}_3$, $-\text{CF}_3$, $-\text{OCH}_3$; or $-\text{cyclopropyl-phenyl}$, optionally substituted with $-\text{F}$, $-\text{Br}$, $-\text{Cl}$, $-\text{CH}_3$, $-\text{CF}_3$, $-\text{OCH}_3$; more preferably $-\text{CH}_2\text{CH}_2\text{-phenyl}$, optionally substituted with one or two substituents independently of one another selected from $-\text{F}$, $-\text{Br}$, $-\text{Cl}$, $-\text{CH}_3$, $-\text{CF}_3$, $-\text{OCH}_3$; $-\text{CH}_2\text{-O-phenyl}$; or $-\text{cyclopropyl-phenyl}$;

and the physiologically acceptable salts thereof.

Another aspect of the invention relates to a compound of general formula 1



wherein

R1 represents $-\text{C}\equiv\text{CH}$;

R3 represents -C₃₋₅-alkyl-OH, -C₃₋₅-alkyl-O-C(=O)C₁₋₆-alkyl, -C₃₋₅-alkyl-P(=O)(OH)₂; -C₃₋₅-alkyl-P(=O)(OC₁₋₆-alkyl)₂; -C₃₋₅-alkyl-P(=O)(OH)(OC₁₋₆-alkyl); -C₃₋₅-alkyl-S(=O)₂(OH); -C₃₋₅-alkyl-S(=O)₂(NH₂); or -C₃₋₅-alkyl-C(=O)(OH); preferably -C₃₋₅-alkyl-P(=O)(OH)₂; more preferably -C₄-alkyl-P(=O)(OH)₂.

R7 represents -C₂₋₁₀-alkyl, optionally substituted with -OH; -C₂₋₁₀-alkyl-C₃₋₁₀-cycloalkyl; or -C₂₋₁₀-alkyl-aryl; preferably -C₂₋₆-alkyl, optionally substituted with -OH; -C₂₋₆-alkyl-C₃₋₆-cycloalkyl; or -C₂₋₆-alkyl-aryl; more preferably -CH₂CH₃, -CH₂CH₂-OH, or -CH₂CH₂CH₃;

R8 represents -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -aryl, -C₁₋₁₀-alkyl-aryl, -C₃₋₆-cycloalkyl-aryl, or -C₁₋₁₀-alkyl-O-aryl; preferably -phenyl, optionally substituted with -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH₂-phenyl, optionally substituted with -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH₂CH₂-phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH=CH-phenyl, optionally substituted with -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH₂-O-phenyl, optionally substituted with -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -cyclopropyl-phenyl, optionally substituted with -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; or -C₃₋₆-cycloalkyl; more preferably -phenyl, optionally substituted with -OCH₃; -CH₂-phenyl, optionally substituted with -Cl; -CH₂CH₂-phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH=CH-phenyl, optionally substituted with -OCH₃; -CH₂-O-phenyl; -cyclopropyl-phenyl; or -cyclopentyl;

and the physiologically acceptable salts thereof.

As used herein, the terms "physiologically acceptable salt" refer to those salts which retain the biological effectiveness and properties of the compound according to general formula 1. Such salts include, but are not restricted to: (1) an acid addition salt which is obtained by reaction of the free base of the compound according to general formula 1 with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perchloric acid and the like, or with organic acids such as acetic acid, oxalic acid, (D)- or (L)- malic acid, maleic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, tartaric acid, citric acid, succinic acid or malonic acid and the like, preferably hydrochloric acid or (L)-malic acid; or (2) salts formed when an acidic proton present in the compound according to general formula 1 either is replaced by a metal ion, e. g., an alkali metal ion, such as sodium or potassium, an alkaline earth ion, such as magnesium or calcium, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

The compound of general Formula 1 may also act as a prodrug. A "prodrug" preferably refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by

oral administration whereas the parent drug is not, or they may penetrate into the central nervous system (CNS) while the parent drug does not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug or otherwise increased lipophilicity to show better solubility in dermal ointments, to penetrate better into the skin, or to permeate into the CNS. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed e.g. to the carboxylic acid or phosphonic acid, the active entity, once inside the cell where water solubility is beneficial. A prodrug may be converted into the parent drug by various mechanisms, including enzymatic processes and metabolic hydrolysis.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2-10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule. The prodrugs of compounds of general formula 1 are within the scope of this invention.

Additionally, it is contemplated that compounds of general formula 1 would be metabolized by enzymes in the body of the organism such as a human being to generate a metabolite that can modulate the activity of the MRGPRX4 receptor. Such metabolites are within the scope of the present invention.

Another aspect of the invention relates to a pharmaceutical composition comprising any of the compounds or salts of the present invention and, optionally, a pharmaceutically acceptable carrier or excipient. This composition may additionally comprise further compounds or medicaments, such as, for example, neuroprotective or antinociceptive, anti-inflammatory or antibiotic agents besides the compounds according to general formula 1.

"Pharmaceutical composition" preferably refers to a mixture of one or more of the compounds described herein, or physiologically/pharmaceutically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically/ pharmaceutically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

As used herein, a "physiologically/pharmaceutically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

A "pharmaceutically acceptable excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients

include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Physiologically or pharmaceutically acceptable carriers and excipients are known to the skilled person. In this regard it can be referred to, e.g., H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende technische Gebiete*, Editio Cantor Aulendorf, 2001.

The pharmaceutical composition according to the invention can be, e.g., solid, liquid or pasty.

A further aspect of the invention relates to a pharmaceutical dosage form comprising the pharmaceutical composition according to the invention.

The pharmaceutical dosage form according to the invention may be adapted for various routes of administration (e.g. systemic, parenteral, subcutaneous, topic, local), such as oral administration, infusion, injection and the like. The pharmaceutical dosage form is preferably adapted for oral, local or subcutaneous administration, or combinations thereof.

Pharmaceutical dosage forms that are adapted for oral administration include tablets, pellets, capsules, powders, granules and the like.

The pharmaceutical dosage form is preferably adapted for administration once daily, twice daily or thrice daily. The pharmaceutical dosage form may release the compound according to general formula 1 immediately (immediate release formulation) or over an extended period of time (retarded release, delayed release, prolonged release, sustained release, and the like).

Another aspect of the invention relates to the compounds according to the invention as described above for use as a medicament.

Another aspect of the invention relates to the compounds according to the invention as described above for use in the prevention or treatment of a condition, disease or disorder that is associated with the MRGPRX4 receptor, preferably that can be prevented or treated by modulating, preferably agonizing the MRGPRX4 receptor, preferably the human wildtype MRGPRX4.

Another aspect of the invention relates to the use of the compounds according to the invention as described above for the manufacture of a medicament for the prevention or treatment of a condition, disease or disorder that is associated with the MRGPRX4 receptor, preferably that can be prevented or

treated by modulating, preferably agonizing the MRGPRX4 receptor, preferably the human wildtype MRGPRX4.

Another aspect of the invention relates to a method for preventing or treating a condition, disease or disorder that is associated with the MRGPRX4, preferably that can be prevented or treated by modulating, preferably agonizing the MRGPRX4, preferably the human wildtype MRGPRX4, the method comprising the step of administering an effective amount of a compound according to the invention as described above to a subject in need thereof.

Preferably, the disease or disorder that is associated with the MRGPRX4 receptor is selected from

- open wounds, e.g. incisions or incised wounds, lacerations, abrasions (grazes), avulsions, puncture wounds, penetration wounds and gunshot wounds; and
- closed wounds, e.g. hematomas and crash injuries; and
- painful states, itching, neuropathic pain, chronic pain.

Preferably, the disease or disorder that is associated with the MRGPRX4 receptor is associated with the wildtype of the MRGPRX4 receptor (not its mutant L83S).

Preferably, the compound, the pharmaceutical composition or the pharmaceutical dosage form is administered topically and/or locally.

The compounds according to the invention show agonistic or antagonistic effects at the MRGPRX4 receptor.

In another aspect, the invention relates to the use of the compounds according to general formula 1 for activating or blocking MRGPRX4 function. In a further aspect, the compounds according to general formula 1 may thus also be used for the prevention, alleviation and/or treatment of a condition, disease or disorder related to MRGPRX4 receptor activity.

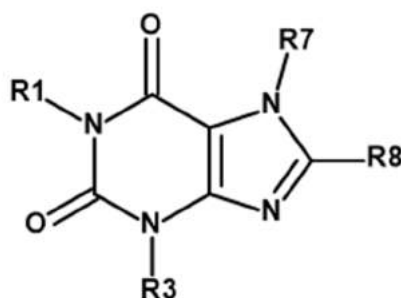
"Treat", "treating" and "treatment" preferably refer to a method of alleviating or abrogating an MRGPRX4 receptor related disease or disorder and/or its attendant symptoms. "Prevent", "preventing" and "prevention" preferably refer to a method of hindering an MRGPRX4 receptor related disease or disorder from occurring, i.e. a prophylactic method.

The compounds according to the invention are useful for treating various organisms. "Organism" preferably refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukaryotic cell or as complex as a mammal, including a human being.

The compounds according to the invention are used in therapeutically effective amounts. "Therapeutically effective amount" preferably refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated.

Preferably, the subject afflicted by a disease treated, alleviated or prevented according to the invented use is a human.

Another aspect of the invention relates to a compound according to general Formula 1



wherein

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

R3 represents -C₁₋₁₀-alkyl-P(=O)(OC₁₋₁₀-alkyl)₂, -C₁₋₁₀-alkyl-P(=O)(OH)(OC₁₋₁₀-alkyl), -C₁₋₁₀-alkyl-S(=O)₂(OH), -C₁₋₁₀-alkyl-S(=O)₂(NH₂), -C₁₋₁₀-alkyl-C(=O)(OH), or -C₁₋₁₀-alkyl-P(=O)(OH)₂;

R7 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl; and

R8 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-O-aryl, -C₃₋₆-cycloalkyl-aryl, -C₃₋₆-cycloalkyl-heteroaryl, -C₁₋₁₀-alkyl-heteroaryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

wherein in each case "C₁₋₁₀-alkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "C₃₋₁₀-cycloalkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "aryl" is a 6-14-membered aryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}\text{-alkyl}$, $-OH$, $-O-C_{1-10}\text{-alkyl}$, $-NH_2$, $-NH-C_{1-10}\text{-alkyl}$, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$;

wherein in each case "heteroaryl" is a 5-14-membered heteroaryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}\text{-alkyl}$, $-OH$, $-O-C_{1-10}\text{-alkyl}$, $-NH_2$, $-NH-C_{1-10}\text{-alkyl}$, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$;

or a physiologically acceptable salt thereof,

for use in the prevention or treatment of a condition, disease or disorder that is associated with the MRGPRX4 receptor, preferably associated with the wildtype of the MRGPRX4 receptor.

The above compounds for use according to the invention may again reflect two substitution patterns, for the purpose of the specification indicated as (iii) and (iv), respectively.

According to substitution pattern (iii) of the compounds for use according to the invention,

R1 represents $-H$, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-}C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, or $-C_{1-10}\text{-alkyl-heteroaryl}$;

R3 represents $-C_{1-10}\text{-alkyl-P(=O)(OH)}_2$;

R7 represents $-H$, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-}C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, or $-C_{1-10}\text{-alkyl-heteroaryl}$; and

R8 represents $-C_{3-6}\text{-cycloalkyl}$, $-aryl$, $-heteroaryl$, $-C_{1-10}\text{-alkyl-aryl}$, or $-C_{1-10}\text{-alkyl-heteroaryl}$ (wherein in case of $-C_2\text{-alkyl-aryl}$, the alkyl-moiety is unsaturated);

According to substitution pattern (iv) of the compounds for use according to the invention,

R1 represents $-H$, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-}C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, $-C_{1-10}\text{-alkyl-heteroaryl}$, $-C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-O-aryl}$, or $-C_{1-10}\text{-alkyl-O-heteroaryl}$;

R3 represents $-C_{10}\text{-alkyl-OH}$; or $-C_{1-10}\text{-alkyl-O-C(=O)C}_{1-10}\text{-alkyl}$;

R7 represents $-H$, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-}C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, $-C_{1-10}\text{-alkyl-heteroaryl}$, $-C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-O-aryl}$, or $-C_{1-10}\text{-alkyl-O-heteroaryl}$; and

R8 represents $-H$, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-}C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, $-C_{1-10}\text{-alkyl-heteroaryl}$, $-C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-O-aryl}$, or $-C_{1-10}\text{-alkyl-O-heteroaryl}$.

Within the above definitions of the compounds for use according to substitution patterns (iii) and (iv), in each case "C₁₋₁₀-alkyl", "C₃₋₁₀-cycloalkyl", "aryl", and "heteroaryl" independently have the same meaning as defined above.

In preferred embodiments of the compounds for use according to substitution pattern (iii),

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl;

R3 represents -C₁₋₆-alkyl-P(=O)(OH)₂;

R7 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl; and

R8 represents -C₃₋₆-cycloalkyl, -aryl, -heteroaryl, -C₁₋₆-alkyl-aryl, or -C₁₋₆-alkyl-heteroaryl (wherein in case of -C₂-alkyl-aryl, the alkyl-moiety is unsaturated).

In preferred embodiments of the compounds for use according to substitution pattern (iv),

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

R3 represents -C₁₋₆-alkyl-OH; or -C₁₋₆-alkyl-O-C(=O)C₁₋₆-alkyl;

R7 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl; and

R8 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl.

In more preferred embodiments of the compounds for use according to substitution pattern (iii),

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl;

R3 represents -CH₂CH₂CH₂CH₂-P(=O)(OH)₂;

R7 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl; and

R8 represents -cyclopentyl, -phenyl, or -CH=CH-phenyl.

In more preferred embodiments of the compounds for use according to substitution pattern (iv),

R1 represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

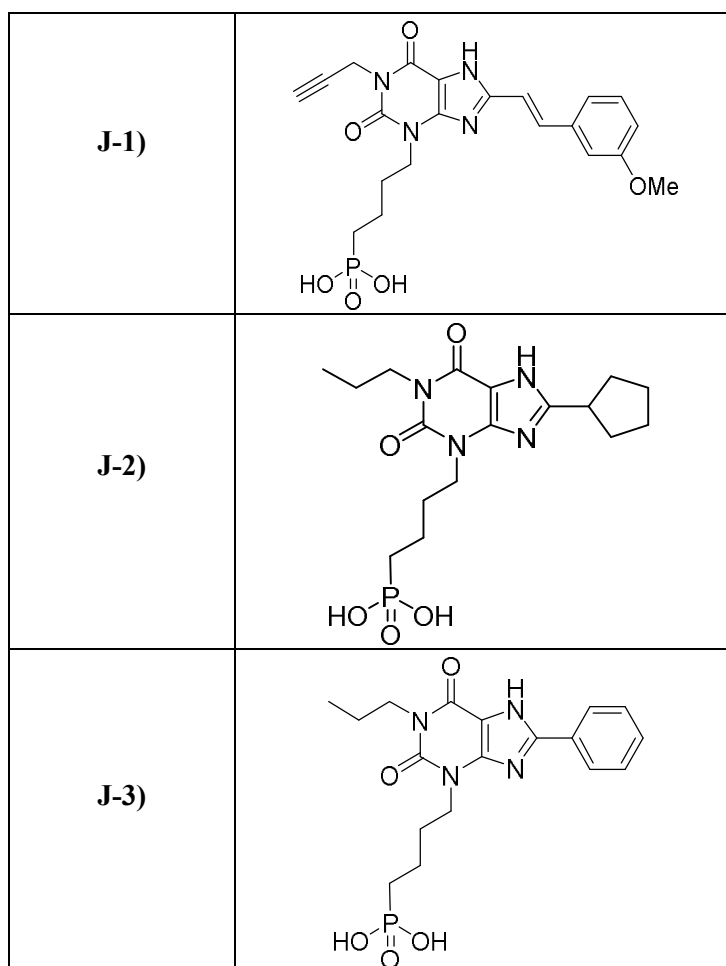
R3 represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{-OH}$; or $-\text{CH}_2\text{CH}_2\text{CH}_2\text{-O-C(=O)C}_{1-6}\text{-alkyl}$;

R7 represents $-\text{H}$, $-\text{C}_{1-10}\text{-alkyl}$, $-\text{C}_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, $-\text{C}_{1-10}\text{-alkyl-aryl}$, $-\text{C}_{1-10}\text{-alkyl-heteroaryl}$, $-\text{C}_{3-10}\text{-cycloalkyl}$, $-\text{C}_{1-10}\text{-alkyl-O-aryl}$, or $-\text{C}_{1-10}\text{-alkyl-O-heteroaryl}$; and

R8 represents $-\text{H}$, $-\text{C}_{1-10}\text{-alkyl}$, $-\text{C}_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, $-\text{C}_{1-10}\text{-alkyl-aryl}$, $-\text{C}_{1-10}\text{-alkyl-heteroaryl}$, $-\text{C}_{3-10}\text{-cycloalkyl}$, $-\text{C}_{1-10}\text{-alkyl-O-aryl}$, or $-\text{C}_{1-10}\text{-alkyl-O-heteroaryl}$.

Within any of the above definitions of the preferred and more preferred embodiments of the compounds for use according to substitution patterns (iii) and (iv), in each case " $\text{C}_{1-6}\text{-alkyl}$ ", " $\text{C}_{3-6}\text{-cycloalkyl}$ ", " aryl ", and " heteroaryl " have the same meaning as defined above.

Particularly preferred compounds for use according to substitution pattern (iii) are compounds J-1 to J-3:



and the physiologically acceptable salts thereof.

Preferably, the disease or disorder that is associated with the MRGPRX4 receptor is selected from

- open wounds, e.g. incisions or incised wounds, lacerations, abrasions (grazes), avulsions, puncture wounds, penetration wounds and gunshot wounds; and
- closed wounds, e.g. hematomas and crash injuries; and
- painful states, itching, neuropathic pain, chronic pain.

Preferably, the disease or disorder that is associated with the MRGPRX4 receptor is associated with the wildtype of the MRGPRX4 receptor (not its mutant L83S).

Preferably, the compound is administered topically and/or locally.

The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the term "includes" shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

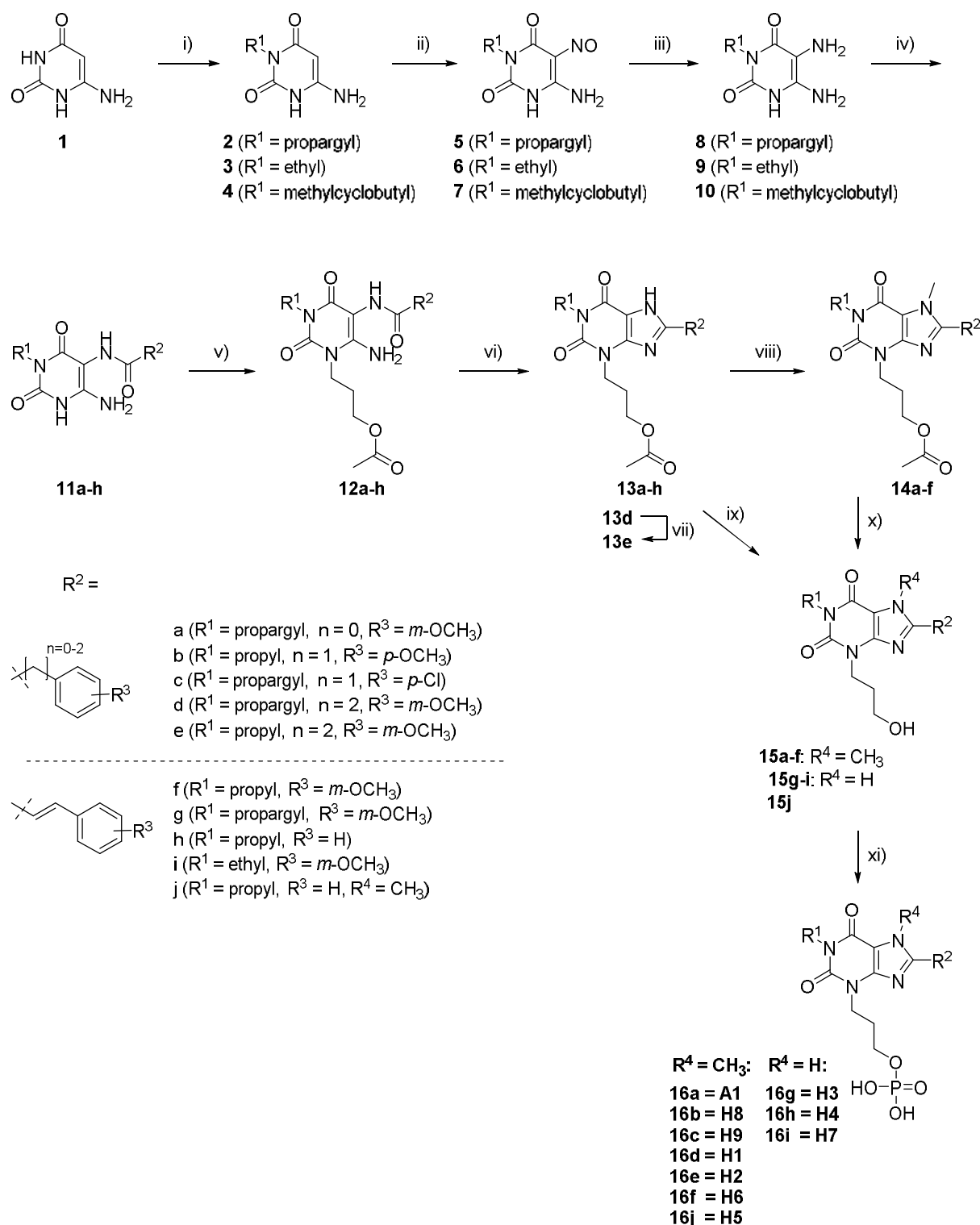
The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

The present inventions will be explained in more detail in the following examples. However, the examples are only used for illustration and do not limit the scope of the present invention.

Experimental Part

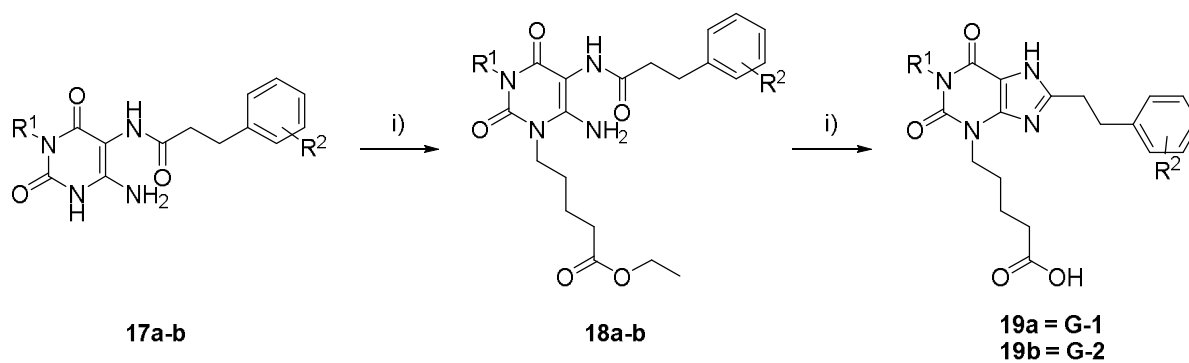
Synthesis of Reference Compounds A-1 and H-1 – H-9):



Reagents and conditions: (i) 2.1 equiv of hexamethyldisilazane (HMDS), reflux, 60–70 °C, I_2 , alkyl halogenide, 120 °C, 2 h, saturated aq. NaHCO_3 solution, 70-75%; (ii) aq. AcOH , HNO_2 , 60-65 °C, 68-75%; (iii) aq NH_3 (15% or 25%), $\text{Na}_2\text{S}_2\text{O}_3$, 70 °C, 50-70 %; (iv) $R^2\text{-COOH}$, $\text{EDC}\cdot\text{HCl}$, methanol, 24 h,

rt, 70-85%; (v) 3-iodopropyl acetate, K_2CO_3 , DMF, rt, 24 h, 60-70%; (vi) P_2O_5 , DMF, 100 °C, 10-30 min, 70-88%; (vii) Pd/C (10%), $H_{2(g)}$, methanol, 5 h, 95%; (viii) CH_3I , K_2CO_3 , DMF, rt, 24 h, 72-90%; (ix) KOH/methanol/ H_2O , 76-100% ; (x) $PO(OMe)_3/POCl_3$, rt, 30 min, H_2O , 41-80%.

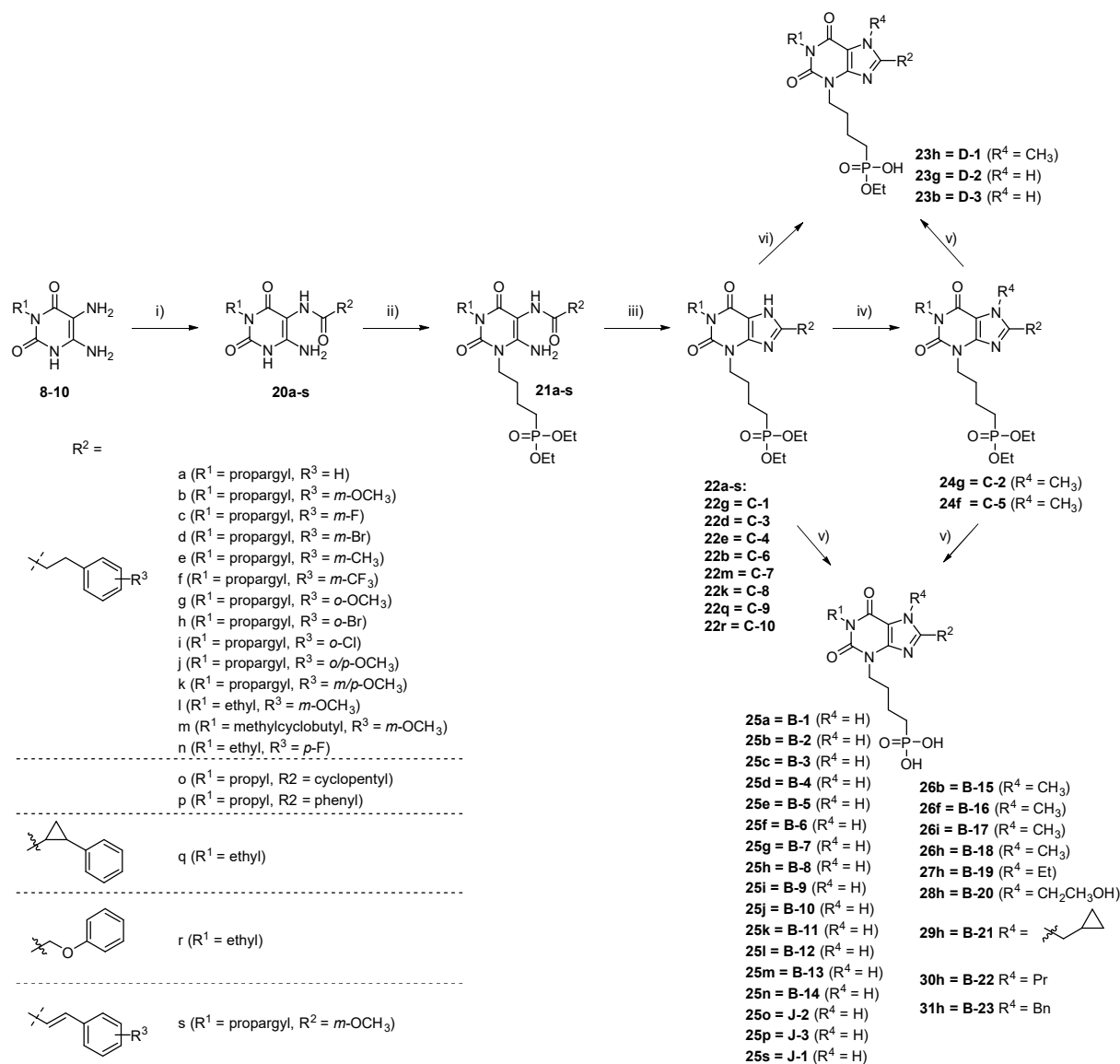
Synthesis of Inventive compounds G-1 – G-2:



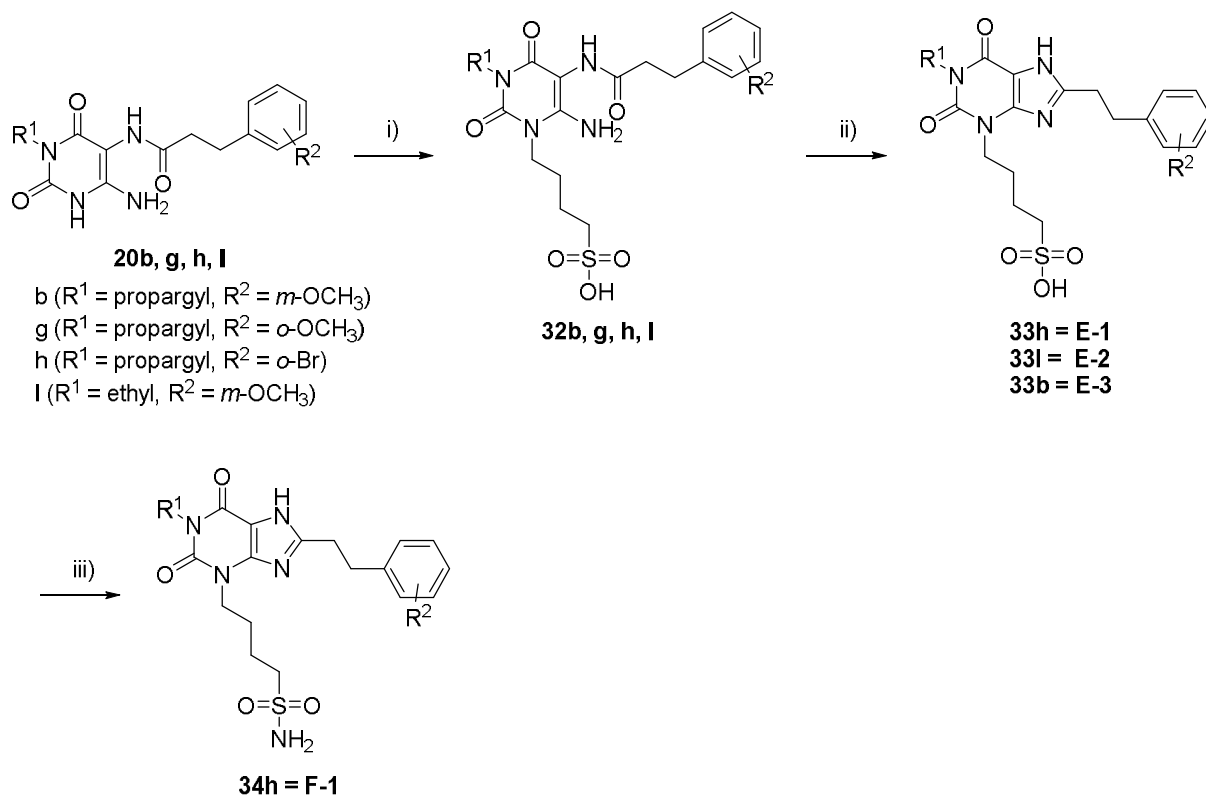
a (R^1 = propargyl, R^2 = *o*- OCH_3)
 b (R^1 = cyclobutylmethyl, R^2 = *m*- CF_3)

Reagents and conditions: (i) ethyl 5-iodopentanoate, K_2CO_3 , DMF, 55 °C, 12 h, (ii) 2N NaOH, 90 °C, 60 min, HCl, 9–12% over 2 steps.

Synthesis of Inventive Compounds B-1 – B-23, C-1 – C-10, D-1 – D-3 and J-1 – J-3):



Reagents and conditions: (i) carboxylic acid, COMU, DIPEA, DMF, rt, H₂O; 72% – 99% (ii) diethyl (4-iodobutyl)phosphonate, K₂CO₃, DMF, 60 °C, 12 h; 24–62% (iii) 2N NaOH, 90 °C, 30 min, HCl; 8–46% (iv) alkyl iodide, DMF, rt, 12 h; 67–99% (v) TMSBr, CH₂Cl₂, rt, 24 h; 49–82% (vi) HMDS, (NH₄)₂SO₄, 150 °C, 16 h, MeOH, 11–24%.

Synthesis of Compounds E-1 – E-3 and F-1):

Reagents and conditions: (i) 1,4-butanedithione, K₂CO₃, DMF, 55 °C, 12 h, (ii) 2N NaOH, 90 °C, 30 min, 8–15% over 2 steps; (iii) 1. SOCl₂, DMF, 0 °C to rt, 2. 25% aq. NH₃, 0 °C, 1 h, 18%.

General Procedures:

The synthesis of 3-substituted 5,6-diaminouracil derivatives (**8–10**) was performed according to literature procedures (Maxwell, L.C.E., and Salivar, C.J. Method of preparing 4-aminouracils. US2715625A, 1952, Müller et al., J. Med. Chem. 1993, 36: 3341-3349)

General procedure A: Amide coupling reaction to produce N3-substituted 5-amino-6-carboxamidouracil derivatives (11a-h, 17a-b, 20a-s)

To a solution of the appropriate carboxylic acid (1.0 equiv) and COMU (1.1 equiv) dissolved in a minimum amount of dimethylformamide (DMF) a mixture of the substituted diaminouracil (**8–10**, 1.1 equiv) and *N,N*-Diisopropylethylamine DIPEA (1.1 equiv) dissolved in DMF was added dropwise. The mixture was stirred at room temperature for 5 – 10 min. After complete conversion of the starting material water (30 ml) was added and the resulting precipitate was filtered off, washed with water and dried under reduced pressure to yield the pure 6-amino-5-carboxamidouracil derivatives **11a-h, 17a-b, 20a-s** in high isolated yields (75-99%).

General procedure B: Alkylation of N1 of the 3-substituted 6-amino-5-carboxamidouracil derivatives (12a-h, 18a-b and 21a-s, 32b, g, h, l)

6-Amino-5-carboxamidouracil derivative (**11a-h, 17a-b, 20a-s**, 1.0 equiv), 1.1 equiv of diethyl (4-iodobutyl)phosphonate or 1,4-butansultone or ethyl 5-bromopentanoate and K₂CO₃ (1.2 equiv) were dissolved in a minimum amount of DMF (1-2 ml). After stirring for 12 h at 55 °C, DMF was removed, water (15 ml) was added, and the resulting mixture extracted with dichloromethane (3 x 15 ml). The combined organic phases were washed with brine (20 ml), dried over MgSO₄ and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography on silica gel 60 (eluent: dichloromethane/methanol, 9:1) to yield the desired substituted uracil derivatives (**12a-h, 18a-b and 21a-s, 32b, g, h, l**).

General procedure D: Ring closure reaction to the corresponding xanthenes using NaOH (13a-h, C-1, C-3, C-4, C-6 to C-10, E-1 to E-3, G-1 to G-2)

The 1,3-disubstituted-6-amino-5-carboxamidouracil derivatives were dissolved in a minimum amount of NaOH (2N) and stirred at 90 °C until complete conversion of the starting material (TLC: dichloromethane/methanol, 9:1) was observed (20-30 min). The mixture was cooled to 0 °C in an ice bath, and the desired product was precipitated by the addition of concentrated HCl solution. The precipitate was filtered off and washed with water to yield the xanthine derivatives as off-white solids.

General procedure E: Alkylation of the xanthine N7-position (14a-f, 23h, g, b, 24g, f, C-2, C-5)

The xanthine derivatives (**13a-h, 22a-s, C-2, C-5**, 1.0 equiv), K₂CO₃ (1.2 equiv) and alkyl iodide/or alkyl bromide (1.1 equiv) were dissolved in a minimum of DMF (1-2 ml) and stirred at room temperature overnight. After complete conversion of the starting material (TLC: dichloromethane/methanol, 9:1) DMF was removed, water was added, and extracted three times with EtOAc (5–10 ml). The combined organic phases were washed with brine, dried over MgSO₄, filtered, and the solvent was removed *in vacuo* to yield the N7-alkylated xanthine derivatives (**14a-f, 23h, g, b, 24g, f, C-2, C-5**).

General procedure F: Deprotection of the phosphonic acid esters using trimethylsilyl bromide (A-1, B-1 to B-23, J-1 to J-3 and H-1 to H-10)

The phosphonic acid ester derivatives were dissolved in dichloromethane (5-10 ml), TMSBr (3 equiv) was added, and the resulting solution was stirred for 12 h at rt. The solvent was removed *in vacuo* and the residue was dissolved in 2N NaOH (2-3 ml), the product was then precipitated by addition of a minimum amount of concentrated HCl solution and washed with water (3 x 5 ml) to yield the final xanthine derivatives (**A-1, B-1 to B-23, J-1 to J-3 and H-1 to H-10**).

A-1 3-(8-(3-Methoxyphenyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 76%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] =

7.49 – 7.44 (m, 1H, H_{arom}), 7.33 (d, $J = 7.6$ Hz, 1H, H_{arom}), 7.31 – 7.27 (m, 1H, H_{arom}), 7.15 – 7.08 (m, 1H, H_{arom}), 4.61 (d, $J = 2.4$ Hz, 2H, N1-CH₂), 4.10 (t, $J = 7.3$ Hz, 2H, N3-CH₂), 3.97 (s, 3H, OCH₃), 3.87 (m, 2H, OCH₂),), 3.82 (s, 3H, N7-CH₃), 3.11 – 3.03 (m, 1H, H_{propargyl}), 2.00-1.98 (m, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.44 (C_{arom}-OCH₃), 153.81 (C8), 151.65 (C6), 149.98 (C2), 147.62 (C4), 130.16 (C_{arom}), 129.48 (C_{arom}), 128.18 (C_{arom}), 125.66 (C_{arom}), 121.62 (C_{arom}), 108.00 (C5), 79.77 (C_{propargyl}), 72.96 (C_{propargyl}), 63.13 (OCH₂), 55.57 (OCH₃), 40.49 (N3-CH₂), 33.88 (N1-CH₂), 30.21 (N7-CH₃), 28.93 (CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 0.27.

B-1) (4-(2,6-Dioxo-8-phenethyl-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: Yield 57%; ¹H NMR (500 MHz, DMSO-*d*₆) δ 13.27 (br s, 1H, N7-H), 7.30 – 7.22 (m, 2H, H_{arom}) 7.12 – 7.11 (m, 3H, H_{arom}), 4.58 (d, $J = 2.5$ Hz, 2H, N1-CH₂), 3.97 (t, $J = 7.2$ Hz, 2H, N3-CH₂), 3.07 (t, $J = 2.1$ Hz, 1H, H_{propargyl}), 3.05 – 2.95 (m, 4H, C8-CH₂CH₂), 1.73-170 (m, 2H, H_{alkyl}), 1.52-1.47 (m, 4H, H_{alkyl}). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 154.14 (C8), 153.02 (C6), 150.12 (C2), 148.14 (C4), 140.58 (C_{arom}), 128.45 (C_{arom}), 128.42 (C_{arom}), 126.22 (C5), 79.89 (C_{propargyl}), 72.81 (C_{propargyl}), 42.91 (N3-CH₂), 33.36 (C8-CH₂CH₂), 30.20 (C8-CH₂CH₂), 28.78, 28.66, 28.12 (1C, PCH₂CH₂), 27.04 1C, PCH₂), 20.28 (C_{alkyl}). ³¹P NMR (243 MHz, DMSO) δ 26.73. ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 26.73. HRMS (ESI-QTOF) calculated for C₂₀H₂₃N₄O₅P [M+H]⁺: 431.1484; found: 431.1480).

B-2) (4-(8-(3-Methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 240–242 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.28 (br s, 1H, N7-H), 7.16 (t, $J = 7.8$ Hz, 1H, H_{arom}), 6.79 – 6.71 (m, 3H, H_{arom}), 4.6 (d, $J = 1.6$ Hz, 2H, N1-CH₂), 3.97 (t, $J = 7.2$ Hz, 2H, N3-CH₂), 3.70 (s, 3H, OCH₃), 3.05 (t, $J = 2.1$ Hz, 1H, H_{propargyl}), 2.99 (s, 4H, C8-CH₂CH₂), 1.76 – 1.70 (m, 2H, H_{alkyl}), 1.58 – 1.44 (m, 4H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.4 (C_{arom}-OCH₃), 153.0 (C8), 150.2 (C2 or C6), 148.2 (C2 or C6), 142.2 (C4), 129.5 (C_{arom}), 120.7 (C_{arom}), 117.5 (C_{arom}), 114.1 (C_{arom}), 111.8 (C_{arom}), 106.0 (C5), 79.9 (C_{propargyl}), 72.9 (C_{propargyl}), 55.0 (OCH₃), 42.9 (N3-CH₂), 33.4 (CH₂), 30.2 (CH₂), 30.1 (N1-CH₂) 28.7 (d, ²J_{C,P} = 14.9 Hz, 1C, PCH₂CH₂), 27.5 (d, ¹J_{C,P} = 138.8 Hz, 1C, PCH₂), 20.3 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.0. HRMS (ESI-QTOF) calculated for C₂₁H₂₅N₄O₆P [M+H]⁺: 461.1584; found: 461.1587.

B-3) (4-(8-(3-Fluorophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 154–156 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.32 (br s, 1H, N7-H), 7.29 (q, $J = 7.8$ Hz, 1H, H_{arom}), 7.07 (d, $J = 10.1$ Hz, 1H, H_{arom}), 7.03 (d, $J = 7.6$ Hz, 1H, H_{arom}), 6.99 (td, $J = 8.7, 2.4$ Hz, 1H, H_{arom}), 4.58 (d, $J = 2.1$ Hz, 2H, N1-CH₂), 3.96 (t, $J = 7.1$ Hz, 2H, N3-CH₂), 3.06 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 3.05 – 2.99 (m, 4H, C8-CH₂CH₂), 1.72 (p, $J = 7.5$ Hz, 2H, H_{alkyl}), 1.58 – 1.51 (m, 2H, H_{alkyl}), 1.47 (dp, $J = 15.6, 9.0, 7.9$ Hz, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 162.4 (d, ¹J_{C,F} = 240.5 Hz, 1C, C_{arom}-F), 154.0 (C_{xanthine}), 153.0 (C_{xanthine}), 150.2 (C_{xanthine}), 148.2 (C_{xanthine}), 143.6 (d, ³J_{C,F} = 7.5 Hz, 1C, C_{arom}), 130.4 (d, ³J_{C,F} = 8.9 Hz, 1C, C_{arom}), 124.7

(d, $^4J_{C,F} = 3.0$ Hz, 1C, C_{arom}), 115.3 (d, $^2J_{C,F} = 20.7$ Hz, 1C, C_{arom}), 113.0 (d, $^2J_{C,F} = 20.7$ Hz, 1C, C_{arom}), 106.0 (C5), 79.9 (C_{propargyl}), 72.9 (C_{propargyl}), 42.9 (N3-CH₂), 33.0 (CH₂), 30.3 (CH₂), 29.8 (N1-CH₂), 28.8 (d, $^2J_{C,P} = 16.1$ Hz, 1C, PCH₂C_HCH₂), 27.4 (d, $^1J_{C,P} = 135.7$ Hz, 1C, PCH₂), 20.2 (d, $^3J_{C,P} = 4.4$ Hz, 1C, PCH₂CH₂C_HCH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₀H₂₂FN₄O₅P [M+H]⁺: 449.1385; found: 449.1389.

B-4 (4-(8-(3-Bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 240–242 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.31 (s, 1H, N7-H), 7.45 (s, 1H, H_{arom}), 7.37 (d, $J = 7.4$ Hz, 1H, H_{arom}), 7.22 (dt, $J = 12.6, 7.5$ Hz, 2H, H_{arom}), 4.59 (s, 2H, N1-CH₂), 3.98 (t, $J = 6.9$ Hz, 2H, N3-CH₂), 3.05 (s, 1H, H_{propargyl}), 3.02 (s, 4H, C8-CH₂CH₂), 1.78 – 1.68 (m, 2H, H_{alkyl}), 1.57 (dt, $J = 16.8, 7.6$ Hz, 2H, H_{alkyl}), 1.52 – 1.44 (m, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.7 (C_{xanthine}), 152.9 (C_{xanthine}), 150.0 (C_{xanthine}), 147.9 (C_{xanthine}), 143.3 (C_{xanthine}), 131.2 (C_{arom}), 130.5 (C_{arom}), 129.0 (C_{arom}), 127.5 (C_{arom}), 121.6 (C_{arom}), 105.9 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 42.7 (C_{alkyl}), 32.6 (C_{alkyl}), 30.1 (C_{alkyl}), 29.6 (C_{alkyl}), 28.5 (d, $^2J_{C,P} = 15.9$ Hz, 1C, PCH₂C_HCH₂), 27.2 (d, $^1J_{C,P} = 136.7$ Hz, 1C, PCH₂), 20.0 (d, $^3J_{C,P} = 4.1$ Hz, 1C, P(CH₂)₂C_HCH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₀H₂₃BrN₄O₅P [M+H]⁺: 509.0584; found: 509.0578.

B-5 (4-(8-(3-Methylphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 244–246 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.29 (s, 1H, N7-H), 7.14 (t, $J = 7.5$ Hz, 1H, H_{arom}), 7.04 (s, 1H, H_{arom}), 6.98 (d, $J = 7.5$ Hz, 2H, H_{arom}), 4.59 (d, $J = 2.2$ Hz, 2H, N1-CH₂), 3.97 (t, $J = 7.2$ Hz, 2H, N3-CH₂), 3.05 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 2.98 (s, 4H, C8-CH₂CH₂), 2.26 (s, 3H, CH₃), 1.74 (p, $J = 7.5$ Hz, 2H, H_{alkyl}), 1.61 – 1.54 (m, 2H, H_{alkyl}), 1.51 – 1.45 (m, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 154.1 (C_{xanthine}), 152.9 (C_{xanthine}), 150.0 (C_{xanthine}), 148.0 (C_{xanthine}), 140.3 (C_{arom}), 137.3 (C_{arom}), 129.0 (C_{arom}), 128.2 (C_{arom}), 126.8 (C_{arom}), 125.3 (C_{arom}), 105.8 (C5), 79.8 (C_{propargyl}), 72.7 (C_{propargyl}), 42.7 (C_{alkyl}), 33.2 (C_{alkyl}), 30.1 (C_{alkyl}), 30.0 (C_{alkyl}), 28.6 (d, $^2J_{C,P} = 15.3$ Hz, 1C, PCH₂C_HCH₂), 27.3 (d, $^1J_{C,P} = 136.3$ Hz, 1C, PCH₂), 21.0 (CH₃), 20.0 (d, $^3J_{C,P} = 3.9$ Hz, 1C, P(CH₂)₂C_HCH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₁H₂₅N₄O₅P [M+H]⁺: 445.1635; found: 445.1650.

B-6 (4-(2,6-Dioxo-1-(prop-2-yn-1-yl)-8-(3-(trifluoromethyl)phenethyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 223–226 °C. ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 13.29 (br s, 1H, N7-H), 7.55 (s, 1H, H_{arom}), 7.51 (q, $J = 6.8$ Hz, 3H, H_{arom}), 4.57 (d, 2H, N1-CH₂), 3.95 (t, 2H, N3-CH₂), 3.14 – 3.10 (m, 2H, C8-CH₂), 3.05 – 3.02 (m, 3H, C8-CH₂CH₂ and H_{propargyl}), 1.71 (dq, $J = 13.1, 6.8$ Hz, 2H, H_{alkyl}), 1.55 – 1.45 (m, 4H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.8 (C8), 153.2 (C2 or C6), 150.1 (C2 or C6), 148.1 (C4), 142.1 (C_{arom}), 132.8 (C_{arom}), 129.5 (C_{arom}), 129.2 (q, $^2J_{C,F} = 32.8$ Hz, 1C, C_{arom}-CF₃), 125.1 (C_{arom}), 124.4 (q, $^1J_{C,F} = 272.1$ Hz, 1C, CF₃), 122.9

(C_{arom}), 123.1 (C5), 79.9 (C_{propargyl}), 72.9 (C_{propargyl}), 43.0 (N3-CH₂), 33.0 (CH₂), 30.3 (N1-CH₂), 29.8 (CH₂), 28.8 (d, ²J_{C,P} = 15.3 Hz, 1C, PCH₂CH₂), 27.7 (d, ¹J_{C,P} = 138.1 Hz, 1C, PCH₂), 20.4 (d, ³J_{C,P} = 4.5 Hz, 1C, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 26.3. HRMS (ESI-QTOF) calculated for C₂₁H₂₂F₃N₄O₅P [M+H]⁺: 499.1280; found: 449.1283.

B-7) (4-(8-(2-Methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 169–172 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.25 (br s, 1H, N7-H), 7.19 – 7.15 (m, 1H, H_{arom}), 7.08 (d, *J* = 7.3 Hz, 1H, H_{arom}), 6.94 (d, *J* = 8.1 Hz, 1H, H_{arom}), 6.82 (t, *J* = 7.3 Hz, 1H, H_{arom}), 4.58 (d, *J* = 2.0 Hz, 2H, N1-CH₂), 3.96 (t, *J* = 7.2 Hz, 2H, N3-CH₂), 3.77 (s, 3H, OCH₃), 3.04 (t, *J* = 2.3 Hz, 1H, H_{propargyl}), 2.93 – 2.97 (m, 4H, C8-CH₂CH₂), 1.72 (p, *J* = 7.4 Hz, 2H, H_{alkyl}), 1.49 – 1.53 (m, 4H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 157.3 (C_{arom}-OCH₃), 154.5 (C_{xanthine}), 153.0 (C_{xanthine}), 150.1 (C_{xanthine}), 148.2 (C_{xanthine}), 129.8 (C_{arom}), 128.3 (C_{arom}), 127.8 (C_{arom}), 120.4 (C_{arom}), 110.8 (C_{arom}), 106.0 (C5), 79.9 (C_{propargyl}), 72.8 (C_{propargyl}), 55.4 (OCH₃), 42.9 (N3-CH₂), 30.2 (N1-CH₂), 28.7 (CH₂), 28.7 (d, ²J_{C,P} = 16.1 Hz, 1C, PCH₂CH₂), 28.6 (CH₂), 27.5 (d, ¹J_{C,P} = 136.6 Hz, 1C, PCH₂), 20.3 (d, ³J_{C,P} = 4.3 Hz, 1C, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 26.9. HRMS (ESI-QTOF) calculated for C₂₁H₂₅N₄O₆P [M+H]⁺: 461.1584; found: 461.1586.

B-8) (4-(8-(2-Bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 209–212 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 13.33 (s, 1H, N7-H), 7.58 (d, *J* = 8.0 Hz, 1H, H_{arom}), 7.29 (d, *J* = 4.4 Hz, 2H, H_{arom}), 7.15 (dt, *J* = 8.7, 4.6 Hz, 1H, H_{arom}), 4.59 (d, *J* = 2.4 Hz, 2H, N1-CH₂), 3.14 (t, *J* = 7.6 Hz, 2H, -CH₂), 3.07 – 2.99 (m, 3H, -CH₂ and H_{propargyl}), 1.74 (p, *J* = 7.5 Hz, 2H, -CH₂), 1.64 – 1.43 (m, 4H, -CH₂CH₂). ¹³C-NMR (126 MHz, DMSO-*d*₆) δ [ppm] = 153.4 (C8 or C4), 152.9 (C8 or C4), 150.0 (CO), 148.0 (CO), 139.3 (C_{arom}), 132.5 (C_{arom}), 130.7 (C_{arom}), 128.4 (C_{arom}), 127.9 (C_{arom}), 123.7 (C_{arom}), 106.0 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 42.7 (N-CH₂), 33.5 (N-CH₂), 30.1 (-CH₂), 28.5 (d, ²J_{C,P} = 15.6 Hz, 1C, PCH₂CH₂), 28.3 (-CH₂), 27.2 (d, ¹J_{C,P} = 136.2 Hz, 1C, PCH₂), 20.0 (d, ³J_{C,P} = 4.3 Hz, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆): δ [ppm] = 27.3. HRMS (ESI-QTOF) calculated for C₂₀H₂₃BrN₄O₅P [M+H]⁺: 509.0584; found: 509.0566.

B-9) (4-(8-(2-Chlorophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 207–209 °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.51 – 12.94 (m, 1H, N7-H), 7.41 (dd, *J* = 7.0, 2.1 Hz, 1H, H_{arom}), 7.29 (dd, *J* = 7.0, 2.4 Hz, 1H, H_{arom}), 7.24 (tt, *J* = 7.4, 5.3 Hz, 2H, H_{arom}), 4.59 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 3.97 (t, *J* = 7.2 Hz, 2H, N3-CH₂), 3.14 (t, *J* = 7.6 Hz, 2H, CH₂), 3.09 – 3.04 (m, 1H, H_{propargyl}), 3.02 (t, *J* = 7.6 Hz, 2H, CH₂), 1.73 (p, *J* = 7.3 Hz, 2H, CH₂), 1.52 (m, 4H, 2 x CH₂). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.5 (C8 or C4), 152.9 (C8 or C4), 150.0 (CO), 148.0 (CO), 137.6 (C_{arom}), 132.9 (C_{arom}), 130.7 (C_{arom}), 129.2 (C_{arom}),

128.2 (C_{arom}), 127.3 (C_{arom}), 105.9 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 42.7 (-CH₂), 31.1 (-CH₂), 30.1 (-CH₂), 28.5 (d, ²J_{C,P} = 15.6 Hz, 1C, PCH₂CH₂), 28.2 (-CH₂), 27.3 (d, ¹J_{C,P} = 137.0 Hz, 1C, PCH₂), 20.0 (d, ³J_{C,P} = 4.2 Hz, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.0. HRMS (ESI-QTOF) n.d.

B-10 (4-(8-(2,4-Dimethoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 255–257 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.02 (d, *J* = 8.1 Hz, 1H, H_{arom}), 6.50 (d, *J* = 2.3 Hz, 1H, H_{arom}), 6.39 (dd, *J* = 8.3, 2.3 Hz, 1H, H_{arom}), 4.58 (d, *J* = 1.9 Hz, 2H, N1-CH₂), 4.01 – 3.92 (m, 2H, N3-CH₂), 3.77 (s, 3H, OCH₃), 3.71 (s, 3H, OCH₃), 3.03 (t, *J* = 2.3 Hz, 1H, H_{propargyl}), 2.93 – 2.83 (m, 4H, C8-CH₂CH₂), 1.77 – 1.68 (m, 2H, H_{alkyl}), 1.57 – 1.48 (m, 2H, H_{alkyl}), 1.40 (dt, *J* = 14.5, 6.7 Hz, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 163.8 (C_{arom}-OCH₃), 160.0 (C_{arom}-OCH₃), 158.9 (C_{xanthine}), 157.9 (C_{xanthine}), 154.6 (C_{xanthine}), 149.9 (C_{xanthine}), 129.8 (C_{arom}), 121.3 (C_{arom}), 105.4 (C5) 104.3 (C_{arom}), 98.3 (C_{arom}), 80.4 (C_{propargyl}), 72.2 (C_{propargyl}), 55.2 (OCH₃), 55.03 (OCH₃), 43.5 (N3-CH₂), 32.1 (-CH₂) 29.8 (N1-CH₂), 29.4 (-CH₂), 27.7 (PCH₂CH₂), 21.0 (PCH₂), 16.7 (P(CH₂)₂CH₂), 15.8 (POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 26.9. HRMS (ESI-QTOF) calculated for C₂₂H₂₇N₄O₇P [M+H]⁺: 491.1690; found: 491.1690.

B-11 (4-(8-(3,4-Dimethoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 254–256 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.02 (d, *J* = 8.1 Hz, 1H, H_{arom}), 6.51 (d, *J* = 2.3 Hz, 1H, H_{arom}), 6.38 (dd, *J* = 8.3, 2.3 Hz, 1H, H_{arom}), 4.58 (d, *J* = 1.9 Hz, 2H, N1-CH₂), 4.02 – 3.93 (m, 2H, N3-CH₂), 3.77 (s, 3H, OCH₃), 3.71 (s, 3H, OCH₃), 3.02 (t, *J* = 2.3 Hz, 1H, H_{propargyl}), 2.93 – 2.83 (m, 4H, C8-CH₂CH₂), 1.77 – 1.68 (m, 2H, H_{alkyl}), 1.57 – 1.48 (m, 2H, H_{alkyl}), 1.40 (dt, *J* = 14.5, 6.7 Hz, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 163.6 (C_{arom}-OCH₃), 160.2 (C_{arom}-OCH₃), 158.9 (C_{xanthine}), 157.9 (C_{xanthine}), 154.6 (C_{xanthine}), 149.9 (C_{xanthine}), 129.8 (C_{arom}), 121.3 (C_{arom}), 105.4 (C5) 104.3 (C_{arom}), 98.3 (C_{arom}), 80.4 (C_{propargyl}), 72.2 (C_{propargyl}), 55.2 (OCH₃), 55.03 (OCH₃), 43.5 (N3-CH₂), 32.1 (-CH₂) 29.7 (N1-CH₂), 29.4 (-CH₂), 27.7 (PCH₂CH₂), 21.0 (PCH₂), 16.7 (P(CH₂)₂CH₂), 15.8 (POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 26.7. HRMS (ESI-QTOF) calculated for C₂₂H₂₇N₄O₇P [M+H]⁺: 491.1690; found: 491.1690.

B-12 (4-(1-Ethyl-8-(3-methoxyphenethyl)-2,6-dioxo-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 243–245 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.15 (t, *J* = 7.8 Hz, 1H, H_{arom}), 6.78 – 6.75 (m, 2H, H_{arom}), 6.73 – 6.69 (m, 1H, H_{arom}), 3.93 (t, *J* = 7.6 Hz, 2H, N1-CH₂), 3.89 – 3.86 (m, 2H, N3-CH₂), 3.69 (s, 3H, OCH₃), 2.96 (s, 4H, C8-CH₂CH₂), 1.69 (dq, *J* = 15.2, 7.9, 6.9 Hz, 2H, H_{alkyl}), 1.51 – 1.44 (m, 2H, H_{alkyl}), 1.40 (dt, *J* = 15.8, 7.4 Hz, 2H, H_{alkyl}), 1.07 (t, *J* = 7.0 Hz, 3H, CH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.4 (C_{arom}-OCH₃), 154.0 (C_{xanthine}), 153.3 (C_{xanthine}), 150.5 (C_{xanthine}), 147.0 (C_{xanthine}), 142.3 (C_{arom}), 129.4 (C_{arom}), 120.7 (C_{arom}), 114.0 (C_{arom}), 111.8 (C_{arom}), 106.7 (C5), 55.0 (OCH₃), 43.0 (N3-CH₂), 35.6 (CH₂) 33.5 (CH₂), 30.1 (N1-CH₂), 29.0 (d,

$^2J_{C,P} = 15.1$ Hz, 1C, PCH₂CH₂), 28.8 (d, $^1J_{C,P} = 135.7$ Hz, 1C, PCH₂), 21.1 (d, $^3J_{C,P} = 4.1$ Hz, 1C, PCH₂CH₂CH₂), 13.3 (N1-CH₂CH₃). $^{31}\text{P-NMR}$ (243 MHz, DMSO-*d*₆) δ [ppm] = 23.8. HRMS (ESI-QTOF) calculated for C₂₀H₂₇N₄O₆P [M+H]⁺: 451.1741; found: 451.1743.

B-13 (4-(1-(Cyclobutylmethyl)-8-(3-methoxyphenethyl)-2,6-dioxo-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 205–207 °C. $^1\text{H-NMR}$ (600 MHz, DMSO-*d*₆) δ [ppm] = 13.16 (br s, 1H, N7-H), 7.17 (t, $J = 8.0$ Hz, 1H, H_{arom}), 6.77 (d, $J = 6.0$ Hz, 2H, H_{arom}), 6.74 – 6.72 (m, 1H, H_{arom}), 3.95 (d, $J = 7.1$ Hz, 2H, N1-CH₂), 3.92 (d, $J = 7.2$ Hz, 2H, N3-CH₂), 3.69 (s, 3H, OCH₃), 2.98 (s, 4H, C8-CH₂CH₂), 2.62 (dt, $J = 15.0, 7.5$ Hz, 1H, H_{alkyl}), 1.91 – 1.86 (m, 2H, H_{alkyl}), 1.72 – 1.76 (m, 6H, H_{alkyl}), 1.55 (dt, $J = 16.8, 7.8$ Hz, 2H, H_{alkyl}), 1.50 – 1.42 (m, 2H, H_{alkyl}). $^{13}\text{C-NMR}$ (151 MHz, DMSO-*d*₆) δ [ppm] = 159.4 (C_{arom}-OCH₃), 154.2 (C_{xanthine}), 153.8 (C_{xanthine}), 151.0 (C_{xanthine}), 147.8 (C_{xanthine}), 142.2 (C_{arom}), 129.5 (C_{arom}), 120.7 (C_{arom}), 114.0 (C_{arom}), 111.8 (C_{arom}), 106.2 (C5), 55.0 (OCH₃), 45.2 (N1-CH₂), 42.6 (N3-CH₂), 34.2 (C_{alkyl}), 33.5 (C_{alkyl}), 30.1 (C_{alkyl}), 28.7 (d, $^2J_{C,P} = 16.2$ Hz, 1C, PCH₂CH₂), 27.4 (d, $^1J_{C,P} = 136.5$ Hz, 1C, PCH₂), 25.7 (2C, C_{cyclobutyle}), 20.2 (d, $^3J_{C,P} = 3.9$ Hz, 1C, P(CH₂)₂CH₂), 17.9 (C_{cyclobutyle}). $^{31}\text{P-NMR}$ (243 MHz, DMSO-*d*₆) δ [ppm] = 27.3. HRMS (ESI-QTOF) calculated for C₂₃H₃₁N₄O₆P [M+H]⁺: 491.2054; found: 491.2068.

B-14 (4-(1-Ethyl-8-(4-fluorophenethyl)-2,6-dioxo-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 256–260 °C. $^1\text{H-NMR}$ (600 MHz, DMSO-*d*₆) δ [ppm] = 13.19 (br s, 1H, N7-H), 7.26 – 7.18 (m, 2H, H_{arom}), 7.10 – 7.03 (m, 2H, H_{arom}), 3.95 – 3.91 (m, 2H, N1-CH₂), 3.90 – 3.85 (m, 2H, N3-CH₂), 3.00 – 2.94 (m, 4H, C8-CH₂CH₂), 1.73 – 1.66 (m, 2H, H_{alkyl}), 1.52 – 1.42 (m, 4H, H_{alkyl}), 1.08 (t, $J = 5.8$ Hz, 3H, CH₂CH₃). $^{13}\text{C-NMR}$ (151 MHz, DMSO-*d*₆) δ [ppm] = 161.2 (d, $^1J = 245$ Hz, 1C, C_{arom}-F), 154.1 (C_{xanthine}), 153.8 (C_{xanthine}), 150.8 (C_{xanthine}), 137.0 (d, $^4J_{C,F} = 3.0$ Hz, 1C, C_{arom}), 130.6 (d, $^3J_{C,F} = 7.8$ Hz, 2C, C_{arom}), 115.5 (d, $^2J_{C,F} = 20.6$ Hz, 2C, C_{arom}), 106.7 (C5), 43.0 (N3-CH₂), 36.1 (CH₂), 32.9 (CH₂), 30.5 (N1-CH₂), 29.1 (d, $^2J_{C,P} = 16.0$ Hz, 1C, P-CH₂CH₂), 28.0 (d, $^1J_{C,P} = 135.5$ Hz, 1C, PCH₂), 20.7 (d, $^3J_{C,P} = 4.5$ Hz, 1C, PCH₂CH₂CH₂), 13.6 (N1-CH₂CH₃). $^{31}\text{P-NMR}$ (243 MHz, DMSO-*d*₆) δ [ppm] = 26.3. HRMS (ESI-QTOF) calculated for C₁₉H₂₄FN₄O₅P [M+H]⁺: 439.1541; found: 439.1543.

B-15 (4-(8-(3-Methoxyphenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: white solid; m.p. n.d.; $^1\text{H-NMR}$ (600 MHz, DMSO-*d*₆) δ [ppm] = 6.82 – 6.79 (m, 2H, H_{arom}), 6.76 – 6.74 (m, 1H, H_{arom}), 6.66 – 6.62 (m, 1H, H_{arom}), 4.58 (d, 2H, N1-CH₂), 3.97 (t, $J = 7.0$ Hz, 2H, N3-CH₂), 3.71 (s, 3H, OCH₃ or NCH₃), 3.70 (s, 3H, OCH₃ or NCH₃), 3.06 – 3.04 (m, 2H, C8-CH₂), 3.04 – 3.02 (m, 1H, H_{propargyl}), 2.99 – 2.96 (m, 2H, C8-CH₂CH₂), 1.74 – 1.69 (m, 2H, H_{alkyl}), 1.58 – 1.52 (m, 2H, H_{alkyl}), 1.51 – 1.45 (m, 2H, H_{alkyl}). $^{13}\text{C-NMR}$ (151 MHz, DMSO-*d*₆) δ [ppm] = 171.6 (C=OCH₃), 159.4 (C8), 154.3 (C2 or C6), 153.4 (C2 or C6), 149.9 (C4), 147.5 (C_{arom}), 142.1 (C_{arom}), 129.5 (C_{arom}), 120.8 (C_{arom}), 114.2 (C_{arom}), 111.9 (C_{arom}), 106.4 (C5), 79.8 (C_{propargyl}), 72.9

(C_{propargyl}), 55.0 (OCH₃), 42.5 (N3-CH₂), 32.8 (CH₂), 31.4 (CH₂), 30.0 (N1-CH₂), 28.7 (d, ²J_{C,P} = 14.9 Hz, 1C, PCH₂CH₂), 27.9 (N7-CH₃) 27.4 (d, ¹J_{C,P} = 137.2 Hz, 1C, PCH₂), 20.2 (d, ³J_{C,P} = 3.9 Hz, 1C, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.0. HRMS (ESI-QTOF) calculated for C₂₂H₂₈N₄O₆P [M+H]⁺: 475.4618; found: 475.4620.

B-16 (4-(7-Methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-8-(3-(trifluoromethyl)phenethyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. n.d. °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.63 (s, 1H, H_{arom}), 7.58 (d, *J* = 7.5 Hz, 1H, H_{arom}), 7.54 (d, *J* = 7.8 Hz, 1H, H_{arom}), 7.50 (t, *J* = 7.6 Hz, 1H, H_{arom}), 4.58 (d, *J* = 2.3 Hz, 2H, N1-CH₂), 3.95 (t, *J* = 7.1 Hz, 2H, N3-CH₂), 3.76 (s, 3H, N7-CH₃), 3.11 (d, *J* = 3.4 Hz, 4H, C8-CH₂CH₂), 3.05 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 1.71 (p, *J* = 7.4 Hz, 2H, C_{alkyl}), 1.58 – 1.52 (m, 2H, C_{alkyl}), 1.50 – 1.43 (m, 2H, C_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 154.0 (C_{xanthine}), 153.5 (C_{xanthine}), 150.0 (C_{xanthine}), 147.5 (C4), 142.1 (C_{arom}), 133.1 (C_{arom}), 129.5 (C_{arom}), 129.2 (q, ²J_{C,F} = 32.9 Hz, 1C, CCF₃), 125.4 (q, ³J_{C,F} = 3.4 Hz, 1C, CHCF₃), 124.5 (q, ¹J_{C,F} = 272.0 Hz, 1C, CF₃), 123.1 (q, ³J_{C,F} = 3.7 Hz, 1C, CHCF₃), 106.5 (C5), 79.9 (C_{propargyl}), 72.9 (C_{propargyl}), 42.5 (N1-CH₂), 32.2 (C_{alkyl}), 31.6 (C_{alkyl}), 30.1 (C_{alkyl}), 28.7 (d, ²J_{C,P} = 16.5 Hz, 1C, PCH₂), 27.6 (N7-CH₃), 27.4 (d, ¹J_{C,P} = 136.6 Hz, 1C, PCH₂), 20.2 (d, ³J_{C,P} = 4.7 Hz, 1C, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.3. HRMS (ESI-QTOF) calculated for C₂₂H₂₅F₃N₄O₅P [M+H]⁺: 513.1509; found: 513.1491.

B-17 (4-(8-(2-Chlorophenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. n.d. °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.42 (dd, *J* = 6.6, 2.5 Hz, 1H), 7.36 (dd, *J* = 6.7, 2.7 Hz, 1H), 7.28 – 7.23 (m, 2H), 4.59 (d, *J* = 2.7 Hz, 2H), 3.96 (t, *J* = 7.1 Hz, 2H), 3.74 (s, 3H), 3.13 (t, *J* = 7.4 Hz, 2H), 3.10 – 3.03 (m, 3H), 1.71 (p, *J* = 7.4 Hz, 2H), 1.59 – 1.50 (m, 2H), 1.51 – 1.43 (m, 2H). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.6 (C8 or C4), 153.3 (C8 or C4), 149.7 (CO), 147.3 (CO), 137.6 (C_{arom}), 133.0 (C_{arom}), 131.0 (C_{arom}), 129.2 (C_{arom}), 128.3 (C_{arom}), 127.3 (C_{arom}), 106.4 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 42.4 (-CH₂), 31.3 (-CH₂), 30.4 (-CH₂), 29.9 (-CH₂), 28.5 (d, ²J_{C,P} = 15.6 Hz, 1C, PCH₂CH₂), 27.3 (d, ¹J_{C,P} = 136.5 Hz, 1C, PCH₂), 26.1 (-CH₂), 20.0 (d, ³J_{C,P} = 3.6 Hz, PCH₂CH₂CH₂). HRMS (ESI-QTOF) n.d.

B-18 (4-(8-(2-Bromophenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. n.d.; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.59 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{arom}), 7.35 (dd, *J* = 7.7, 1.8 Hz, 1H, H_{arom}), 7.31 (td, *J* = 7.4, 1.3 Hz, 1H, H_{arom}), 7.17 (td, *J* = 7.6, 1.8 Hz, 1H, H_{arom}), 4.59 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 3.96 (t, *J* = 7.1 Hz, 2H, N3-CH₂), 3.74 (s, 3H, N7-CH₃), 3.13 (t, *J* = 7.1 Hz, 2H, -CH₂), 3.10 – 3.02 (m, 3H, -CH₂ and H_{propargyl}), 1.76 – 1.67 (m, 2H, -CH₂), 1.59 – 1.51 (m, 2H, -CH₂), 1.51 – 1.43 (m, 2H, -CH₂). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.5 (C8 or C4), 153.3 (C8 or C4), 149.8 (CO), 147.3 (CO), 139.3 (C_{arom}), 132.5

(C_{arom}), 131.0 (C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 123.7 (C_{arom}), 106.4 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 42.4 (N-CH₂), 33.0 (N-CH₂), 31.3 (N7-CH₃), 29.9 (CH₂), 28.5 (d, ²J_{C,P} = 15.6 Hz, 1C, PCH₂CH₂), 27.2 (d, ¹J_{C,P} = 137.5 Hz, 1C, PCH₂), 26.3 (CH₂), 20.0 (d, ³J_{C,P} = 4.4 Hz, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.1.

B-19 (4-(8-(2-Bromophenethyl)-7-ethyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 192 – 195 °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.59 (d, *J* = 7.9 Hz, 1H, H_{arom}), 7.36 (d, *J* = 7.5 Hz, 1H, H_{arom}), 7.30 (t, *J* = 7.4 Hz, 1H, H_{arom}), 7.16 (t, *J* = 7.5 Hz, 1H, H_{arom}), 4.59 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 4.18 (q, *J* = 7.1 Hz, 2H, N7-CH₂), 3.96 (t, *J* = 7.2 Hz, 2H, N3-CH₂), 3.15 (t, *J* = 7.6 Hz, 2H, -CH₂), 3.09 – 3.05 (m, 3H, -CH₂ and H_{propargyl}), 1.72 (p, *J* = 7.4 Hz, 2H, -CH₂), 1.58 – 1.44 (m, 4H, -CH₂CH₂), 1.22 (t, *J* = 7.1 Hz, 3H, N7-CH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 152.9 (C8 or C4), 152.7 (C8 or C4), 149.8 (CO), 147.7 (CO), 139.3 (C_{arom}), 132.5 (C_{arom}), 131.1 (C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 123.8 (C_{arom}), 105.5 (C5), 79.7 (C_{propargyl}), 72.8 (C_{propargyl}), 42.5 (N-CH₂), 39.5 (N-CH₂), 33.3 (N-CH₂), 30.0 (-CH₂), 28.6 (d, ²J_{C,P} = 15.7 Hz, 1C, PCH₂CH₂), 27.5 (d, ¹J_{C,P} = 136.1 Hz, 1C, PCH₂), 26.1 (-CH₂), 20.2 (d, ³J_{C,P} = 4.2 Hz, PCH₂CH₂CH₂), 15.9 (N7-CH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆): δ [ppm] = 26.6. HRMS (ESI-QTOF) calculated for C₂₂H₂₆BrN₄O₅P [M+H]⁺: 537.0897; found: 537.0888.

B-20 (4-(8-(2-Bromophenethyl)-7-(2-hydroxyethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 157–160 °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.59 (d, *J* = 7.7 Hz, 1H, H_{arom}), 7.38 (dd, *J* = 7.6, 1.7 Hz, 1H, H_{arom}), 7.33 – 7.28 (m, 1H, H_{arom}), 7.17 (td, *J* = 7.7, 1.8 Hz, 1H, H_{arom}), 4.59 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 4.19 (t, *J* = 5.3 Hz, 2H, N-CH₂), 3.98 (t, *J* = 7.2 Hz, 2H, N-CH₂), 3.67 – 3.66 (m, 1H, -CH₂OH), 3.18 – 3.13 (m, 2H, -CH₂), 3.14 – 3.09 (m, 2H, -CH₂), 3.06 (q, *J* = 2.6 Hz, 1H, H_{propargyl}), 1.73 (p, *J* = 7.3 Hz, 2H, -CH₂), 1.61 – 1.53 (m, 2H, -CH₂), 1.53 – 1.45 (m, 2H, -CH₂). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 154.3 (C8 or C4), 153.0 (C8 or C4), 149.8 (CO), 147.7 (CO), 139.6 (C_{arom}), 132.5 (C_{arom}), 131.0 (C_{arom}), 128.5 (C_{arom}), 127.9 (C_{arom}), 123.8 (C_{arom}), 105.7 (C5), 79.7 (C_{propargyl}), 72.8 (C_{propargyl}), 60.1 (-CH₂OH), 47.3 (N7-CH₂), 42.4 (N-CH₂), 33.1 (N-CH₂), 30.0 (-CH₂), 28.5 (d, ²J_{C,P} = 15.8 Hz, 1C, PCH₂CH₂), 27.2 (d, ¹J_{C,P} = 136.5 Hz, 1C, PCH₂), 26.5 (-CH₂), 20.0 (d, ³J_{C,P} = 4.1 Hz, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆): δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₂H₂₆BrN₄O₆P [M+H]⁺: 553.0846; found: 553.0829.

B-21 (4-(8-(2-Bromophenethyl)-7-(cyclopropylmethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 181–184 °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.59 (dd, *J* = 8.1, 1.2 Hz, 1H, H_{arom}), 7.39 (dd, *J* = 7.7, 1.7 Hz, 1H, H_{arom}), 7.31 (td, *J* = 7.4, 1.2 Hz, 1H, H_{arom}), 7.16 (td, *J* = 7.6, 1.7 Hz, 1H, H_{arom}), 4.59 (d, *J* = 2.4 Hz, 2H, N1-CH₂), 4.08 (d, *J* = 7.1 Hz, 2H, N7-CH₂), 3.98 (t, *J* = 7.2 Hz, 2H, N3-CH₂), 3.18 (t, *J* = 7.5 Hz, 2H, -CH₂), 3.10 (dd, *J*

= 8.0, 6.6 Hz, 2H, -CH₂), 3.06 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 1.74 (p, $J = 7.4$ Hz, 2H, -CH₂), 1.61 – 1.46 (m, 4H, -CH₂CH₂), 1.23 – 1.14 (m, 1H, N7-CH₂CH), 0.45 – 0.41 (m, 2H, -CH₂_{cyclopropyl}), 0.40 – 0.36 (m, 2H, -CH₂_{cyclopropyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.1 (C8 or C4), 153.0 (C8 or C4), 149.8 (CO), 147.7 (CO), 139.4 (C_{arom}), 132.5 (C_{arom}), 131.1 (C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 123.8 (C_{arom}), 105.8 (C5), 79.7 (C_{propargyl}), 72.8 (C_{propargyl}), 48.3 (N-CH₂), 42.5 (N-CH₂), 33.1 (N-CH₂), 30.0 (-CH₂), 28.6 (d, $^2J_{C,P} = 15.7$ Hz, 1C, PCH₂CH₂), 27.3 (d, $^1J_{C,P} = 136.7$ Hz, 1C, PCH₂), 26.3 (-CH₂), 20.1 (d, $^3J_{C,P} = 4.0$ Hz, PCH₂CH₂CH₂), 11.7 (-CH_{cyclopropyl}), 3.3 (-CH₂_{cyclopropyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₄H₂₉BrN₄O₅P [M+H]⁺: 563.1053; found: 563.1038.

B-22) (4-(8-(2-Bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-7-propyl-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 178–181 °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.59 (d, $J = 7.9$ Hz, 1H, H_{arom}), 7.37 (d, $J = 7.6$ Hz, 1H, H_{arom}), 7.30 (t, $J = 7.4$ Hz, 1H, H_{arom}), 7.16 (t, $J = 7.5$ Hz, 1H, H_{arom}), 4.59 (d, $J = 2.4$ Hz, 2H, N1-CH₂), 4.09 (t, $J = 7.4$ Hz, 2H, N-CH₂), 3.98 (t, $J = 7.1$ Hz, 2H, N-CH₂), 3.20 – 3.14 (m, 2H, -CH₂), 3.10 – 3.04 (m, 3H, -CH₂ and H_{propargyl}), 1.74 (p, $J = 7.3$ Hz, 2H, -CH₂), 1.66 – 1.53 (m, 4H, 2 x -CH₂), 1.53 – 1.45 (m, 2H, -CH₂), 0.81 (t, $J = 7.4$ Hz, 3H, -CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.0 (C8 or C4), 152.9 (C8 or C4), 149.8 (CO), 147.7 (CO), 139.3 (C_{arom}), 132.5 (C_{arom}), 131.1 (C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 123.8 (C_{arom}), 105.8 (C5), 79.7 (C_{propargyl}), 72.8 (C_{propargyl}), 46.0 (N-CH₂), 42.4 (N-CH₂), 33.3 (N-CH₂), 30.0 (-CH₂), 28.5 (d, $^2J_{C,P} = 15.8$ Hz, 1C, PCH₂CH₂), 27.3 (d, $^1J_{C,P} = 136.8$ Hz, 1C, PCH₂), 26.2 (-CH₂), 23.6 (-CH₂), 20.1 (d, $^3J_{C,P} = 4.1$ Hz, PCH₂CH₂CH₂), 10.6 (-CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆): δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₃H₂₉BrN₄O₅P [M+H]⁺: 551.1053; found: 553.1038.

B-23) (4-(7-Benzyl-8-(2-bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; m.p. 126–129 °C; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.55 (d, $J = 7.9$ Hz, 1H), 7.32 (t, $J = 7.4$ Hz, 2H), 7.29 – 7.25 (m, 3H), 7.17 – 7.10 (m, 3H), 5.50 (s, 2H), 4.59 (d, $J = 2.5$ Hz, 2H), 4.00 (t, $J = 7.2$ Hz, 2H), 3.08 – 3.04 (m, 3H), 3.01 (dd, $J = 8.2, 5.8$ Hz, 2H), 1.75 (p, $J = 7.4$ Hz, 2H), 1.62 – 1.46 (m, 4H). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.6 (C8 or C4), 153.3 (C8 or C4), 149.8 (CO), 147.7 (CO), 139.2 (C_{arom}), 136.4 (C_{arom}), 132.5 (C_{arom}), 130.9 (C_{arom}), 128.8 (2C, C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 127.7 (C_{arom}), 126.6 (2C, C_{arom}), 123.7 (C_{arom}), 106.0 (C5), 79.6 (C_{propargyl}), 72.8 (C_{propargyl}), 47.2 (N7-CH₂), 42.5 (N-CH₂), 32.9 (N-CH₂), 30.0 (-CH₂), 28.5 (d, $^2J_{C,P} = 15.5$ Hz, 1C, PCH₂CH₂), 27.2 (d, $^1J_{C,P} = 137.1$ Hz, 1C, PCH₂), 26.3 (-CH₂), 20.1 (d, $^3J_{C,P} = 4.2$ Hz, PCH₂CH₂CH₂). ³¹P-NMR (243 MHz, DMSO-*d*₆): δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₇H₂₈BrN₄O₅P [M+H]⁺: 599.1053; found: 599.1051.

C-1) Diethyl (4-(8-(2-Methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. 104–106 °C. ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] =

13.26 (s, 1H, N7-H), 7.18 (td, $J = 7.8, 1.7$ Hz, 1H, H_{arom}), 7.09 (dd, $J = 7.5, 1.7$ Hz, 1H, H_{arom}), 6.96 (dd, $J = 8.3, 1.0$ Hz, 1H, H_{arom}), 6.83 (td, $J = 7.4, 1.1$ Hz, 1H, H_{arom}), 4.59 (d, $J = 2.5$ Hz, 2H, N1-CH₂), 4.00 (t, $J = 6.8$ Hz, 2H, N3-CH₂), 3.98 – 3.90 (m, 4H, POCH₂), 3.78 (s, 3H, OCH₃), 3.05 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 3.01 – 2.91 (m, 4H, CH₂CH₂), 1.83 – 1.73 (m, 4H, CH₂CH₂), 1.48 (m, 2H, CH₂), 1.19 (t, $J = 7.0$ Hz, 6H, POCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 157.1 (C_{arom}-OCH₃), 154.4 (C8), 152.9 (CO), 150.0 (CO), 148.1 (C4), 129.5 (C_{arom}), 128.1 (C_{arom}), 127.6 (C_{arom}), 120.2 (C_{arom}), 110.6 (C_{arom}), 105.9 (C5), 79.7 (C_{propargyl}), 72.6 (C_{propargyl}), 60.7 (d, $^2J_{C,P} = 6.7$ Hz, 1C, POCH₂), 55.2 (OCH₃), 42.4 (N3-CH₂), 30.0 (N1-CH₂), 28.4 (CH₂), 28.3 (CH₂), 28.2 (d, $^2J_{C,P} = 15.1$ Hz, 1C, PCH₂CH₂), 24.1 (d, $^1J_{C,P} = 138.8$ Hz, 1C, PCH₂), 19.3 (d, $^3J_{C,P} = 5.2$ Hz, 1C, P(CH₂)₂CH₂), 16.2 (d, $^3J_{C,P} = 5.6$ Hz, 2C, POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₅H₃₃N₄O₆P [M+H]⁺: 517.2210 ; found: 517.2213.

C-2) Diethyl (4-(8-(2-methoxyphenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. 124–146 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.20 (ddd, $J = 8.2, 7.4, 1.7$ Hz, 1H, H_{arom}), 7.11 (dd, $J = 7.4, 1.7$ Hz, 1H, H_{arom}), 6.95 (dd, $J = 8.3, 1.1$ Hz, 1H, H_{arom}), 6.84 (td, $J = 7.3, 1.1$ Hz, 1H, H_{arom}), 4.57 (d, $J = 2.4$ Hz, 2H, N1-CH₂), 4.00 – 3.89 (m, 6H, N3-CH₂, POCH₂), 3.77 (s, 3H, OCH₃), 3.70 (s, 3H, N7-CH₃), 3.05 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 2.99 – 2.92 (m, 4H, CH₂CH₂), 1.81 – 1.71 (m, 4H, CH₂CH₂), 1.51 – 1.42 (m, 2H, CH₂), 1.18 (t, $J = 7.0$ Hz, 6H, POCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 157.3 (C_{arom}-OCH₃), 154.5 (C8), 153.4 (CO), 150.0 (CO), 147.6 (C6), 130.0 (C_{arom}), 128.1 (C_{arom}), 128.0 (C_{arom}), 120.4 (C_{arom}), 110.8 (C_{arom}), 106.4 (C5), 79.8 (C_{propargyl}), 72.8 (C_{propargyl}), 60.9 (d, $^2J_{C,P} = 6.4$ Hz, 2C, POCH₂), 55.4 (OCH₃), 42.3 (N3-CH₂), 31.4 (N7-CH₃), 30.0 (N1-CH₂), 28.3 (d, $^2J_{C,P} = 15.3$ Hz, 1C, PCH₂CH₂), 28.0 (CH₂), 26.6 (CH₂), 24.2 (d, $^1J_{C,P} = 138.9$ Hz, 1C, PCH₂), 19.4 (d, $^3J_{C,P} = 4.7$ Hz, 1C, P(CH₂)₂CH₂), 16.4 (d, $^3J_{C,P} = 5.6$ Hz, POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₆H₃₅N₄O₆P [M+H]⁺: 531.2367; found: 531.2361.

C-3) Diethyl (4-(8-(3-bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. 133–135 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.28 (br s, 1H), 7.45 (s, 1H, H_{arom}), 7.38 (d, $J = 7.8$ Hz, 1H, H_{arom}), 7.23 (t, $J = 7.7$ Hz, 1H, H_{arom}), 7.20 (d, $J = 7.7$ Hz, 1H, H_{arom}), 4.59 (s, 2H, N1-CH₂), 4.01 – 3.90 (m, 6H, N3-CH₂ and 2 x OCH₂), 3.05 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 3.05 – 2.95 (m, 4H, C8-CH₂CH₂), 1.82 – 1.74 (m, 4H, H_{alkyl}), 1.52 – 1.42 (m, 2H, H_{alkyl}), 1.19 (t, $J = 7.0$ Hz, 6H, 2 x CH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.7 (C8), 152.9 (CO), 150.0 (CO), 148.0 (C6), 143.3 (C_{arom}), 131.1 (C_{arom}), 130.4 (C_{arom}), 129.0 (C_{arom}), 127.4 (C_{arom}), 121.6 (C_{arom}), 106.0 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 60.8 (d, $^2J_{C,P} = 6.6$ Hz, POCH₂), 42.4 (N3-CH₂), 32.7 (CH₂), 30.1 (N1-CH₂), 29.7 (CH₂), 28.1 (d, $^2J_{C,P} = 16.4$ Hz, 1C, PCH₂CH₂), 24.1 (d, $^1J_{C,P} = 139.2$ Hz, 1C, PCH₂), 19.3 (d, $^3J_{C,P} = 5.0$ Hz, 1C, P(CH₂)₂CH₂), 16.2 (d, $^3J_{C,P} = 6.5$ Hz,

POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₄H₃₀BrN₄O₅P [M+H]⁺: 565.1210; found: 565.1226.

C-4 Diethyl (4-(8-(3-methylphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonate: White solid; m.p. 111–113 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.31 (br s, 1H, N7-H), 7.15 (t, *J* = 7.5 Hz, 1H, H_{arom}), 7.04 (s, 1H, H_{arom}), 6.98 (t, *J* = 7.7 Hz, 2H, H_{arom}), 4.59 (d, *J* = 2.2 Hz, 2H, N1-CH₂), 4.02 – 3.98 (m, 2H, N3-CH₂), 3.97 – 3.91 (m, 4H, 2 x OCH₂), 3.06 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 2.98 (s, 4H, C8-CH₂CH₂), 2.26 (s, 3H, C_{arom}CH₃), 1.83 – 1.74 (m, 4H, H_{alkyl}), 1.53 – 1.43 (m, 2H, H_{alkyl}), 1.19 (t, *J* = 7.0 Hz, 6H, 2 x OCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 154.1 (C8), 152.9 (CO), 150.0 (CO), 148.1 (C4), 140.3 (C_{arom}), 137.3 (C_{arom}), 128.9 (C_{arom}), 128.2 (C_{arom}), 126.8 (C_{arom}), 125.2 (C_{arom}), 105.8 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 60.8 (d, ²*J*_{C,P} = 6.1 Hz, POCH₂), 42.4 (N3-CH₂), 33.2 (CH₂), 30.0 (N1-CH₂), 28.1 (d, ²*J*_{C,P} = 15.4 Hz, 1C, PCH₂CH₂), 24.1 (d, ¹*J*_{C,P} = 138.5 Hz, 1C, PCH₂), 21.0 (CH₂), 19.3 (d, ³*J*_{C,P} = 4.4 Hz, 1C, P(CH₂)₂CH₂), 16.3 (d, ³*J*_{C,P} = 5.6 Hz, POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₅H₃₃N₄O₅P [M+H]⁺: 501.2261; found: 501.2262.

C-5 Diethyl (4-(7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-8-(3-(trifluoromethyl)phenethyl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonate: white solid; m.p. 121–124 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.62 (t, *J* = 1.7 Hz, 1H, H_{arom}), 7.59 – 7.49 (m, 3H, H_{arom}), 4.57 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 3.99 – 3.88 (m, 6H, N3-CH₂, POCH₂), 3.76 (s, 3H, N7-CH₃), 3.13 – 3.10 (m, 4H, CH₂CH₂), 3.05 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 1.81 – 1.69 (m, 4H, CH₂CH₂), 1.50 – 1.41 (m, 2H, CH₂), 1.18 (t, *J* = 7.0 Hz, 6H, POCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.7 (C8), 153.3 (CO), 149.8 (CO), 147.3 (C6), 141.9 (C_{arom}), 132.8 (C_{arom}), 129.2 (C_{arom}), 129.0 (q, ²*J*_{C,F} = 32.1 Hz, 1C, C_{arom}-CF₃), 125.1 (q, ³*J*_{C,F} = 3.9 Hz, 1C, C_{arom}), 124.3 (q, ¹*J*_{C,F} = 272.3 Hz, 1C, CF₃), 122.9 (q, ³*J*_{C,F} = 3.8 Hz, 1C, C_{arom}), 106.3 (C5), 79.6 (C_{propargyl}), 72.7 (C_{propargyl}), 60.8 (d, ²*J*_{C,P} = 6.2 Hz, 2C, POCH₂), 42.0 (N3-CH₂), 32.0 (N7-CH₃), 30.8 (N1-CH₂), 29.9 (CH₂), 28.1 (d, ²*J*_{C,P} = 16.5 Hz, 1C, PCH₂CH₂), 27.3 (CH₂), 24.1 (d, ¹*J*_{C,P} = 137.8 Hz, 1C, PCH₂), 19.2 (d, ³*J*_{C,P} = 4.7 Hz, 1C, P(CH₂)₂CH₂), 16.2 (d, ³*J*_{C,P} = 5.9 Hz, POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₆H₃₃F₃N₄O₅P [M+H]⁺: 569.2135; found: 569.2103.

C-6 Diethyl (4-(8-(3-methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonate: White solid; m.p. 135–137 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 13.29 (s, 1H, N7-H), 7.18 (t, *J* = 7.8 Hz, 1H, H_{arom}), 6.79 – 6.72 (m, 3H, H_{arom}), 4.59 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 4.01 – 3.98 (m, 2H, N3-CH₂), 3.98 – 3.90 (m, 4H, POCH₂), 3.71 (s, 3H, OCH₃), 3.05 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 3.00 (s, 4H, CH₂CH₂), 1.84 – 1.70 (m, 4H, CH₂CH₂), 1.54 – 1.40 (m, 2H, CH₂), 1.19 (t, *J* = 7.0 Hz, 6H, POCH₂CH₃). ¹³C-NMR (126 MHz, DMSO-*d*₆) δ [ppm] = 159.2 (C_{arom}-OCH₃), 154.0 (C8), 152.8 (CO), 150.0 (CO), 148.1 (C4), 142.0 (C_{arom}), 129.3 (C_{arom}), 120.4 (C_{arom}), 113.9 (C_{arom}),

111.6 (C_{arom}), 105.8 (C5), 79.7 (C_{propargyl}), 72.6 (C_{propargyl}), 60.7 (d, $^2J_{C,P}$ = 6.3 Hz, 1C, POCH₂), 54.8 (OCH₃), 42.4 (N3-CH₂), 33.2 (CH₂), 30.0 (N1-CH₂), 29.9 (CH₂), 28.2 (d, $^2J_{C,P}$ = 15.4 Hz, 1C, PCH₂CH₂), 24.1 (d, $^1J_{C,P}$ = 138.9 Hz, 1C, PCH₂), 19.3 (d, $^3J_{C,P}$ = 4.7 Hz, 1C, P(CH₂)₂CH₂), 16.2 (d, $^3J_{C,P}$ = 5.7 Hz, 2C, POCH₂CH₃). $^{31}\text{P-NMR}$ (243 MHz, DMSO-*d*₆) δ [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₅H₃₃N₄O₆P [M+H]⁺: 517.2210; found: 517.2221.

C-7) Diethyl (4-(1-(cyclobutylmethyl)-8-(3-methoxyphenethyl)-2,6-dioxo-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. 82–84 °C. $^1\text{H-NMR}$ (600 MHz, DMSO-*d*₆) δ [ppm] = 13.17 (br s, 1H, N7-H), 7.17 (t, J = 8.0 Hz, 1H, H_{arom}), 6.77 – 6.72 (m, 3H, H_{arom}), 3.97 (t, J = 7.0 Hz, 2H, N1-CH₂), 3.96 – 3.93 (m, 2H, N3-CH₂), 3.93 – 3.91 (m, 4H, POCH₂), 3.69 (s, 3H, OCH₃), 2.99 – 2.95 (m, 4H, C8-CH₂CH₂), 2.66 – 2.58 (m, 1H, H_{alkyl}), 1.91 – 1.85 (m, 2H, H_{alkyl}), 1.79 – 1.71 (m, 8H, H_{alkyl}), 1.44 (dq, J = 15.0, 8.2, 6.2 Hz, 2H, H_{alkyl}), 1.17 (t, J = 7.0 Hz, 6H, H_{alkyl}). $^{13}\text{C-NMR}$ (151 MHz, DMSO-*d*₆) δ [ppm] = 159.4 (C_{arom}-OCH₃), 154.2 (C8), 153.7 (CO), 151.0 (CO), 147.9 (C4), 142.2 (C_{arom}), 129.5 (C_{arom}), 120.6 (C_{arom}), 114.0 (C_{arom}), 111.8 (C_{arom}), 106.2 (C5), 60.9 (d, $^2J_{C,P}$ = 6.5 Hz, POCH₂), 55.0 (OCH₃), 45.1 (N3-CH₂), 42.4 (C_{alkyl}), 34.2 (C_{alkyl}), 33.5 (C_{alkyl}), 30.1 (N1-CH₂), 28.4 (d, $^2J_{C,P}$ = 16.4 Hz, 1C, PCH₂CH₂), 25.7 (2C, C_{cyclobutyle}), 24.3 (d, $^1J_{C,P}$ = 138.6 Hz, 1C, PCH₂), 19.4 (d, $^3J_{C,P}$ = 5.4 Hz, 1C, P(CH₂)₂CH₂), 17.9 (C_{cyclobutyle}), 16.4 (d, $^3J_{C,P}$ = 5.9 Hz, POCH₂CH₃). $^{31}\text{P-NMR}$ (243 MHz, DMSO-*d*₆) δ [ppm] = 32.8. HRMS (ESI-QTOF) calculated for C₂₇H₃₉N₄O₆P [M+H]⁺: 547.2680; found: 547.2678.

C-8) Diethyl (4-(8-(3,4-dimethoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. n.d. $^1\text{H NMR}$ (600 MHz, DMSO-*d*₆) δ 13.27 (s, 1H, N7-H), 6.83 (d, J = 8.2 Hz, 1H, H_{arom}), 6.78 (d, J = 2.0 Hz, 1H, H_{arom}), 6.69 (dd, J = 8.2, 2.0 Hz, 1H, H_{arom}), 4.59 (d, J = 2.5 Hz, 2H, N-CH₂), 4.00 (t, J = 6.9 Hz, 2H, N-CH₂), 3.97 – 3.91 (m, 4H, 2 x OCH₂), 3.70 (s, 6H, 2 x OCH₃), 3.06 (t, J = 2.4 Hz, 1H, H_{propargyl}), 2.99 – 2.93 (m, 4H, CH₂-CH₂), 1.82 – 1.74 (m, 4H, H_{alkyl}), 1.52 – 1.44 (m, 2H, H_{alkyl}), 1.19 (t, J = 7.0 Hz, 6H, 2 x OCH₂CH₃). $^{13}\text{C NMR}$ (151 MHz, DMSO) δ 154.2 (C_{xanthine}), 152.8 (C_{xanthine}), 150.0 (C_{xanthine}), 148.6 (C_{xanthine}), 148.1 (C_{xanthine}), 147.2 (C_{xanthine}), 132.8 (C_{arom}), 120.0 (C_{arom}), 112.2 (C_{arom}), 111.9 (C_{arom}), 105.8 (C5), 79.7 (C_{propargyl}), 72.6 (C_{propargyl}), 60.8 (d, $^2J_{C,P}$ = 6.6 Hz, POCH₂), 55.5 (2 x OCH₃), 42.4 (C_{alkyl}), 32.9 (C_{alkyl}), 30.3 (C_{alkyl}), 30.0 (C_{alkyl}), 28.2 (d, $^2J_{C,P}$ = 16.1 Hz, 1C, PCH₂CH₂), 24.0 (d, $^1J_{C,P}$ = 138.4 Hz, 1C, PCH₂), 19.3 (d, $^3J_{C,P}$ = 4.8 Hz, 1C, P(CH₂)₂CH₂), 16.2 (d, $^3J_{C,P}$ = 5.6 Hz, POCH₂CH₃). $^{31}\text{P NMR}$ (243 MHz, DMSO) δ 32.8.

C-9) Diethyl (4-(1-ethyl-2,6-dioxo-8-(2-phenylcyclopropyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: Off-white solid; m.p. 124-126 °C. $^1\text{H NMR}$ (600 MHz, DMSO-*d*₆): δ [ppm] = 13.10 (s, 1H, N7-H), 7.31 – 7.26 (m, 2H, H_{arom}), 7.21 – 7.16 (m, 3H, H_{arom}), 3.99 – 3.95 (m, 2H, N1-CH₂ or N3-CH₂), 3.95 – 3.87 (m, 6H, 2 x OCH₂ and N1-CH₂ or N3-CH₂), 2.25 – 2.20 (m, 1H, CH_{cyclopropyle}), 1.84 – 1.67 (m, 5H, CH_{cyclopropyle} and 2 x H_{alkyl}), 1.62 – 1.54 (m, 1H, CH_{cyclopropyle}), 1.48 – 1.37 (m, 2H,

H_{alkyl}), 1.16 (td, $J = 7.0, 2.7$ Hz, 6H, 2 x OCH₂CH₃), 1.10 (t, $J = 7.0$ Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO): δ [ppm] = 154.7 (C_{xanthine}), 153.5 (C_{xanthine}), 150.6 (C_{xanthine}), 148.1 (C_{xanthine}), 140.7 (C_{xanthine}), 128.6 (C_{arom}), 126.3 (C_{arom}), 125.9 (C_{arom}), 106.1 (C5), 60.9 (d, $^2J_{C,P} = 6.1$ Hz, POCH₂CH₂), 42.2 (C_{alkyl}), 35.7 (C_{alkyl}), 28.3 (d, $^2J_{C,P} = 16.1$ Hz, 1C, PCH₂CH₂), 27.1 (C_{cyclopropyle}), 24.1 (d, $^1J_{C,P} = 139.0$ Hz, 1C, PCH₂), 21.4 (C_{cyclopropyle}), 19.4 (d, $^3J_{C,P} = 5.1$ Hz, 1C, P(CH₂)₂CH₂), 17.5 (C_{cyclopropyl}), 16.4 (d, $^3J_{C,P} = 6.2$ Hz, POCH₂CH₃), 13.3 (CH₃). ³¹P NMR (243 MHz, DMSO): δ [ppm] = 32.9. LC-MS: positive mode [m/z] = 490.2 ([M+H]⁺). ESI-MS purity: 93.5%.

C-10) Diethyl (4-(1-ethyl-2,6-dioxo-8-(phenoxyethyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: Off-white solid; m.p. n.d. ¹H NMR (600 MHz, DMSO-*d*₆) δ 13.80 (s, 1H, N7-H), 7.34 – 7.29 (m, 1H, H_{arom}), 7.07 – 7.03 (m, 1H, H_{arom}), 7.00 – 6.96 (m, 1H, H_{arom}), 5.13 (s, 2H, C8-CH₂), 4.00 (t, $J = 6.9$ Hz, 2H, N-CH₂), 3.98 – 3.90 (m, 3H, H_{propargyl}), 1.81 – 1.73 (m, 4H, H_{alkyl}), 1.52 – 1.44 (m, 2H, H_{alkyl}), 1.18 (t, $J = 7.0$ Hz, 6H, 2 x OCH₂CH₃), 1.12 (t, $J = 7.0$ Hz, 3H, CH₂CH₃). ¹³C NMR (151 MHz, DMSO) δ 157.7 (C_{xanthine}), 153.9 (C_{xanthine}), 150.4 (C_{xanthine}), 148.6 (C_{xanthine}), 147.5 (C_{xanthine}), 129.5 (2C, C_{arom}), 121.3 (C_{arom}), 114.8 (2C, C_{arom}), 62.7 (OCH₂), 60.7 (2 x OCH₂), 42.3 (C_{alkyl}), 35.7 (C_{alkyl}), 28.3 (C_{alkyl}), 28.2 (d, $^2J_{C,P} = 15.6$ Hz, 1C, PCH₂CH₂), 24.0 (d, $^1J_{C,P} = 140.0$ Hz, 1C, PCH₂), 19.3 (d, $^3J_{C,P} = 4.8$ Hz, 1C, P(CH₂)₂CH₂), 16.2 (d, $^3J_{C,P} = 5.7$ Hz, POCH₂CH₃), 13.1 (C_{alkyl}). ³¹P NMR (243 MHz, DMSO) δ 32.8.

Synthesis of compounds class D (**D-1**) – (**D-3**)

D-1) Ethyl hydrogen (4-(8-(2-bromophenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. n.d.; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.60 (dd, $J = 7.9, 1.2$ Hz, 1H, H_{arom}), 7.35 (dd, $J = 7.7, 1.8$ Hz, 1H, H_{arom}), 7.31 (td, $J = 7.4, 1.2$ Hz, 1H, H_{arom}), 7.20 – 7.14 (m, 1H, H_{arom}), 4.59 (d, $J = 2.5$ Hz, 2H, N1-CH₂), 3.97 (t, $J = 7.0$ Hz, 2H, N3-CH₂), 3.92 – 3.85 (m, 2H, POCH₂), 3.74 (s, 3H, N7-CH₃), 3.13 (t, $J = 7.2$ Hz, 2H, -CH₂), 3.09 – 3.04 (m, 3H, -CH₂ and H_{propargyl}), 1.77 – 1.70 (m, 2H, -CH₂), 1.65 (ddd, $J = 18.0, 9.3, 6.5$ Hz, 2H, -CH₂), 1.52 – 1.41 (m, 2H, -CH₂), 1.18 (t, $J = 7.0$ Hz, 3H, POCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.5 (C8 or C4), 153.3 (C8 or C4), 149.8 (CO), 147.4 (CO), 139.3 (C_{arom}), 132.5 (C_{arom}), 131.0 (C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 123.7 (C_{arom}), 106.4 (C5), 79.6 (C_{propargyl}), 72.7 (C_{propargyl}), 59.8 (d, $^2J_{C,P} = 6.2$ Hz, 1C, POCH₂CH₃), 42.2 (N-CH₂), 32.9 (N-CH₂), 31.3 (N7-CH₃), 29.9 (-CH₂), 28.3 (d, $^2J_{C,P} = 15.8$ Hz, 1C, PCH₂CH₂), 26.3 (-CH₂), 25.4 (d, $^1J_{C,P} = 138.2$ Hz, 1C, PCH₂), 19.6 (d, $^3J_{C,P} = 4.4$ Hz, PCH₂CH₂CH₂), 16.4 (d, $^3J_{C,P} = 6.1$ Hz, POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO) δ [ppm] = 29.7.

D-2) Ethyl hydrogen (4-(8-(2-methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. 172–174 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm]

= 13.26 (br s, 1H, N7-H), 7.21 – 7.17 (m, 1H, H_{arom}), 7.16 – 7.13 (m, 1H, H_{arom}), 6.96 (d, $J = 8.2$ Hz, 1H, H_{arom}), 6.84 (t, $J = 7.4$ Hz, 1H, H_{arom}), 4.58 (d, $J = 2.5$ Hz, 2H, N1-CH₂), 4.17 (q, $J = 7.1$ Hz, 2H, POCH₂), 3.96 (t, $J = 7.1$ Hz, 2H, N3-CH₂), 3.77 (s, 3H, OCH₃), 3.05 (t, $J = 2.3$ Hz, 1H, H_{propargyl}), 2.96 (dt, $J = 11.4, 5.7$ Hz, 4H, C8-CH₂CH₂), 1.73 (p, $J = 7.4$ Hz, 2H, H_{alkyl}), 1.59 – 1.52 (m, 2H, H_{alkyl}), 1.51 – 1.45 (m, 2H, H_{alkyl}), 1.23 (t, $J = 7.1$ Hz, 3H, POCH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 157.3 (C_{arom}-OCH₃), 153.8 (C_{xanthine}), 153.0 (C_{xanthine}), 150.0 (C_{xanthine}), 147.9 (C_{xanthine}), 130.1 (C_{arom}), 128.1 (C_{arom}), 128.0 (C_{arom}), 120.4 (C_{arom}), 110.8 (C_{arom}), 105.6 (C5), 79.9 (C_{propargyl}), 72.9 (C_{propargyl}), 55.4 (2C, POCH₂ and OCH₃), 42.6 (N3-CH₂), 39.6 (POCH₂), 30.1 (N1-CH₂), 28.7 (d, ² $J_{C,P} = 16.5$ Hz, 1C, PCH₂CH₂), 28.6 (CH₂), 27.4 (d, ¹ $J_{C,P} = 136.0$ Hz, 1C, PCH₂), 26.4 (CH₂), 20.2 (d, ³ $J_{C,P} = 4.2$ Hz, 1C, PCH₂CH₂CH₂), 16.0 (POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.1. HRMS (ESI-QTOF) calculated for C₂₃H₃₀N₄O₆P [M+H]⁺: 489.1903; found: 489.1904.

D-3) Ethyl hydrogen (4-(8-(3-methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate: White solid; m.p. 118–120 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.19 (t, $J = 7.8$ Hz, 1H, H_{arom}), 6.85 – 6.82 (m, 2H, H_{arom}), 6.76 (dd, $J = 8.2, 2.3$ Hz, 1H, H_{arom}), 4.60 (d, $J = 2.2$ Hz, 2H, N1-CH₂), 4.18 (q, $J = 7.1$ Hz, 4H, POCH₂), 4.00 (t, $J = 7.1$ Hz, 2H, N3-CH₂), 3.71 (s, 3H, OCH₃), 3.10 – 3.08 (m, 1H, H_{propargyl}), 3.08 – 3.06 (m, 2H, C8-CH₂), 3.02 (t, $J = 7.4$ Hz, 2H, C8-CH₂CH₂), 1.79 – 1.73 (m, 2H, H_{alkyl}), 1.62 – 1.55 (m, 2H, H_{alkyl}), 1.51 (dq, $J = 15.5, 8.6, 7.3$ Hz, 2H, H_{alkyl}), 1.20 (t, $J = 7.1$ Hz, 3H, CH₂CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.8 (C_{arom}-OCH₃), 153.9 (C_{xanthine}), 153.3 (C_{xanthine}), 150.3 (C_{xanthine}), 148.2 (C_{xanthine}), 142.5 (C_{arom}), 129.8 (C_{arom}), 121.2 (C_{arom}), 114.6 (C_{arom}), 112.2 (C_{arom}), 105.9 (C5), 80.2 (C_{propargyl}), 73.2 (C_{propargyl}), 55.4 (OCH₃), 42.9 (N3-CH₂), 39.6 (POCH₂), 33.5 (CH₂), 30.4 (N1-CH₂), 29.0 (d, ² $J_{C,P} = 15.4$ Hz, 1C, PCH₂CH₂), 28.0 (CH₂), 27.7 (d, ¹ $J_{C,P} = 135$ Hz, 1C, PCH₂), 20.5 (d, ³ $J_{C,P} = 4.7$ Hz, 1C, PCH₂CH₂CH₂), 16.3 (POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 27.2. HRMS (ESI-QTOF) calculated for C₂₃H₃₀N₄O₆P [M+H]⁺: 489.1903; found: 489.1905.

E-1) 4-(8-(2-Bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butane-1-sulfonic acid: ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.58 (d, $J = 7.9$ Hz, 1H, H_{arom}), 7.31 – 7.29 (m, 2H, H_{arom}), 7.15 (dt, $J = 8.0, 4.5$ Hz, 1H, H_{arom}), 4.60 (d, $J = 2.5$ Hz, 2H, CH₂), 3.97 (t, $J = 7.2$ Hz, 2H, CH₂), 3.19 – 3.10 (m, 2H, CH₂), 3.07 – 3.00 (m, 3H, CH₂ and H_{propargyl}), 1.80 – 1.69 (m, 2H, H_{alkyl}), 1.64 – 1.54 (m, 2H, H_{alkyl}). ¹³C NMR (126 MHz, DMSO) δ 153.3 (C_{xanthine}), 152.9 (C_{xanthine}), 149.9 (C_{xanthine}), 147.9 (C_{xanthine}), 139.3 (C_{arom}), 132.5 (C_{arom}), 130.7 (C_{arom}), 128.4 (C_{arom}), 127.9 (C_{arom}), 123.7 (C_{arom}), 106.0 (C5), 79.7 (C_{propargyl}), 72.7 (C_{propargyl}), 51.1 (C_{alkyl}), 43.0 (C_{alkyl}), 33.5 (C_{alkyl}), 30.0 (C_{alkyl}), 28.3 (C_{alkyl}), 26.9 (C_{alkyl}), 22.2 (C_{alkyl}).

E-2) 4-(1-Ethyl-8-(3-methoxyphenethyl)-2,6-dioxo-1,2,6,7-tetrahydro-3H-purin-3-yl)butane-1-sulfonic acid: White solid; m.p. 179–181 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.17 (t, $J = 7.8$ Hz, 1H, H_{arom}), 6.81 – 6.77 (m, 2H, H_{arom}), 6.74 (dd, $J = 8.2, 2.5$ Hz, 1H, H_{arom}), 3.95 (t, $J = 7.1$ Hz, 2H,

N1-CH₂), 3.91 (q, $J = 7.0$ Hz, 2H, N3-CH₂), 3.71 (s, 3H, OCH₃), 3.02 – 2.96 (m, 4H, CH₂CH₂), 2.49 – 2.44 (m, 2H, CH₂), 1.78 – 1.67 (m, 2H, CH₂), 1.58 (q, $J = 7.7, 7.1$ Hz, 2H, CH₂), 1.11 (t, $J = 7.0$ Hz, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.4 (C_{arom}-OCH₃), 153.8 (C8), 153.6 (CO), 150.5 (CO), 147.7 (C6), 142.2 (C_{arom}), 129.5 (C_{arom}), 120.7 (C_{arom}), 114.0 (C_{arom}), 111.8 (C_{arom}), 106.4 (C5), 55.0 (OCH₃), 51.3 (CH₂SO₃H), 43.0 (N3-CH₂), 35.8 (CH₂), 33.5 (CH₂), 30.1 (CH₂), 27.2 (CH₂), 22.5 (CH₂), 13.4 (CH₃). HRMS (ESI-QTOF) calculated for C₂₀H₂₇N₄O₆S [M+H]⁺: 451.1646; found: 451.1639.

E-3) 4-(8-(3-Methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butane-1-sulfonic acid: White solid; m.p. 175–177 °C. ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 7.20 – 7.15 (m, 1H, H_{arom}), 6.81 – 6.76 (m, 2H, H_{arom}), 6.76 – 6.72 (m, 1H, H_{arom}), 4.60 (d, $J = 2.5$ Hz, 2H, N1-CH₂), 3.97 (t, $J = 7.2$ Hz, 2H, N3-CH₂), 3.71 (s, 3H, OCH₃), 3.04 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 3.02 – 2.99 (m, 4H, CH₂CH₂), 2.50 – 2.48 (m, 2H, CH₂), 1.78 – 1.68 (m, 2H, CH₂), 1.64 – 1.54 (m, 2H, CH₂). ¹³C-NMR (126 MHz, DMSO-*d*₆) δ [ppm] = 159.4 (C_{arom}-OCH₃), 154.1 (C8), 153.0 (CO), 150.1 (CO), 148.1 (C6), 142.2 (C_{arom}), 129.5 (C_{arom}), 120.7 (C_{arom}), 114.0 (C_{arom}), 111.8 (C_{arom}), 106.0 (C5), 79.9 (C_{propargyl}), 72.8 (C_{propargyl}), 55.0 (OCH₃), 51.2 (CH₂SO₃H), 43.2 (N3-CH₂), 33.4 (CH₂), 30.2 (CH₂), 30.1 (CH₂), 27.1 (CH₂), 22.4 (CH₂). HRMS (ESI-QTOF) calculated for C₂₁H₂₅N₄O₆S [M+H]⁺: 561.1489; found: 461.1482.

F-1) 4-(8-(2-Bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butane-1-sulfonamide: White solid; m.p. 216–219 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 7.59 (d, $J = 7.9$ Hz, 1H, H_{arom}), 7.31 – 7.28 (m, 2H, H_{arom}), 7.19 – 7.13 (m, 1H, H_{arom}), 6.72 (s, 2H, NH₂), 4.60 (d, $J = 2.5$ Hz, 2H, N-CH₂), 4.00 (t, $J = 7.0$ Hz, 2H, N-CH₂), 3.15 (dd, $J = 8.5, 6.7$ Hz, 2H, CH₂), 3.09 – 2.97 (m, 5H, H_{propargyl}, CH₂), 1.85 – 1.75 (m, 2H, CH₂), 1.76 – 1.65 (m, 2H, CH₂). ¹³C-NMR (126 MHz, DMSO-*d*₆) δ [ppm] = 153.7 (C8 or C4), 153.1 (C8 or C4), 150.2 (CO), 148.2 (CO), 139.5 (C_{arom}), 132.6 (C_{arom}), 130.8 (C_{arom}), 128.6 (C_{arom}), 128.0 (C_{arom}), 123.8 (C_{arom}), 106.3 (C5), 79.9 (C_{propargyl}), 72.8 (C_{propargyl}), 54.1 (CH₂), 42.6 (CH₂), 33.7 (CH₂), 30.2 (CH₂), 28.5 (CH₂), 26.4 (CH₂), 20.9 (CH₂).

G-1) 5-(8-(2-Methoxyphenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3*H*-purin-3-yl)pentanoic acid: White solid; m.p. 196–198 °C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 7.18 (td, $J = 7.9, 1.8$ Hz, 1H, H_{arom}), 7.08 (dd, $J = 7.4, 1.7$ Hz, 1H, H_{arom}), 6.95 (dd, $J = 8.3, 1.1$ Hz, 1H, H_{arom}), 6.82 (td, $J = 7.4, 1.1$ Hz, 1H, H_{arom}), 4.59 (d, $J = 2.5$ Hz, 2H, N1-CH₂), 3.98 (t, $J = 7.1$ Hz, 2H, N3-CH₂), 3.04 (t, $J = 2.4$ Hz, 1H, H_{propargyl}), 3.01 – 2.90 (m, 4H, CH₂CH₂), 2.26 (t, $J = 7.3$ Hz, 2H, CH₂), 1.75 – 1.63 (m, 2H, CH₂), 1.56 – 1.45 (m, 2H, CH₂). ¹³C-NMR (126 MHz, DMSO-*d*₆) δ [ppm] = 174.2 (COOH), 157.1 (C8), 154.3 (CO), 152.8 (CO), 150.0 (C6), 148.0 (C_{arom}), 129.6 (C_{arom}), 128.1 (C_{arom}), 127.6 (C_{arom}), 120.2 (C_{arom}), 110.6 (C_{arom}), 105.9 (C5), 79.8 (C_{propargyl}), 72.6 (C_{propargyl}), 55.2 (OCH₃), 42.6 (N3-CH₂),

33.2 (CH₂), 30.0 (CH₂), 28.4 (CH₂), 28.3 (CH₂), 27.0 (CH₂), 21.6 (CH₂). HRMS (ESI-QTOF) calculated for C₂₂H₂₅N₄O₅ [M+H]⁺: 425.1819; found: 425.1830.

G-2) 5-(1-(Cyclobutylmethyl)-2,6-dioxo-8-(3-(trifluoromethyl)phenethyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)pentanoic acid: Off-white solid; m.p. 210–212 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.04 (br s, 1H, COOH), 7.48 – 7.52 (m, 4H, H_{arom}), 3.97 – 3.89 (m, 4H, N1-CH₂, N3-CH₂), 3.11 (t, *J* = 7.6 Hz, 2H, CH₂), 3.02 (t, *J* = 7.5 Hz, 2H, CH₂), 2.62 (m, 1H, N1-CH₂CH), 2.23 (t, *J* = 7.4 Hz, 2H, H_{alkyl}), 1.88 (td, *J* = 8.0, 7.4, 3.4 Hz, 2H, H_{alkyl}), 1.73 – 1.77 (m, 6H, H_{alkyl}), 1.65 (p, *J* = 7.3 Hz, 2H, H_{alkyl}), 1.47 (p, *J* = 7.5 Hz, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 174.4 (C=O), 154.2 (C8), 153.3 (CO), 151.0 (CO), 147.8 (C6), 142.1 (C_{arom}), 132.7 (C_{arom}), 129.4 (C_{arom}), 129.1 (q, ²*J*_{C,F} = 31.5 Hz, 1C, C_{arom}-CF₃), 124.4 (q, ¹*J*_{C,F} = 275 Hz, 1C, CF₃), 125.1 (q, ³*J*_{C,F} = 4.0 Hz, 1C, C_{arom}), 123.0 (q, *J*_{C,F} = 4.1 Hz, 1C, C_{arom}), 106.3 (C5), 45.1 (N1-CH₂), 42.6 (N3-CH₂), 34.2 (C_{alkyl}), 33.3 (C_{alkyl}), 33.0 (C_{alkyl}), 29.8 (C_{alkyl}), 27.1 (C_{alkyl}), 25.7 (2C, C_{cyclobutyle}), 21.6 (C_{alkyl}), 17.9 (C_{alkyl}). HRMS (ESI-QTOF) calculated for C₂₄H₂₇F₃N₄O₄ [M+H]⁺: 493.2057; found: 493.2077.

H-1) 3-(8-(3-Methoxyphenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 60%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.18 (t, *J* = 7.8 Hz, 1H, H_{arom}), 6.87 – 6.60 (m, 3H, H_{arom}), 4.57 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 4.12 – 3.95 (m, 2H, N3-CH₂), 3.88 (s, 3H, N8-CH₃), 3.71 (m, 5H, OCH₂, OCH₃), 3.08 – 2.98 (m, 4H, C_{8alkyl}), 2.97 (m, 1H, H_{propargyl}), 1.96 (m, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.46 (C_{arom}-OCH₃), 154.32 (C8), 153.42 (C6), 149.95 (C2), 147.40 (C4), 142.13 (C_{arom}), 129.50 (C_{arom}), 120.86 (C_{arom}), 114.17 (C5), 111.97 (C_{arom}), 106.48 (C_{arom}), 79.83 (C_{propargyl}), 72.89 (C_{propargyl}), 63.29 (N7-CH₃), 55.09 (OCH₃), 32.93 (N1-CH₃), 31.46 (N2-CH₃), 30.06, 28.89, 27.92 (C_{alkyls}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] = 0.01. (HRMS (ESI-QTOF) calculated for C₂₁H₂₅N₄O₇P [M+H]⁺: 499.1359; found: 499.1353).

H-2) 3-(8-(3-Methoxyphenethyl)-7-methyl-2,6-dioxo-1-propyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 64%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.17 (t, *J* = 8.0 Hz, 1H, H_{arom}), 6.84 – 6.78 (m, 2H, H_{arom}), 6.79 – 6.53 (m, 1H, H_{arom}), 4.03 (t, *J* = 7.3 Hz, 2H, N1-CH₂), 3.86 (q, *J* = 6.6 Hz, 2H, N3-CH₂), 3.84 (s, 3H, N8-CH₃), 3.70-3.86 (m, 5H, H_{alkyl}, OCH₃), 3.02 (m, 2H, H_{alkyl}), 2.95-2.94 (m, 2H, H_{alkyl}), 1.95-1.93 (m, 2H, H_{alkyl}), 1.54 (m, 2H, H_{alkyl}), 0.84 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.77 (C_{arom}-OCH₃), 154.70 (C8), 154.15 (C6), 150.82 (C2), 147.42 (C4), 142.51 (C5), 129.80, 121.17, 114.46, 112.27, 106.92 (C_{arom}), 63.51 (N7-CH₃), 55.39 (OCH₃), 42.31, 33.26, 31.68, 29.27, 28.22, 21.28, 11.64 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.22. (HRMS (ESI-QTOF) calculated for C₂₁H₂₉N₄O₇P [M+Na]⁺: 503.1672; found: 503.1666).

H-3) (E)-3-(8-(3-Methoxystyryl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 41%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.77 (br s, NH), 7.65 (d, *J* = 16.4 Hz, 1H, CH=CH), 7.32-7.30 (m, 1H, H_{arom}), 7.22 – 7.16 (m, 2H, H_{arom}, CH=CH), 7.12 (d, *J* = 16.4 Hz, 1H, CH=CH), 6.96 – 6.90 (m, 1H, H_{arom}), 4.61 (d, *J* = 2.5 Hz, 2H, N1-CH₂), 4.14 – 4.08 (m, 2H, N3-CH₂), 3.92 (q, *J* = 6.7 Hz, 2H, OCH₂), 3.80 (s, 3H, OCH₃), 3.09 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 2.02 (p, *J* = 6.8 Hz, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.84 (C_{arom}), 153.06 (C8), 150.20 (C6), 136.98 (C2), 135.68 (C4), 130.15 (C=C), 130.10 (C=C), 119.86 (C_{arom}), 116.25 (C_{arom}), 115.36 (C_{arom}), 112.18 (C_{arom}), 79.84 (C_{propargyl}), 73.00 (C_{propargyl}), 63.43 (OCH₂), 51.23 (OCH₂), 30.36, 28.90 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.03. HRMS (ESI-QTOF) calculated for C₂₀H₂₁N₄O₇P [M+H]⁺: 461.1226; found: 461.1221).

H-4) (E)-3-(2,6-Dioxo-1-propyl-8-styryl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 61%; ¹H NMR (500 MHz, DMSO-*d*₆) δ 13.56 (s, 1H, 7-NH), 7.82 – 7.56 (m, 3H, H_{arom}, CH=C-), 7.38 (dt, *J* = 31.0, 7.3 Hz, 3H, H_{arom}), 7.04 (d, *J* = 16.4 Hz, 1H, C=CH), 4.13 – 4.04 (m, 2H, N1CH₂), 3.91 (q, *J* = 6.7 Hz, 2H, N3CH₂), 3.87 – 3.81 (m, 2H, OCH₂), 2.01 (p, *J* = 6.7 Hz, 2H, H_{alkyl}), 1.66 – 1.35 (m, 2H, H_{alkyl}), 0.86 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 154.02 (C8), 150.76 (C6), 149.62 (C2), 148.27 (C4), 135.56 (C=C), 135.36 (C=C), 129.23 (C5), 129.10 (C_{arom}), 127.27 (C_{arom}), 115.93 (C_{arom}), 107.42 (C_{arom}), 63.38 (OCH₂), 40.23 (N1_{alkyl}), 28.95 23 (N3_{alkyl}), 28.89, 20.98, 11.33 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.03. HRMS (ESI-QTOF) calculated for C₁₉H₂₃N₄O₆P [M+H]⁺: 457.1253; found: 457.1247).

H-5) (E)-3-(7-Methyl-2,6-dioxo-1-propyl-8-styryl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; 42%; ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.71 (d, *J* = 7.5 Hz, 2H, H_{arom}), 7.58 (d, *J* = 15.7 Hz, 1H, CH=CH), 7.40 – 7.30 (m, 3H, H_{arom}), 7.23 (d, *J* = 15.7 Hz, 1H, CH=CH), 4.05 (t, *J* = 7.5 Hz, 2H, N1-CH₂), 3.97 (s, 3H, N-CH₃), 3.85 (q, *J* = 6.6 Hz, 2H, N3-CH₂), 3.73 (d, *J* = 2.5 Hz, 2H, OCH₂), 1.97-1.95 (m, 2H, H_{alkyl}), 1.51-1.49 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 154.02, (C8) 150.76 (C6), 149.62 (C2), 148.27 (C4), 135.56 (CH=CH), 135.36 (CH=CH), 129.23 (C5), 129.10, 127.27, 115.93, 107.40 (C_{arom}), 63.38 (N-CH₃), 63.34, (OCH₂) 28.95, 28.89, 20.98, 11.33 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.68. HRMS (ESI-QTOF) calculated for C₂₀H₂₅N₄O₆P [M+Na]⁺: 471.1409; found: 471.1404).

H-6) (E)-3-(8-(3-Methoxystyryl)-7-methyl-2,6-dioxo-1-propyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 52%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.63 (d, *J* = 15.1 Hz, 1H, CH=CH), 7.34-7.31 (m, 4H, H_{arom}, CH=CH), 7.00 – 6.88 (m, 1H, H_{arom}), 4.12-4.11 (m, 2H, N1-CH₂), 4.03 (s, 3H, N7-CH₃), 3.91.3.90 (m, 2H, N3CH₂), 3.81 (s, 3H, OCH₃), 2.02-2.00 (m, 2H, H_{alkyl}), 1.56-1.55 (m, 2H, H_{alkyl}), 0.86 (t, *J* = 7.5 Hz, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.81 (C_{arom}), 154.36 (C8), 150.59 (C6), 149.80 (C2),

147.78 (C4), 137.13 (C=C), 136.87 (C=C), 129.95 (C5), 120.42 (C_{arom}), 115.37 (C_{arom}), 113.24 (C_{arom}), 112.64 (C_{arom}), 107.56 (C_{arom}), 63.44 (N-CH₃), 55.39 (OCH₃), 31.61, 20.98, 11.35 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.02. HRMS (ESI-QTOF) calculated for C₂₁H₂₇N₄O₇P [M+Na]⁺: 501.1515; found: 501.1510).

H-7) (E)-3-(1-Ethyl-8-(3-methoxystyryl)-2,6-dioxo-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 46%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.64 (d, *J* = 15.7 Hz, 1H, CH=CH), 7.40 – 7.36 (m, 2H, H_{arom}), 7.35 – 7.29 (m, 2H, H_{arom}, CH=CH), 6.93 (d, *J* = 7.7, 1H, H_{arom}), 4.14 – 4.06 (m, 2H, N1CH₂), 3.91-3.90 (m, 4H, N3-CH₂, OCH₂), 3.81 (s, 3H, OCH₃), 2.02-2.01 (m, 2H, H_{alkyl}), 1.12 (t, *J* = 7.0 Hz, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.82 (C_{arom}), 154.17 (C8), 150.40 (C6), 149.81 (C2), 137.14 (C4), 136.90 (C=C), 129.95 (C=C), 120.43 (C5), 115.40 (C_{arom}), 113.28 (C_{arom}), 112.63 (C_{arom}), 107.62 (C_{arom}), 63.46 (OCH₂), 55.42 (OCH₃), 35.72, 31.65, 13.31 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.20. HRMS (ESI-QTOF) calculated for C₁₉H₂₃N₄O₇P [M+Na]⁺: 473.1202; found: 473.1197).

H-8) 3-(8-(3-Methoxybenzyl)-2,6-dioxo-1-propyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 46%; ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.12 (d, *J* = 8.1 Hz, 2H, H_{arom}), 6.84 (d, *J* = 8.1 Hz, 2H, H_{arom}), 4.08 (s, 2H, C8CH₂), 4.01 (t, *J* = 7.5 Hz, 2H, N1-CH₂), 3.73 (s, N7-CH₃), 3.67-3.62 (m, 7H, N3-CH₂, OCH₃, OCH₂), 1.59 – 1.38 (m, 2H, H_{alkyl}), 1.13 (t, *J* = 7.1 Hz, 2H, CH₂), 0.80 (t, *J* = 7.5 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO) δ 158.18 (C_{arom}), 154.39 (C8), 153.08 (C6), 150.41 (C2), 147.14 (C4), 129.56 (C8), 127.81 (C_{arom}), 114.23 (C_{arom}), 106.91 (C5), 55.14 (OCH₂), 45.59 (OCH₃), 31.75 (N7-CH₃), 31.37, 20.91, 11.29 (C_{alkyls}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.08. HRMS (ESI-QTOF) calculated for C₂₀H₂₇N₄O₇P [M+Na]⁺: 489.1515; found: 489.1510).

H-9): 3-(8-(4-Chlorobenzyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl dihydrogen phosphate: White solid; Yield 51%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.35 (d, *J* = 8.0 Hz, 2H, H_{arom}), 7.30 (d, *J* = 8.3 Hz, 2H, H_{arom}), 4.55 (s, 3H, N7-CH₃), 4.02 (t, *J* = 7.4 Hz, 2H, N1-CH₂), 3.83-3.82 (m, 2H, N3-CH₂), 3.03 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 1.93-1.92 (m, 2H, H_{alkyl}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 153.19 (C8), 152.54 (C6), 150.09 (C2), 148.21 (C4), 136.02 (C_{arom}), 131.54 (C_{arom}), 130.59 (C_{arom}), 128.63 (C_{arom}), 79.83 (C_{propargyl}), 72.82 (C_{propargyl}), 62.76 (OCH₂), 33.65 (N7-CH₃), 30.22, 28.95, 28.94 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 0.37. HRMS (ESI-QTOF) calculated for C₁₈H₁₈ClN₄O₆P [M+H]⁺: 475.0550; found: 475.0545.

J-1): (E)-(4-(8-(3-Methoxystyryl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonic acid: White solid; Yield 58; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 12.0 (s, 1H, NH), 7.62 (d, 1H, H_{vinyl}), 7.29-7.33 (m, 1H), 7.15-7.24 (m, 2H), 7.07 (d, *J* = 16.35 Hz, 1H, H_{vinyl}), 6.93 (m, 1H, H_{arom}), 4.60 (d, *J* = 2.4 Hz, C_{propargyl}), 4.02 (t, *J* = 6.62, 7.14 Hz, 2H, NCH₂), 3.80 (d, *J* = 8.20, 3H,

OCH₃), 3.07 (t, $J = 2.40$, 1H, H_{propargyl}), 1.79 (m, 2H, PCH₂), 1.59 (m, 2H), 1.53 (m, 2H). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 159.8, 153.0, 150.1, 150.0, 148.7, 136.9, 135.7, 130.1, 119.8, 116.2, 115.4, 112.1, 107.1, 79.8, 72.9, 55.3, 43.0, 30.3, 28.8, 28.0, 26.9, 20.3, 20.2. ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 27.17.

J-2): (4-(8-Cyclopentyl-2,6-dioxo-1-propyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; Yield 13%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.07 (br s, 1H, 7-NH), 3.95 (t, $J = 7.1$ Hz, 2H, N1-CH₂), 3.81 (d, $J = 6.3$ Hz, 2H, N3-CH₂), 3.12 (m, 1H, H_{cyclopentyl}), 2.03 – 1.89 (m, 2H, H_{alkyl}), 1.83 – 1.64 (m, 6H, H_{alkyl}, H_{cyclopentyl}), 1.53-1.51 (m, 8H, H_{alkyl}, H_{cyclopentyl}), 0.84 (t, $J = 7.4$ Hz, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 158.02 (C8), 154.00 (C6), 150.76 (C2), 147.83 (C4), 106.23 (C5), 42.56 (N1-CH₂), 42.12 (N3-CH₂), 32.08 (C_{cyclopentyl}), 28.81, 28.00, 27.09, 25.19, 20.99, 20.21, 11.31 (C-alkyl, C_{cyclopentyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 29.21. HRMS (ESI-QTOF): n.d.

J-3) (4-(2,6-Dioxo-8-phenyl-1-propyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)butyl)phosphonic acid: White solid; Yield 17%; ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = ¹H NMR (600 MHz, DMSO-*d*₆) δ 13.82 (br s, 1H, 7-H), 8.25 – 7.86 (m, 2H, H_{arom}), 7.50-7.48 (m, 3H, H_{arom}), 4.23 – 3.99 (m, 2H, N1-CH₂), 3.92 – 3.79 (m, 2H, N3-CH₂), 1.96 – 1.75 (m, 2H, H_{alkyl}), 1.63 – 1.47 (m, 6H, H_{alkyl}), 1.08 – 0.70 (m, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = ¹³C NMR (151 MHz, DMSO-*d*₆) δ 154.22 (C8), 150.78 (C6), 150.06 (C2), 148.40 (C4), 130.37 (C5), 129.09, 126.65, 107.91 (C_{arom}), 28.80, 27.88, 26.98, 21.00, 20.21, 11.34 (C_{alkyl}). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ [ppm] 27.21. HRMS (ESI-QTOF) calculated for C₁₈H₂₃N₄O₅P [M+Na]⁺: 429.1304; found: 429.1298).

The potency of the xanthine derivatives was determined at the human wild-type MRGPRX4 (Table 1) and, in addition, at its rare variant L83S (containing a single point mutation, see Table 2), in β -arrestin recruitment as well as calcium mobilization assays. For β -arrestin recruitment assays, recombinant Chinese hamster ovary (CHO) cells expressing the human wildtype MRGPRX4 were used (DiscoverX/Eurofins) which allowed to measure β -galactosidase complementation upon β -arrestin recruitment. For calcium mobilization assays, recombinant CHO cells expressing the wildtype MRGPXR, or LN229 glioblastoma cells natively expressing the MRGPRX4 L83S mutant were used.

The assays were performed as previously described for other rhodopsin-like GPCRs (Müller, C.E.; 8-Benzamidochromen-4-one-2-carboxylic acids: potent and selective agonists for the orphan G protein-coupled receptor GPR35. *J. Med. Chem.* 2013, 56:5182-97; Müller, C.E. Molecular Recognition of Agonists and Antagonists by the Nucleotide-Activated G Protein-Coupled P2Y 2 Receptor. *J. Med. Chem.* 2017, 60:8425-8440).

Table 1. Potency of selected compounds in activating the human MRGPRX4

	β-arrestin assay (CHO β-arrestin ARMS1-ProLink™ 2 human MRGPRX4 wild type)			Calcium assay (human MRGPRX4 wild type)		
	EC₅₀ ± SEM (μM)^a	E_{max}^b (%)	IC₅₀ ± SEM (μM)^{a,c}	EC₅₀ ± SEM (μM)^a	E_{max}^d (%)	IC₅₀ ± SEM (μM)^{a,e}
A-1)	>10	n.d.	>10	>10	n.d.	>10
B-1)	>10	n.d.	>10	3.25 ± 0.61	110	n.d.
B-2)	5.71 ± 1.23	91	n.d.	0.435 ± 0.078	102	n.d.
B-3)	7.94 ± 1.45	89	n.d.	0.601 ± 0.103	110	n.d.
B-4)	2.14 ± 0.85	103	n.d.	0.0808 ± 0.0168	100	n.d.
B-5)	15.2 ± 0.52	99	n.d.	0.492 ± 0.038	91	n.d.
B-6)	11.9 ± 1.8	114	n.d.	0.267 ± 0.066	99	n.d.
B-7)	3.12 ± 1.20	103	n.d.	0.397 ± 0.032	108	n.d.
B-8)	0.243 ± 0.045	98	n.d.	0.0227 ± 0.0072	114	n.d.
B-9)	1.26 ± 0.27	107	n.d.	0.0552 ± 0.00170	107	n.d.
B-10)	14.5 ± 2.4	100	n.d.	0.425 ± 0.187	113	n.d.
B-11)	>10	n.d.	50.5 ± 1.4	28.0 ± 3.3	100	n.d.
B-12)	>10	n.d.	>10 (-27 ± 9)	3.80 ± 0.19	104	n.d.
B-13)	48.3 ± 14.1	96	n.d.	1.60 ± 0.46	94	n.d.
B-14)	>10	n.d.	>10 (3 ± 15)	9.45 ± 3.23	99	n.d.
B-15)	6.28 ± 1.38	141	n.d.	0.333 ± 0.007	113	n.d.
B-16)	0.370 ± 0.050	100	n.d.	0.0324 ± 0.0056	100	n.d.
B-17)	0.169 ± 0.060	114	n.d.	0.00223 ± 0.00013	109	n.d.
B-18)	0.0935 ± 0.0054	132	n.d.	0.00635 ± 0.00167	112	n.d.
B-19)	0.0259 ± 0.0079	144	n.d.	0.00426 ± 0.00056	115	n.d.
B-20)	0.0329 ± 0.0075	124	n.d.	0.00186 ± 0.00045	103	n.d.
B-21)	0.0898 ± 0.0209	163	n.d.	0.00202 ± 0.00062	109	n.d.

B-22)	0.208 ± 0.049	176	n.d.	0.0178 ± 0.0062	119	n.d.
B-23)	0.0599 ± 0.0128	164	n.d.	0.00503 ± 0.00159	103	n.d.
C-1)	>10	n.d.	>10	>10	n.d.	>10
C-2)	>10	n.d.	>10	>10	n.d.	>10
C-3)	>10	n.d.	>10	>10	n.d.	>10
C-4)	>10	n.d.	>10	>10	n.d.	>10
C-5)	>10	n.d.	>10	>10	n.d.	>10
C-6)	>10	n.d.	>10	>10	n.d.	>10
C-7)	>10	n.d.	>10	>10	n.d.	>10
C-8)	>10	n.d.	>10	>10	n.d.	>10
C-9)	>10	n.d.	>10	>10	n.d.	>10
C-10)	>10	n.d.	>10	>10	n.d.	>10
D-1)	0.395 ± 0.126	108	n.d.	0.0555 ± 0.0090	115	n.d.
D-2)	0.113 ± 0.033	122	n.d.	3.32 ± 0.61	103	n.d.
D-3)	0.407 ± 0.71	112	n.d.	0.0363 ± 0.0018	114	n.d.
E-1)	>10	n.d.	>10	>10	n.d.	>10
E-2)	>10	n.d.	>10	>10	n.d.	>10
E-3)	>10	n.d.	>10	>10	n.d.	>10
F-1)	>10	n.d.	>10	>10	n.d.	>10
G-1)	>10	n.d.	>10	>10	n.d.	>10
G-2)	>10	n.d.	>10	>10	n.d.	>10
H-1)	13.0 ± 4.1	97	n.d.	0.574 ± 0.179	107	n.d.
H-2)	>10	n.d.	>10	2.50 ± 0.73	113	n.d.
H-3)	>10	n.d.	>10	>10	n.d.	>10
H-4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-6)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-9)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-10)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
J-1)	>10	n.d.	inhibition	>10	n.d.	inhibition
J-2)	>10	n.d.	>10	>50	n.d.	>50
J-3)	>10	n.d.	>10	>10	n.d.	>10

^aThe results represent means of 3-4 independent experiments.

^bEfficacies are based on the maximal effect of 100 nM B-16 (set as 100%).

^cInhibition of the effect of 2.6 μM B-16 (corresponding to its EC₈₀ value)

^dEfficacy based on the maximal effect of 1 μM B-16 (set as 100%).

^eInhibition of the effect of 100 nM B-16 (corresponding to its EC₈₀ value)

Table 2. Effects of compounds on MRGPRX4 natural variant receptor L83S determined in β -arrestin recruitment and calcium mobilization assays.

	β -arrestin assay (CHO β -arrestin human MRGPRX4 natural variant L83S)			Calcium assay (human glioblastoma LN229 cells expressing the MPGPRX4 natural variant L83S)		
	EC ₅₀ \pm SEM (μ M) ^a	E _{max} ^b (%)	IC ₅₀ \pm SEM (μ M) ^{a,c}	EC ₅₀ \pm SEM (μ M) ^a	E _{max} (%) ^d	IC ₅₀ (%) ^{a,e}
A-1)	n.d.		n.d.	>10		>10
B-1)	0.399 \pm 0.115	84	n.d.	0.134 \pm 0.017	92	n.d.
B-2)	0.0320 \pm 0.0110	98	n.d.	0.0112 \pm 0.0013	99	n.d.
B-3)	0.0339 \pm 0.0051	93	n.d.	0.0631 \pm 0.0186	118	n.d.
B-4)	0.00324 \pm 0.00116	110	n.d.	0.00469 \pm 0.00158	109	n.d.
B-5)	0.0343 \pm 0.0076	103	n.d.	0.0181 \pm 0.0015	96	n.d.
B-6)	0.0205 \pm 0.0024	100	n.d.	0.0382 \pm 0.0109	150	n.d.
B-7)	0.00894 \pm 0.00138	101	n.d.	0.0103 \pm 0.0013	132	n.d.
B-8)	0.00315 \pm 0.00082	143	n.d.	n.d.	n.d.	n.d.
B-9)	0.00345 \pm 0.00028	116	n.d.	n.d.	n.d.	n.d.
B-10)	0.0714 \pm 0.0193	87	n.d.	0.0306 \pm 0.0031	82	n.d.
B-11)	n.d.	n.d.	9.72 \pm 1.05	n.d.	n.d.	n.d.
B-12)	1.19 \pm 0.05	102	n.d.	0.214 \pm 0.051	94	n.d.
B-13)	0.0689 \pm 0.0170	93	n.d.	0.0599 \pm 0.0107	91	n.d.
B-14)	0.735 \pm 0.131	81	n.d.	0.500 \pm 0.113	74	n.d.
B-15)	0.0300 \pm 0.0058	140	n.d.	0.0192 \pm 0.0035	132	n.d.
B-16)	0.00228 \pm 0.00016	100	n.d.	0.00317 \pm 0.00060	137	n.d.
B-18)	0.000296 \pm 0.000033	128	n.d.	0.000501 \pm 0.000076	108	n.d.
C-1)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-2)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-3)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-4)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-5)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-6)	>10	n.d.	n.d.	n.d.	n.d.	n.d.

C-7)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-8)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-9)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
C-10)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
D-1)	0.114 ± 0.023	120	n.d.	0.134 ± 0.012	105	n.d.
D-2)	0.000493 ± 0.000104	141	n.d.	n.d.	n.d.	n.d.
D-3)	0.00580 ± 0.00141	110	n.d.	0.00265 ± 0.00072	111	n.d.
E-1)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
E-2)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
E-3)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
F-1)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
G-1)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
G-2)	>10	n.d.	n.d.	n.d.	n.d.	n.d.
H-1)	0.374 ± 0.111	86	n.d.	0.238 ± 0.011	98	n.d.
H-2)	0.674 ± 0.050	n.d.	n.d.	0.801 ± 0.233	88	n.d.
H-3)	>10	n.d.	n.d.	>10	n.d.	>10
J-1)	0.200 ± 0.059	65	n.d.	0.0116 ± 0.0013		n.d.
J-2)	6.36 ± 1.63	76	n.d.	>10		4.45 ± 0.68
J-3)	>10		>10	>10		>10

^aThe results represent means of 3-4 independent experiments.

^bEfficacy based on 100 nM B-16 (set as 100%)

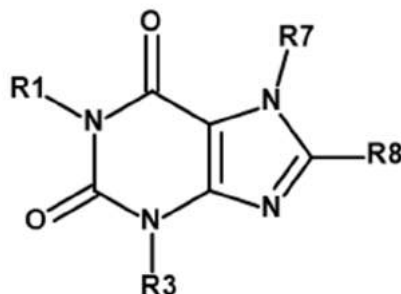
^cInhibition of the effect of 6 nM B-16 (corresponding to its EC₈₀ value) or of 500 nM H-1 (corresponding to its EC₈₀ value).

^dEfficacy based on 30 μM H-1 (set as 100%)

^eInhibition of 1 μM H-1 (corresponding to its EC₈₀).

Patent claims:

1. A compound according to general Formula 1



wherein

- R1** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;
- R3** represents -C₁₋₁₀-alkyl-P(=O)(OC₁₋₁₀-alkyl)₂, -C₁₋₁₀-alkyl-P(=O)(OH)(OC₁₋₁₀-alkyl), -C₁₋₁₀-alkyl-S(=O)₂(OH), -C₁₋₁₀-alkyl-S(=O)₂(NH₂), -C₁₋₁₀-alkyl-C(=O)(OH), or -C₁₋₁₀-alkyl-P(=O)(OH)₂;
- R7** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl; and
- R8** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-O-aryl, -C₃₋₆-cycloalkyl-aryl, -C₃₋₆-cycloalkyl-heteroaryl, -C₁₋₁₀-alkyl-heteroaryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

wherein in each case "C₁₋₁₀-alkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

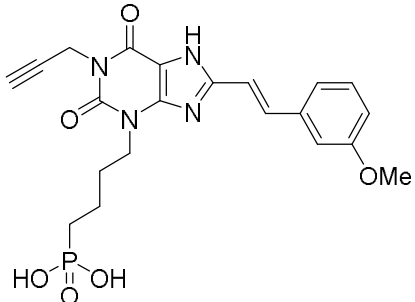
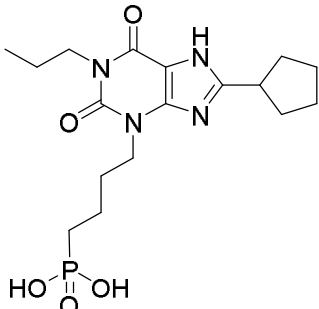
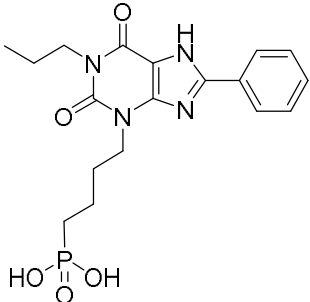
wherein in each case "C₃₋₁₀-cycloalkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "aryl" is a 6-14-membered aryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from -C≡CH, -CF₃, -CCl₃, -CBr₃, -Cl₃, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "heteroaryl" is a 5-14-membered heteroaryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from $-C\equiv CH$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-Cl_3$, $-C(=O)OH$, $-C(=O)O-C_{1-10}\text{-alkyl}$, $-OH$, $-O-C_{1-10}\text{-alkyl}$, $-NH_2$, $-NH-C_{1-10}\text{-alkyl}$, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$;

or a physiologically acceptable salt thereof;

with the proviso that the compound is not a compound selected from the group consisting of compounds J-1 to J-3:

J-1)	
J-2)	
J-3)	

2. The compound according to claim 1, wherein

(i) **R3** represents $-C_{1-10}\text{-alkyl-P(=O)(OH)}_2$ and **R8** represents $-C_{3-6}\text{-cycloalkyl-aryl}$, $-C_{3-6}\text{-cycloalkyl-heteroaryl}$, $-C_{1-10}\text{-alkyl-aryl}$ or $-C_{1-10}\text{-alkyl-heteroaryl}$ (wherein in case of $-C_2\text{-alkyl-aryl}$, the alkyl-moiety is saturated);

and wherein in each case **R1** and **R7** independently from one another are selected from $-H$, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, and $-C_{1-10}\text{-alkyl-heteroaryl}$; or

wherein

(ii) **R3** represents $-C_{1-10}\text{-alkyl-P(=O)(OC}_{1-10}\text{-alkyl)}_2$; or

R3 represents $-C_{1-10}\text{-alkyl-P(=O)(OH)(OC}_{1-10}\text{-alkyl)}$; or

R3 represents $-C_{1-10}\text{-alkyl-S(=O)}_2\text{(OH)}$; or

R3 represents $-C_{1-10}\text{-alkyl-S(=O)}_2\text{(NH}_2\text{)}$; or

R3 represents $-C_{1-10}\text{-alkyl-C(=O)(OH)}$;

and wherein in each case **R1**, **R7**, and **R8** independently from one another are selected from -H, $-C_{1-10}\text{-alkyl}$, $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-aryl}$, $-C_{1-10}\text{-alkyl-heteroaryl}$, $-C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-O-aryl}$, and $-C_{1-10}\text{-alkyl-O-heteroaryl}$.

3. The compound according to claim 1 or 2, wherein

R1 represents $-C_{1-10}\text{-alkyl}$, optionally substituted with $-C\equiv CH$; or $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$; preferably $-C_{1-6}\text{-alkyl}$, optionally substituted with $-C\equiv CH$; or $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$; more preferably $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH_2C\equiv CH$, or $-CH_2\text{-cyclobutyl}$;

and/or

R3 represents $-C_{3-5}\text{-alkyl-P(=O)(OH)}_2$; $-C_{3-5}\text{-alkyl-O-P(=O)(OH)}_2$; $-C_{3-5}\text{-alkyl-P(=O)(OC}_{1-6}\text{-alkyl)}_2$; $-C_{3-5}\text{-alkyl-P(=O)(OH)(OC}_{1-6}\text{-alkyl)}$; $-C_{3-5}\text{-alkyl-S(=O)}_2\text{(OH)}$; $-C_{3-5}\text{-alkyl-S(=O)}_2\text{(NH}_2\text{)}$; or $-C_{3-5}\text{-alkyl-C(=O)(OH)}$; preferably $-C_{3-5}\text{-alkyl-P(=O)(OH)}_2$; more preferably $-C_4\text{-alkyl-P(=O)(OH)}_2$;

and/or

R7 represents -H; $-C_{1-10}\text{-alkyl}$, optionally substituted with -OH; $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$; or $-C_{1-10}\text{-alkyl-aryl}$; preferably -H; $-C_{1-6}\text{-alkyl}$, optionally substituted with -OH; $-C_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$; or $-C_{1-6}\text{-alkyl-aryl}$; more preferably -H, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2\text{-OH}$, $-CH_2CH_2CH_3$, $-CH_2\text{-cyclopropyl}$, or $-CH_2\text{-phenyl}$;

and/or

R8 represents $-C_{3-10}\text{-cycloalkyl}$, $-C_{1-10}\text{-alkyl-C}_{3-10}\text{-cycloalkyl}$, -aryl, $-C_{1-10}\text{-alkyl-aryl}$, $-C_{3-6}\text{-cycloalkyl-aryl}$, or $-C_{1-10}\text{-alkyl-O-aryl}$; preferably -phenyl, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2\text{-phenyl}$, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2CH_2\text{-phenyl}$, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH=CH\text{-phenyl}$, optionally substituted with -F, -Br, -Cl, $-CH_3$, $-CF_3$, $-OCH_3$; $-CH_2\text{-O-phenyl}$, optionally substituted with -F, -Br, -Cl, -

CH₃, -CF₃, -OCH₃; -cyclopropyl-phenyl, optionally substituted with -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; or -C₃₋₆-cycloalkyl;

more preferably -phenyl, optionally substituted with -OCH₃; -CH₂-phenyl, optionally substituted with -Cl; -CH₂CH₂-phenyl, optionally substituted with one or two substituents independently of one another selected from -F, -Br, -Cl, -CH₃, -CF₃, -OCH₃; -CH=CH-phenyl, optionally substituted with -OCH₃; -CH₂-O-phenyl; -cyclopropyl-phenyl; or -cyclopentyl.

4. The compound according to any of the preceding claims,

wherein

- (i) **R3** represents -C₁₋₆-alkyl-P(=O)(OH)₂ and **R8** represents -C₃₋₆-cycloalkyl-phenyl or -C₁₋₆-alkyl-phenyl (wherein in case of -C₂-alkyl-phenyl, the alkyl-moiety is saturated);

and wherein in each case **R1** and **R7** independently from one another are selected from -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, and -C₁₋₆-alkyl-heteroaryl; or

wherein

- (ii) **R3** represents -C₁₋₆-alkyl-P(=O)(OC₁₋₆-alkyl)₂; or

R3 represents -C₁₋₆-alkyl-P(=O)(OH)(OC₁₋₆-alkyl); or

R3 represents -C₁₋₆-alkyl-S(=O)₂(OH); or

R3 represents -C₁₋₆-alkyl-S(=O)₂(NH₂); or

R3 represents -C₁₋₆-alkyl-C(=O)(OH);

and wherein in each case **R1**, **R7**, and **R8** independently from one another are selected from -H, -C₁₋₆-alkyl, -C₁₋₆-alkyl-C₃₋₆-cycloalkyl, -C₁₋₆-alkyl-aryl, -C₁₋₆-alkyl-heteroaryl, -C₃₋₆-cycloalkyl, -C₁₋₆-O-aryl, and -C₁₋₆-O-heteroaryl;

wherein in each case "C₁₋₆-alkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "C₃₋₆-cycloalkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

or a physiologically acceptable salt thereof.

5. The compound according to any of the preceding claims,

wherein

(i) **R3** represents $-C_4\text{-alkyl-P(=O)(OH)}_2$ and **R8** represents $-C_3\text{-cycloalkyl-aryl}$ or $-C_{1-6}\text{-alkyl-phenyl}$ (wherein in case of $-C_2\text{-alkyl-phenyl}$, the alkyl-moiety is saturated);

and wherein in each case **R1** and **R7** are independently from one another are selected from $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-}C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, and $-C_{1-6}\text{-alkyl-heteroaryl}$; or

wherein

(ii) **R3** represents $-C_4\text{-alkyl-P(=O)(OC}_{1-6}\text{-alkyl)}_2$; or

R3 represents $-C_4\text{-alkyl-P(=O)(OH)(OC}_{1-6}\text{-alkyl)}$; or

R3 represents $-C_4\text{-alkyl-S(=O)}_2\text{(OH)}$; or

R3 represents $-C_4\text{-alkyl-S(=O)}_2\text{(NH}_2\text{)}$; or

R3 represents $-C_4\text{-alkyl-C(=O)(OH)}$;

and wherein in each case **R1**, **R7**, and **R8** independently from one another are selected from $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-}C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, $-C_{1-6}\text{-alkyl-heteroaryl}$, $-C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-O-aryl}$, and $-C_{1-6}\text{-O-heteroaryl}$;

wherein in each case " $C_{1-6}\text{-alkyl}$ " may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from $-C\equiv CH$, $-C(=O)OH$, $-C(=O)O-C_{1-10}\text{-alkyl}$, $-OH$, $-O-C_{1-10}\text{-alkyl}$, $-NH_2$, $-NH-C_{1-10}\text{-alkyl}$, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$;

wherein in each case " $C_{3-6}\text{-cycloalkyl}$ " may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from $-C\equiv CH$, $-C(=O)OH$, $-C(=O)O-C_{1-6}\text{-alkyl}$, $-OH$, $-O-C_{1-10}\text{-alkyl}$, $-NH_2$, $-NH-C_{1-10}\text{-alkyl}$, $-N(C_{1-10}\text{-alkyl})_2$, $-N_3$, $-F$, $-Cl$, $-Br$, and $-I$;

or a physiologically acceptable salt thereof.

6. The compound according any of the preceding claims,

wherein

(i) **R3** represents $-CH_2CH_2CH_2CH_2\text{-P(=O)(OH)}_2$ and **R8** represents -cyclopropyl-aryl , $-CH_2\text{-phenyl}$ or $-CH_2CH_2\text{-phenyl}$ (wherein in case of $-CH_2CH_2\text{-phenyl}$, the ethyl-moiety is saturated);

and wherein in each case **R1** and **R7** independently from one another are selected from $-H$, $-C_{1-6}\text{-alkyl}$, $-C_{1-6}\text{-alkyl-}C_{3-6}\text{-cycloalkyl}$, $-C_{1-6}\text{-alkyl-aryl}$, and $-C_{1-6}\text{-alkyl-heteroaryl}$; or

wherein

(ii) **R3** represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-P(=O)(OC}_{1-6}\text{-alkyl)}_2$; or

R3 represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-P(=O)(OH)(OC}_{1-6}\text{-alkyl)}$; or

R3 represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-S(=O)}_2\text{(OH)}$; or

R3 represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-S(=O)}_2\text{(NH}_2\text{)}$; or

R3 represents $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C(=O)(OH)}$;

and wherein in each case **R1**, **R7**, and **R8** independently from one another are selected from $-\text{H}$, $-\text{C}_{1-6}\text{-alkyl}$, $-\text{C}_{1-6}\text{-alkyl-C}_{3-6}\text{-cycloalkyl}$, $-\text{C}_{1-6}\text{-alkyl-aryl}$, $-\text{C}_{1-6}\text{-alkyl-heteroaryl}$, $-\text{C}_{3-6}\text{-cycloalkyl}$, $-\text{C}_{1-6}\text{-O-aryl}$, and $-\text{C}_{1-6}\text{-O-heteroaryl}$;

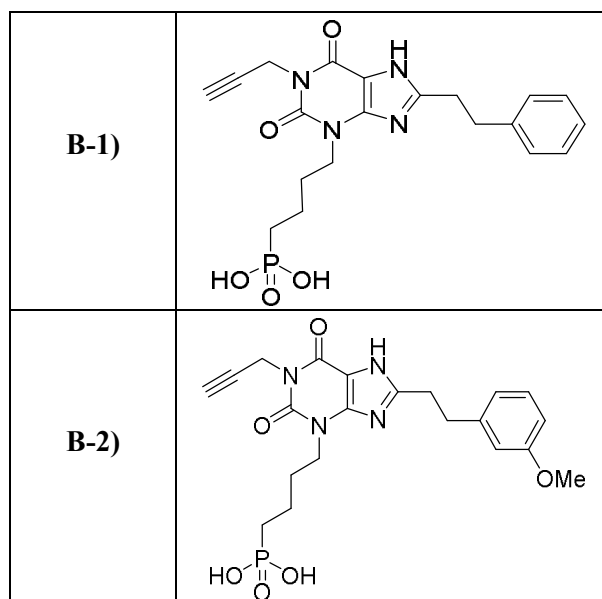
wherein in each case " $\text{C}_{1-6}\text{-alkyl}$ " may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from $-\text{C}\equiv\text{CH}$, $-\text{C(=O)OH}$, $-\text{C(=O)O-C}_{1-10}\text{-alkyl}$, $-\text{OH}$, $-\text{O-C}_{1-10}\text{-alkyl}$, $-\text{NH}_2$, $-\text{NH-C}_{1-10}\text{-alkyl}$, $-\text{N(C}_{1-10}\text{-alkyl)}_2$, $-\text{N}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, and $-\text{I}$;

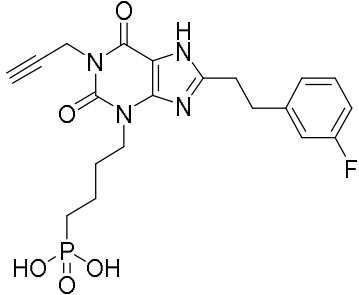
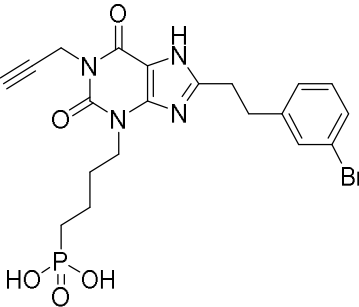
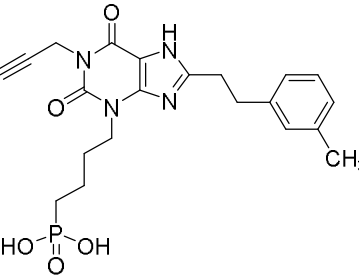
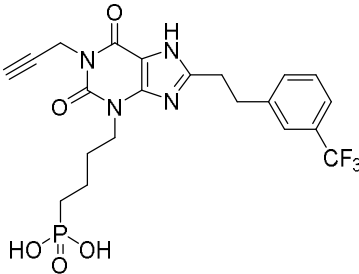
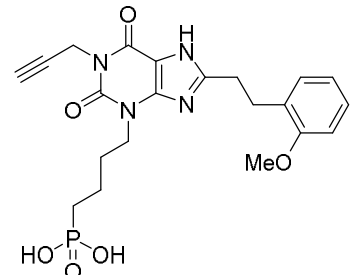
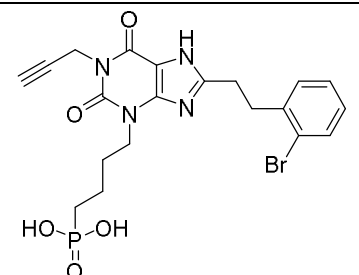
wherein in each case " $\text{C}_{3-6}\text{-cycloalkyl}$ " may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from $-\text{C}\equiv\text{CH}$, $-\text{C(=O)OH}$, $-\text{C(=O)O-C}_{1-6}\text{-alkyl}$, $-\text{OH}$, $-\text{O-C}_{1-10}\text{-alkyl}$, $-\text{NH}_2$, $-\text{NH-C}_{1-10}\text{-alkyl}$, $-\text{N(C}_{1-10}\text{-alkyl)}_2$, $-\text{N}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, and $-\text{I}$;

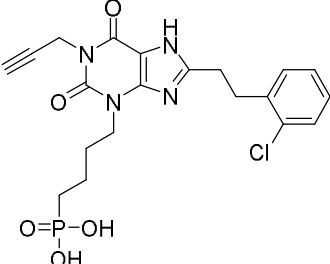
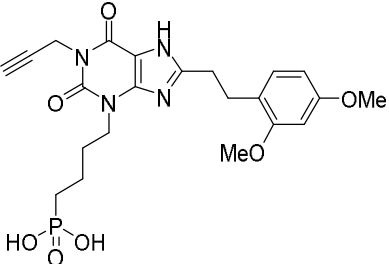
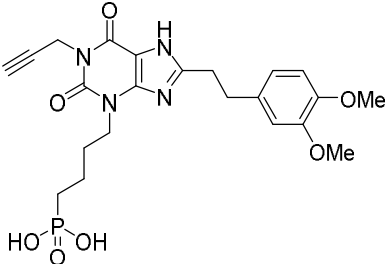
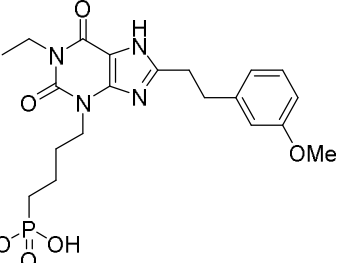
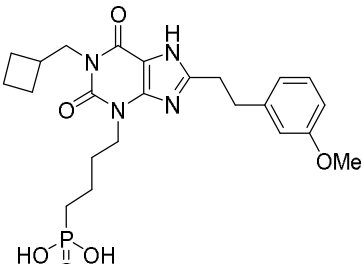
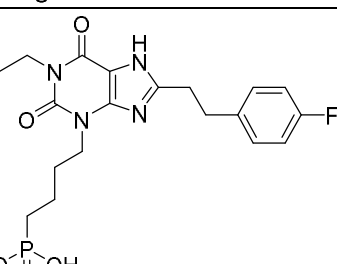
or a physiologically acceptable salt thereof.

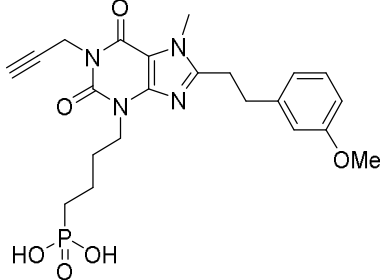
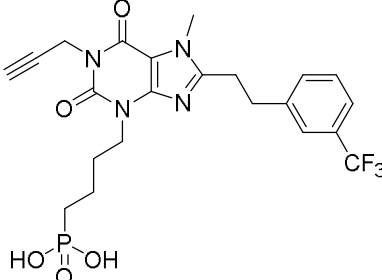
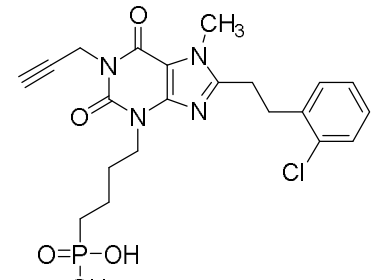
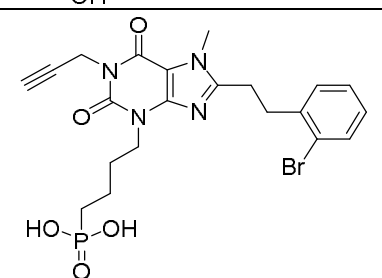
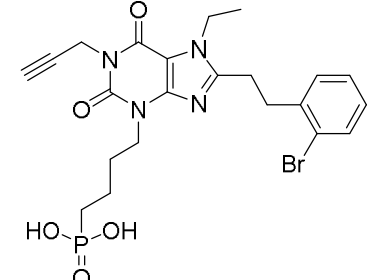
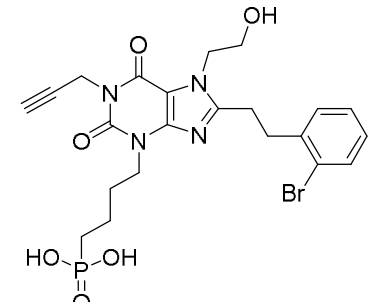
7. The compound according any of the preceding claims which is selected from compounds

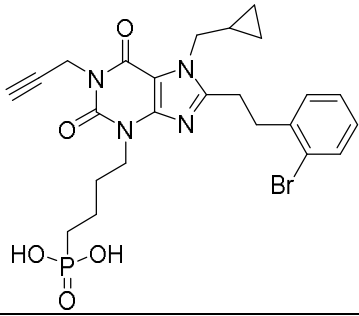
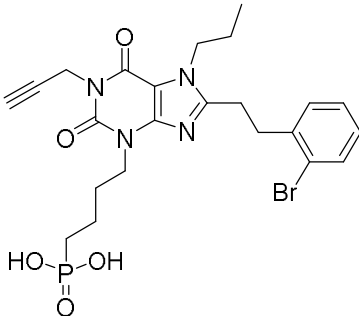
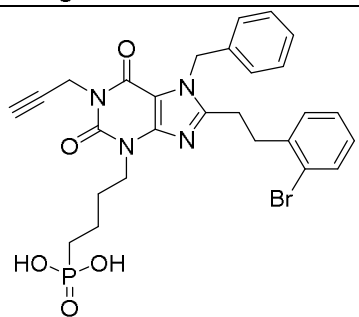
(I) B-1 to B-23 and the physiologically acceptable salts thereof:



B-3)	
B-4)	
B-5)	
B-6)	
B-7)	
B-8)	

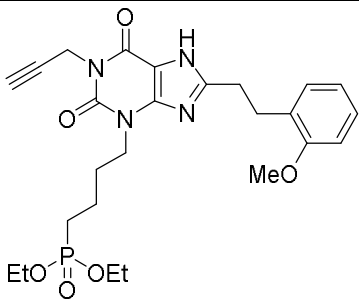
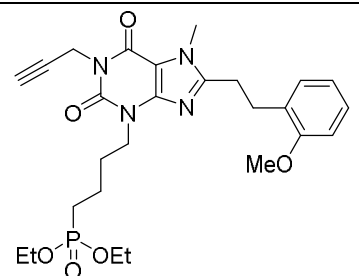
B-9)	 <chem>CC#CC1=NC(=O)N(CCCOP(=O)(O)O)C(=O)N1C2=NC=CC=C2Cl</chem>
B-10)	 <chem>CC#CC1=NC(=O)N(CCCOP(=O)(O)O)C(=O)N1C2=CC(OC)=CC(OC)=C2</chem>
B-11)	 <chem>CC#CC1=NC(=O)N(CCCOP(=O)(O)O)C(=O)N1C2=CC(OC)=CC(OC)=C2</chem>
B-12)	 <chem>CCN1C(=O)N(CCCOP(=O)(O)O)C(=O)N1C2=CC=C(C=C2)OC</chem>
B-13)	 <chem>C1CC1CN1C(=O)N(CCCOP(=O)(O)O)C(=O)N1C2=CC=C(C=C2)OC</chem>
B-14)	 <chem>CCN1C(=O)N(CCCOP(=O)(O)O)C(=O)N1C2=CC=C(C=C2)F</chem>

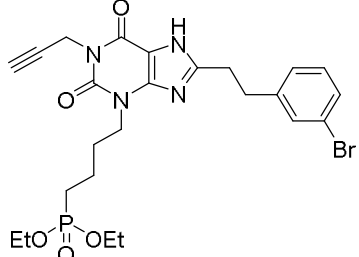
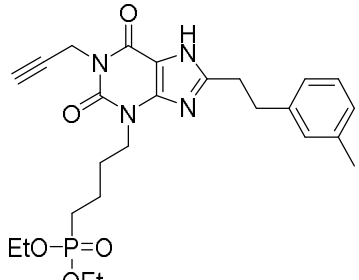
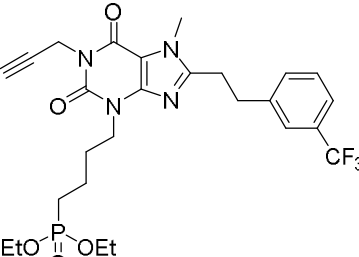
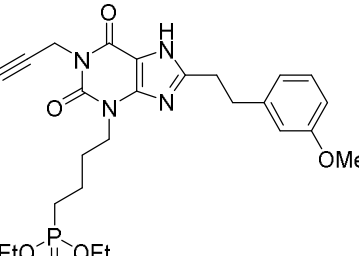
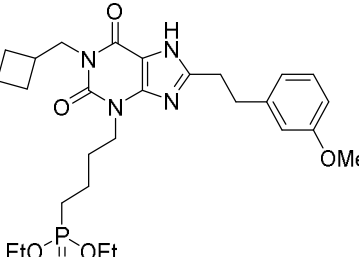
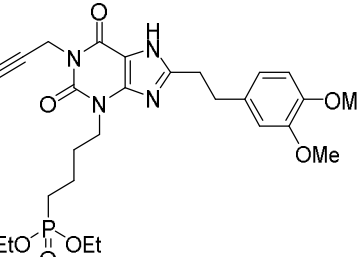
B-15)	 <chem>C#CCN1C(=O)N(C)C(=O)N1C(=O)N(C)C2=CN(C)C=C2C3=CC=C(C=C3)OCOP(=O)(O)O</chem>
B-16)	 <chem>C#CCN1C(=O)N(C)C(=O)N1C(=O)N(C)C2=CN(C)C=C2C3=CC=C(C=C3)C(F)(F)FOP(=O)(O)O</chem>
B-17)	 <chem>C#CCN1C(=O)N(C)C(=O)N1C(=O)N(C)C2=CN(C)C=C2C3=CC=C(C=C3)ClOP(=O)(O)O</chem>
B-18)	 <chem>C#CCN1C(=O)N(C)C(=O)N1C(=O)N(C)C2=CN(C)C=C2C3=CC=C(C=C3)BrOP(=O)(O)O</chem>
B-19)	 <chem>C#CCN1C(=O)N(CC)C(=O)N1C(=O)N(CC)C2=CN(C)C=C2C3=CC=C(C=C3)BrOP(=O)(O)O</chem>
B-20)	 <chem>C#CCN1C(=O)N(CO)C(=O)N1C(=O)N(CO)C2=CN(C)C=C2C3=CC=C(C=C3)BrOP(=O)(O)O</chem>

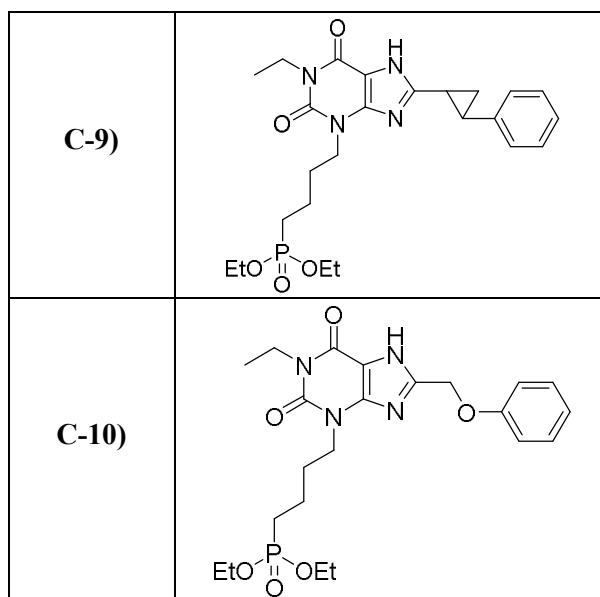
B-21)	
B-22)	
B-23)	

and

(II) C-1 to C-10 and the physiologically acceptable salts thereof:

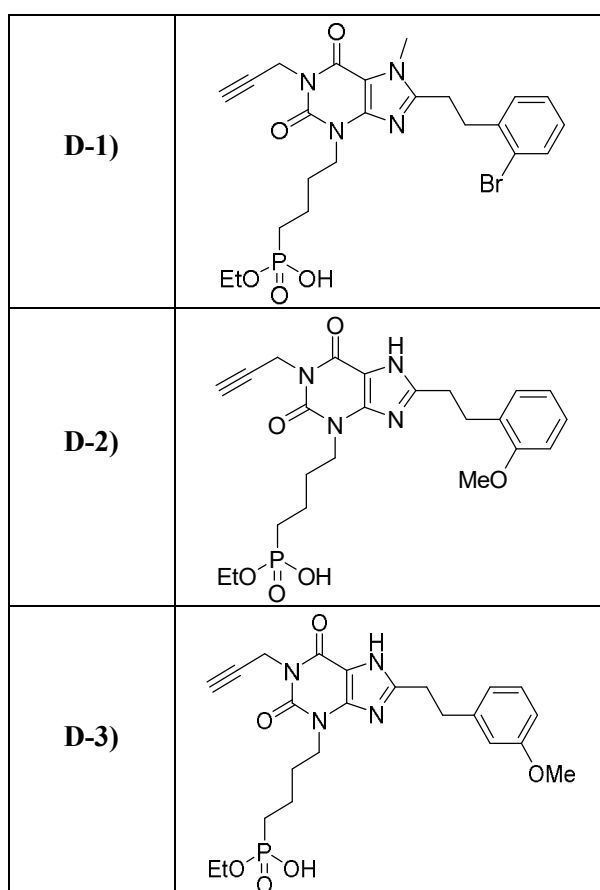
C-1)	
C-2)	

C-3)	 <chem>CCOP(=O)(OCC)CCCCN1C(=O)N(C#CC)C(=O)N1C2=NC=CC=C2Br</chem>
C-4)	 <chem>CCOP(=O)(OCC)CCCCN1C(=O)N(C#CC)C(=O)N1C2=NC=CC=C2C</chem>
C-5)	 <chem>CCOP(=O)(OCC)CCCCN1C(=O)N(C#CC)C(=O)N1C2=NC=CC=C2C(F)(F)F</chem>
C-6)	 <chem>CCOP(=O)(OCC)CCCCN1C(=O)N(C#CC)C(=O)N1C2=NC=CC=C2OC</chem>
C-7)	 <chem>CCOP(=O)(OCC)CCCCN1C(=O)N(C1CCC1)C(=O)N1C2=NC=CC=C2OC</chem>
C-8)	 <chem>CCOP(=O)(OCC)CCCCN1C(=O)N(C#CC)C(=O)N1C2=NC(OC)C(OC)=C2</chem>



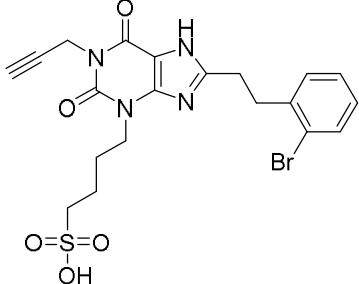
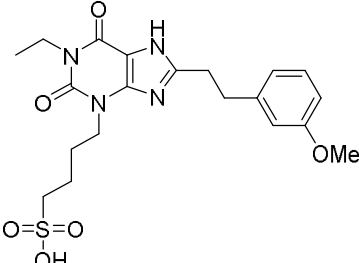
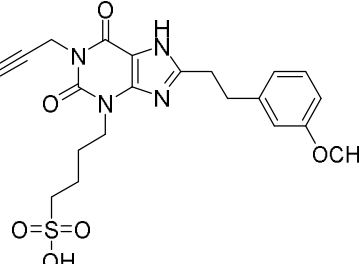
and

(III) D-1 to D-3 and the physiologically acceptable salts thereof:



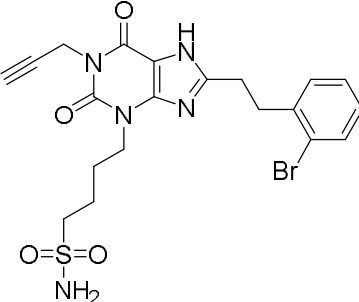
and

(IV) E-1 to E-3 and the physiologically acceptable salts thereof:

E-1)	
E-2)	
E-3)	

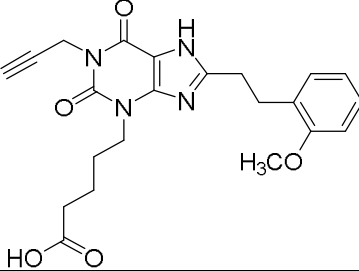
and

(V) F-1 and the physiologically acceptable salts thereof:

F-1)	
-------------	--

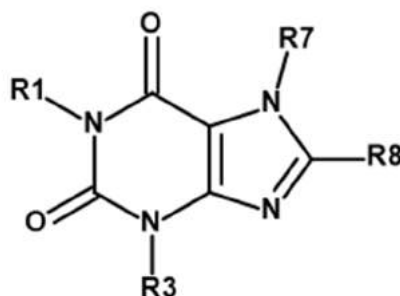
and

(VI) G-1 and G-2 and the physiologically acceptable salts thereof:

G-1)	
-------------	--

G-2)	
-------------	--

8. The compound according to any of the preceding claims for use as a medicament.
9. The compound according to any of claims 1 to 7 for use in the prevention or treatment of a condition, disease or disorder that is associated with the MRGPRX4 receptor, preferably associated with the wildtype of the MRGPRX4 receptor.
10. A compound according to general Formula 1



wherein

- R1** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl;
- R3** represents -C₁₋₁₀-alkyl-P(=O)(OC₁₋₁₀-alkyl)₂, -C₁₋₁₀-alkyl-P(=O)(OH)(OC₁₋₁₀-alkyl), -C₁₋₁₀-alkyl-S(=O)₂(OH), -C₁₋₁₀-alkyl-S(=O)₂(NH₂), -C₁₋₁₀-alkyl-C(=O)(OH), or -C₁₋₁₀-alkyl-P(=O)(OH)₂;
- R7** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, or -C₁₋₁₀-alkyl-O-heteroaryl; and
- R8** represents -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-O-aryl, -C₃₋₆-cycloalkyl-aryl, -C₃₋₆-cycloalkyl-heteroaryl, -C₁₋₁₀-alkyl-heteroaryl, or -C₁₋₁₀-alkyl-O-heteroaryl;

wherein in each case "C₁₋₁₀-alkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "C₃₋₁₀-cycloalkyl" may be linear or branched, unless expressly stated otherwise saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -C≡CH, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "aryl" is a 6-14-membered aryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from -C≡CH, -CF₃, -CCl₃, -CBr₃, -Cl₃, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

wherein in each case "heteroaryl" is a 5-14-membered heteroaryl moiety which may be unsubstituted, mono- or disubstituted with a substituent independently selected from -C≡CH, -CF₃, -CCl₃, -CBr₃, -Cl₃, -C(=O)OH, -C(=O)O-C₁₋₁₀-alkyl, -OH, -O-C₁₋₁₀-alkyl, -NH₂, -NH-C₁₋₁₀-alkyl, -N(C₁₋₁₀-alkyl)₂, -N₃, -F, -Cl, -Br, and -I;

or a physiologically acceptable salt thereof,

for use in the prevention or treatment of a condition, disease or disorder that is associated with the MRGPRX4 receptor, preferably associated with the wildtype of the MRGPRX4 receptor.

11. The compound for use according to claim 10, wherein the compound is according to any of claims 2 to 7.

12. The compound according to claim 10, wherein

(iii) **R3** represents -C₁₋₁₀-alkyl-P(=O)(OH)₂ and **R8** represents -C₃₋₆-cycloalkyl, -aryl, -heteroaryl, -C₁₋₁₀-alkyl-aryl, or -C₁₋₁₀-alkyl-heteroaryl (wherein in case of -C₂-alkyl-aryl, the alkyl-moiety is unsaturated);

and wherein in each case **R1** and **R7** independently from one another are selected from -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, and -C₁₋₁₀-alkyl-heteroaryl; or

wherein

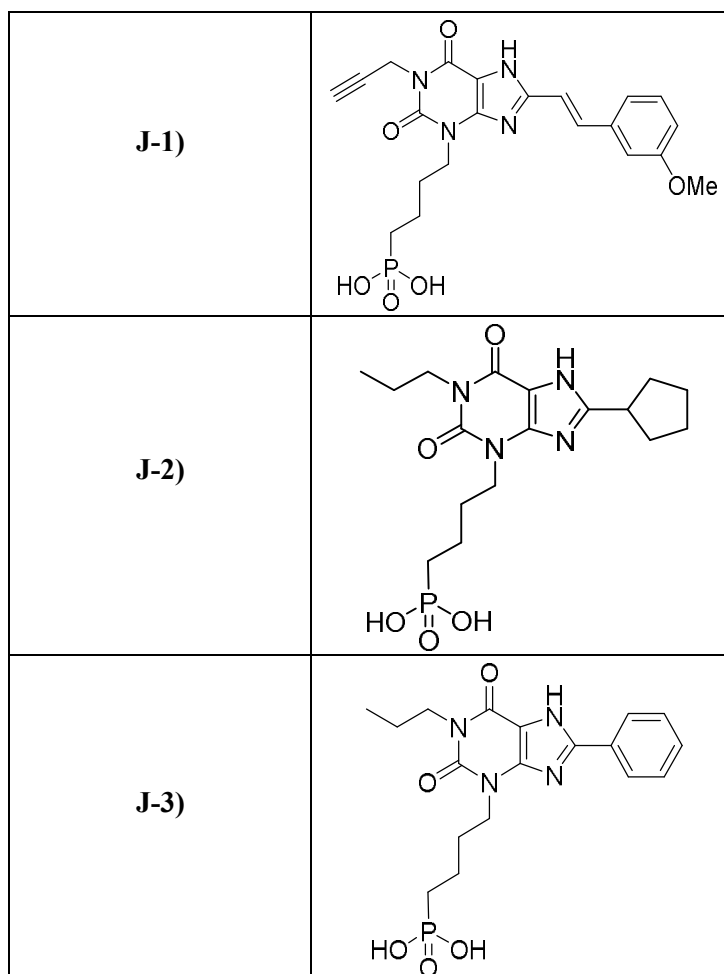
(iv) **R3** represents -C₁₋₁₀-alkyl-OH; or

R3 represents -C₁₋₁₀-alkyl-O-C(=O)C₁₋₁₀-alkyl;

and wherein in each case **R1**, **R7**, and **R8** independently from one another are selected from -H, -C₁₋₁₀-alkyl, -C₁₋₁₀-alkyl-C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-aryl, -C₁₋₁₀-alkyl-heteroaryl, -C₃₋₁₀-cycloalkyl, -C₁₋₁₀-alkyl-O-aryl, and -C₁₋₁₀-alkyl-O-heteroaryl;

for use in the prevention or treatment of a condition, disease or disorder that is associated with the MRGPRX4 receptor, preferably associated with the wildtype of the MRGPRX4 receptor.

13. The compound for use according to any of claims 10 or 11, which is selected from compounds (VII) J-1 to J-3 and the physiologically acceptable salts thereof:



14. The compound for use according to any of claims 8 to 13, wherein the disease or disorder that is associated with the MRGPRX4 receptor, preferably associated with the wildtype of the MRGPRX4 receptor, is selected from
- open wounds; preferably selected from the group consisting of incisions or incised wounds, lacerations, abrasions (grazes), avulsions, puncture wounds, penetration wounds and gunshot wounds; and
 - closed wounds; preferably selected from the group consisting of hematomas and crash injuries; and
 - painful states, itching, neuropathic pain, chronic pain.
15. The compound for use according to any of claims 8 to 14, wherein the compound is administered topically and/or locally.

Abstract:

The invention relates to MRGPRX4 receptor agonists and antagonists useful for treating, alleviating and/or preventing diseases and disorders related to MRGPRX4 receptor function as well as pharmaceutical compositions comprising such compounds and methods for preparing such compounds. The invention is further directed to the use of these compounds, alone or in combination with other therapeutic agents, for alleviating, preventing and/or treating diseases and disorders, especially the use as wound healing medicaments and for the treatment of chronic pain and itch.

6. Homology Modeling and Docking Studies of the MRGX4 receptor

6.1 Homology model of the human MRGPRX4

To elucidate the binding modes of the newly synthesized xanthine derivatives which act as agonists for the human MRGPRX4 and their interactions with the receptor, a homology model of the human MRGPRX4 was generated using Modeller9.16 (see Figure 1).¹⁻² Among the different available crystal structures of GPCRs, the same δ -branch of the class A family and activation of pathway (G_q) justified the selection of the P2Y₁ receptor as template for generating the homology model of the receptor. From the available two crystal structures of P2Y₁ receptor, the high resolution (2.2 Å) receptor in complex with the antagonist 1-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)-3-(4-(trifluoromethoxy)phenyl)urea (BPTU, PDB 4XNV) was selected.³ The sequence of the human MRGPRX4 and P2Y₁ receptor was aligned using EMBOSS Needle.⁴ The gaps in the transmembrane and loop regions were adjusted on the basis of motifs identified for GPCRs.⁵ The manual alignment was necessary for improving the quality of the homology models. The final sequence alignment between both receptors is depicted in Figure 2. Among the 500 generated models, the best model was selected on the basis of Discrete Optimized Protein Energy (DOPE) score and visual inspection of the receptor. The selected homology model was verified using the Ramachandran plot assessment called RAMPAGE (Figure 3). The number of residues in the favored region was 223 (84.5%) and in the allowed region 35 (13.3%). There were 6 residues in the outlier region (2.3%).⁶

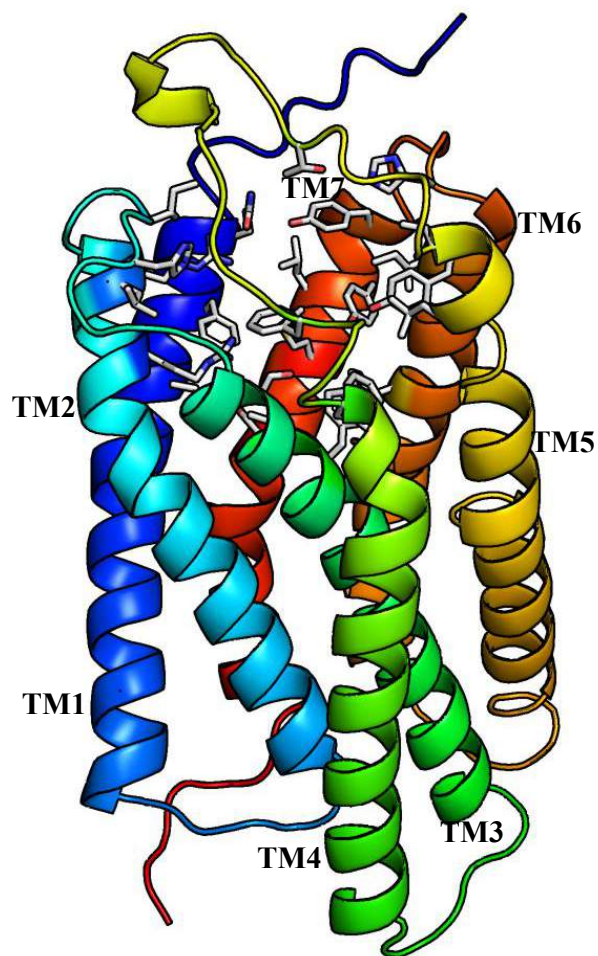


Figure 1: Homology model of MRGPRX4. The homology model of the human MRGPRX4 based on the P2Y₁ crystal structure (PDB 4XNV) is shown in cartoon representation and in spectrum colors. The important amino acids in the putative orthosteric binding site is represented in stick model and colored in grey. The oxygen atoms are colored in red, the nitrogen atoms in blue and sulfur atoms in yellow.

```

sp|Q96LA9|MRGX4_HUMAN -----MDPTVPVFGTKLTPI-NGREETPCYNQTLSTFTVLTCTI
sp|P47900|P2RY1_HUMAN MTEVLWPAVPNGTDA AFLAGPGSSWGNSTVASTAAVSSSFKCALTKTGFQFYLLPAVYIL
* * * * *
sp|Q96LA9|MRGX4_HUMAN ISLVGLTGNVAVLWLLGYRMRR-NAVSIYILNLAADFLFLSFQIIRLPLRLINISHLI-
sp|P47900|P2RY1_HUMAN VFII GFLGNSVAIWVVFHMKPWSGISVYMFN LALADFLYVLTLPALIFYYFNKTDWIFG
* * * * * * * * * *
GX3N LX3DX7P

--R-KILVSVMTFPYFTGLSMLSIASTERCLSVLWPIWYRCRPTHLSAVVCVLLWGLSL
sp|Q96LA9|MRGX4_HUMAN
sp|P47900|P2RY1_HUMAN DAMCKLQRFIFHVNLVGSILFLTCISAHRYSGVVYPLKSLGRLKKNNAICISVLVWLIIVV
* * * * * * * * * *
SX3LX2IX2DRY

LFSMLEWRFCDFLFGADSSW-C-E-TSDFIPVAWLIF-LCVVLCVSSVLLVLRIL
sp|Q96LA9|MRGX4_HUMAN
sp|P47900|P2RY1_HUMAN VAISPIL-FYSGTGVRKNKTITCYDTSDEYLRSYFIYSMCTVAMFCVPLVLIIG
* * * * * * * * * *
WX7,8P FX2PX7Y

CGSRKMPLTRL-----YVTILLTVL-VFLLCGLPFGILGALIYRMHL-N-----
sp|Q96LA9|MRGX4_HUMAN
sp|P47900|P2RY1_HUMAN CYGLIVRAL-IYKDLNSPLRRKSIYLVIIIVLTVFAVSYIIPFHVMTMNLRLRDFQTPAM
* * * * * * * * * *
FX2CWXP

--L-EVLYCHVYLVCMSSLNSSANPIIYFFVGSFRQRQNRQNLKLVLQR--A-LQDKPE
sp|Q96LA9|MRGX4_HUMAN
sp|P47900|P2RY1_HUMAN CAFNDRVY-ATYQVTRGLASLNSCVDPILYFLAGDTFRRLSRATRKASRRSEANLQSKSE
* * * * * * * * * *
LX5NPX2Y

VDKGGQLPEESLELSGSRIGP
sp|Q96LA9|MRGX4_HUMAN
sp|P47900|P2RY1_HUMAN -DMTLNILEPFKQNG-DTSL--
* * * *

```

Figure 2: Manual sequence alignment of MrgX-4 and P2Y₁.

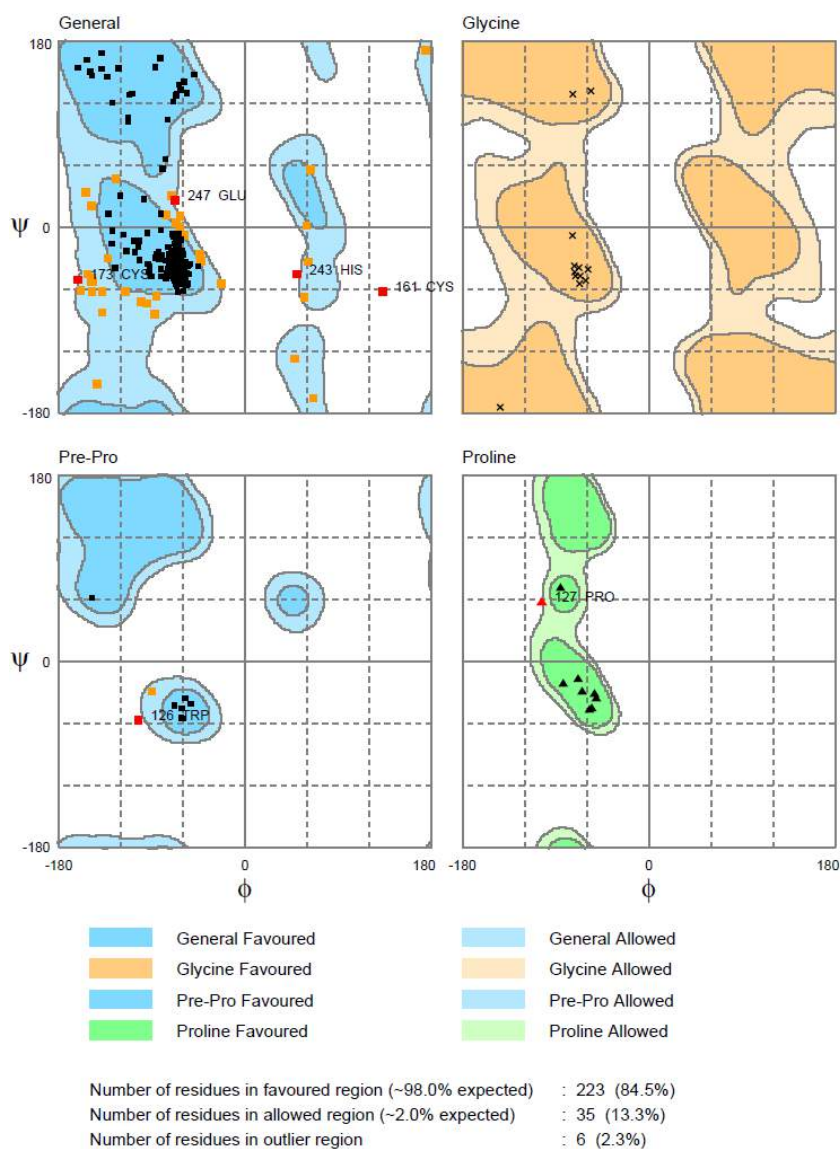


Figure 3: Ramachandran Plot of the MRGPRX4 homology model.

6.2 Docking studies of MRGPRX4 agonists

In order to explore the ligand-receptor interactions of the new xanthine derivatives as MRGPRX4 agonists, compound **B-16** and **D-2** were selected for molecular docking experiments using AutoDock.⁷ The putative orthosteric binding pocket of the receptor was identified using the SiteFinder module implemented in Molecular Operating Environment (MOE) 2018.01.⁸ The selected binding pose from docking simulations suggests that the

xanthine derivatives anchored inside the putative orthosteric binding pocket through the strong electrostatic interactions between the phosphonate group and the amino acid residues Arg82 and Arg86 (TM2) of the receptor. Furthermore, the phosphonate group of **B-16** possibly introduces an additional weak electrostatic interaction with Met258 (TM7) located at a distance of 3.5 Å which is not present in the case of **D-2** due the distance is increased to 4.9 Å. The mono-ester substitution (ethoxy) at the phosphonate group resulted in dramatic improvement in the activity. Whereas the di-ester substitution (diethoxy) resulted in complete loss of activity against the receptor (**C-1** to **C-10**). Additionally, bioisosteric replacements of the phosphonate by carboxylic acid (**G-1** and **G-2**) or sulfonic acid (**E-1** to **E-3**) resulted in complete loss of activity.

The xanthine core moiety is surrounded by hydrophobic residues include Phe104, Trp158, Phe163, Leu184, Tyr240, and Met258 and possibly provide a strong hydrophobic interaction. The compounds are further stabilized by hydrogen bond interactions formed between the carbonyl group (C2) of the xanthine moiety and the residue Arg82. The keto group at position 6 of the xanthine moiety might introduce a water-mediated interaction with Ser100 or Thr103 which located at a distance of 4-5 Å.

The electron rich propargyl moiety fits inside the binding pocket surrounded by polar amino acids Arg82 (TM2), Ser100 (TM2) and Thr103 (TM2) in distances less than 3 Å. The *N*1-propargyl substitution was highly preferable in comparison to a methylcyclobutyl (**B-13**) and other inactive substitutions include ethyl (**B-14**). The larger non-polar methylcyclobutyl moiety which reduces the activity which resulted in complete loss of activity are due to clashes with the amino acids residues in the binding pocket of the receptor. Alternatively, an ethyl group seems to be too small for tight binding of the molecule in the pocket and resulted inactive against the receptor. The size, the angle and the electronic properties of the propargyl group makes a well-tolerated side chain at the *N*1-position.

The *N*7-methyl group of **B-16** is directed towards a hydrophobic subpocket formed by Leu184 (TM5), Trp158 (TM4) and Phe104 (TM3). This is supported by the increase in potency for **B-16** ($EC_{50} = 0.540 \mu\text{M}$) compared to **B-6** ($EC_{50} = 9.63 \mu\text{M}$).

The docked pose of **D-2** and **B-16** shows that the phosphonic acid ester (**D-2**) or phosphonate group (**B-16**) and the xanthine core moiety of both derivatives positioned in the same orientation in the binding pocket. However the substituted phenyl group at position *C*8 of the xanthine core positioned in the subpocket formed by residues Thr175, Asp177, Val181, Leu184, Ile239,

Tyr240, His 243 and Tyr250. As shown in Figure 4 (A) and (B), the position of the phenyl group in the subpocket is depend on the different substituents *o*-OCH₃ (**B-16**) or *m*-CF₃ (**D-2**). The activity can be greatly improved with the optimal substitution at the phenyl group and the *N*3- or *N*7- position of the xanthine core.

In order to explore the selectivity of the MRGPRX4 agonists against other MRGPRX subtypes (X1-X3) a multiple sequence alignment using Clustal Omega⁹ was prepared (See Figure 6). The important amino acids inside the MRGPRX4 binding pocket were analyzed and compared to other receptor subtypes. The amino acid Arg82 which forms a hydrogen bond interaction with the phosphonate moiety is only present in the MRGPRX4. Additionally the three tyrosine amino acids from the transmembrane region 7 (Tyr240, Tyr250 and Tyr254) that stabilized the aromatic group of the MRGPRX4 agonists are also not present in other MRGX receptor subtypes (X1-X3). Arg86 which forms a hydrogen bond with the phosphonate group in our model is only present in the MRGX3 receptor subtype but not in MRGPRX1 or -X2. These differences of amino acids inside the binding pocket explains the selectivity of the new synthesized MRGPRX4 agonists.

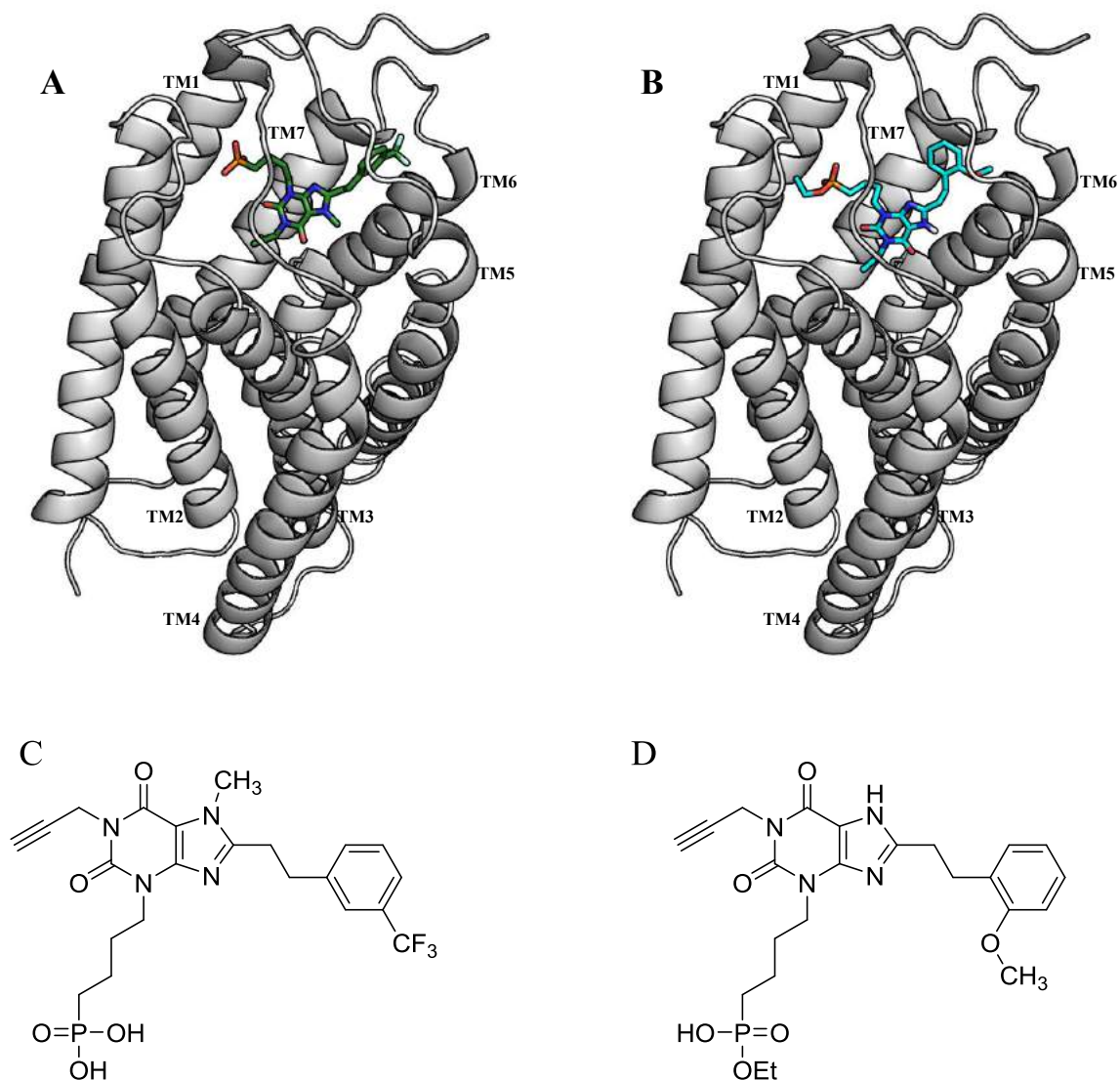


Figure 4: A: The docked pose of (A) **B-16** (carbon colored dark green) and (B) **D-2** (carbon colored cyan) in the homology model of the human MRGPRX4. The oxygen atoms are colored in red, the nitrogen atoms in blue, the fluorine atoms in light green and the phosphorus atoms in orange. The ligands are shown in stick models and the receptors as cartoon representation. (C) Structure of **B-16**, (D) structure of **D-2**.

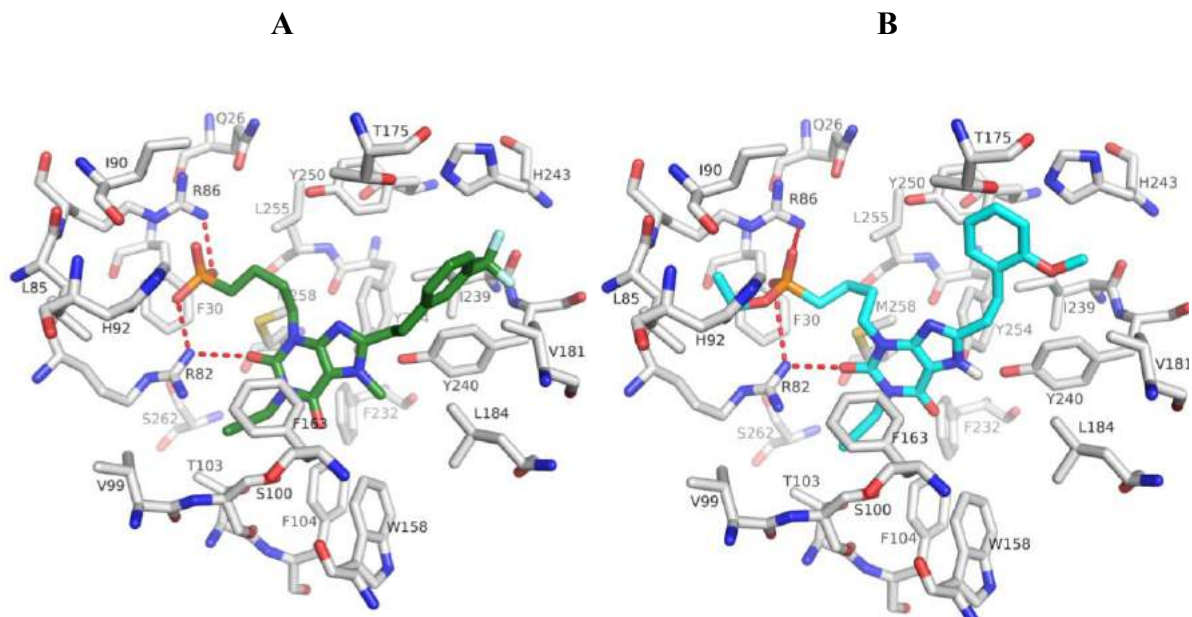


Figure 5: The putative binding pose of (A) **B-16** and (B) **D-2** with the important amino acids in the binding pocket of the human MRGPRX4 model. The electrostatic interactions between the xanthine derivatives and the receptor are depicted as dashed red lines. For representation and color coding see Figure 4.

sp Q96LB1 MRGX2_HUMAN	MDPTTPAWGTESTTVNGNDQALLLCCGKETLIPVFLILFIALVGLVGNFVLWLLGFRMR
sp Q96LA9 MRGX4_HUMAN	MDPTVPVFGTKLTPINGREET---PCYNQTLSTVLTLCIISLVGLTGNVAVLWLLGYRMR
sp Q96LB2 MRGX1_HUMAN	MDPTISTLDTELTPINGTEET---LCYKQTLSTVLTLCIVSLVGLTGNVAVLWLLGCRMR
sp Q96LB0 MRGX3_HUMAN	MDSTIPVLGTELTPINGREET---PCYKQTLSTGLTLCIVSLVALTGNVAVLWLLGCRMR
sp Q96LB1 MRGX2_HUMAN	RNAFSVYVLSLAGADFLFLCFQIINCLVYLSNFFCSISINFPSSFVTTVMTCAYLAGLSML
sp Q96LA9 MRGX4_HUMAN	RNAVSIYILNLAADDFLFLSFQIIRLPLRLI----NISHLIRKILVSVMTFPYFTGLSML
sp Q96LB2 MRGX1_HUMAN	RNAFSIYILNLAADDFLFLSGRLIYSLLSFI----SIPHTISKILYPVMMFSYFAGLSFL
sp Q96LB0 MRGX3_HUMAN	RNAVSIYILNLAADDFLFLSGHIICSPRLRLI----NIRHPISKILSPVMTFPYFIGLSML
sp Q96LB1 MRGX2_HUMAN	STVSTERCLSVLWPIWYRCRRPRHLSAVVCVLLWALSLLLSILEGKFCGFLFSDGDSGWC
sp Q96LA9 MRGX4_HUMAN	SAISTERCLSVLWPIWYRCRRPTHLSAVVCVLLWGLSLLFMSLEWRFCDFLFSGADSSWC
sp Q96LB2 MRGX1_HUMAN	SAVSTERCLSVLWPIWYRCHRPHTLSAVVCVLLWALSLLRSILEWMLCGFLFSGADSAWC
sp Q96LB0 MRGX3_HUMAN	SAISTERCLSILWPIWYHCRPRYLSSVMCVLLWALSLLRSILEWMLCDFLFSGANSVWC
sp Q96LB1 MRGX2_HUMAN	QTFDFITAAWLIFLFMVLCGSSLALLVRLCGSRGLPLTRLYLTILLTVLVFLLCGLPFG
sp Q96LA9 MRGX4_HUMAN	ETSDFIPVAWLIIFLCVVLCSLVLLVRLICGSRKMPTRLYVTILLTVLVFLLCGLPFG
sp Q96LB2 MRGX1_HUMAN	QTSDFITVAWLIFLCVVLCSLVLLIRILCGSRKIPLTRLYVTILLTVLVFLLCGLPFG
sp Q96LB0 MRGX3_HUMAN	ETSDFITIAWLVLFCVVLCSLVLLVRLICGSRKMPTRLYVTILLTVLVFLLCGLPFG
sp Q96LB1 MRGX2_HUMAN	IQWFLILWIWKDSVLFCHIHVPVSVLSSLNSSANPIIYFFVGSFRKQWRLQQPILKLAL
sp Q96LA9 MRGX4_HUMAN	ILGALIVYRMHLNLEVLVYCHVYLVCMSSLNSSANPIIYFFVGSFRQRQN--RQNLKLVL
sp Q96LB2 MRGX1_HUMAN	IQFFFLFWIHVDREVLVFCVHVLVSIFLSALNSSANPIIYFFVGSFRQRQN--RQNLKLVL
sp Q96LB0 MRGX3_HUMAN	IQWALFSRIHLDWVLFCHVHVLVSIFLSALNSSANPIIYFFVGSFRQRQN--RQNLKLVL
sp Q96LB1 MRGX2_HUMAN	QRALQDIAEVDHSEGCFRQGTPEMSRSSLV-
sp Q96LA9 MRGX4_HUMAN	QRALQDKPEVDKGEQQLPEESLELSGSRLGP
sp Q96LB2 MRGX1_HUMAN	QRALQDASEVDEGGGQLPEEILELSGSRLAQ
sp Q96LB0 MRGX3_HUMAN	QRALQDTPPEVDEGGGWLPEETLELSGSRLAQ

Numbering is based on MRGPRX4

Figure 6: Multiple sequence alignment of MRGPRX1-X4

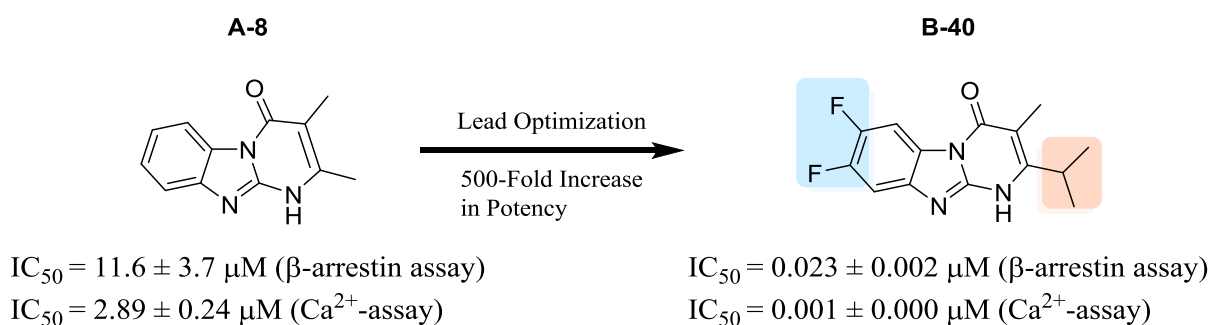
- (1) Šali, A.; Blundell, T. L. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.* **1993**, *234* (3), 779-815.
- (2) Webb, B.; Sali, A. Comparative protein structure modeling using MODELLER. *Curr. Protoc. Bioinformatics* **2014**, *47* (1), 5.6. 1-5.6. 32.
- (3) Zhang, D.; Gao, Z.-G.; Zhang, K.; Kiselev, E.; Crane, S.; Wang, J.; Paoletta, S.; Yi, C.; Ma, L.; Zhang, W. Two disparate ligand-binding sites in the human P2Y 1 receptor. *Nature* **2015**, *520* (7547), 317.
- (4) Chojnacki, S.; Cowley, A.; Lee, J.; Foix, A.; Lopez, R. Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Res.* **2017**, *45* (W1), W550-W553.
- (5) Costanzi, S., Homology modeling of class a G protein-coupled receptors. In *Methods. Mol. Biol.*, Springer: 2011; pp 259-279.
- (6) Lovell, S. C.; Davis, I. W.; Arendall, W. B.; de Bakker, P. I.; Word, J. M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C. Structure validation by C α geometry: phi,psi and C β deviation. *Proteins* **2002**, *50*, 437-450.
- (7) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30* (16), 2785-2791.
- (8) *Molecular Operating Environment (MOE)*, 2013.08, Chemical Computing Group ULC: 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7,, 2019.
- (9) Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T. J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Söding, J. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **2011**, *7* (1), 539.

7. Patent II: MRGX Receptor Antagonists – US 16/670,149

Müller, C.; Alnouri, W.; Riedel, Y.; Thimm, D.; Marx, D.; Namasivayam, V.; Gattner, S.; Herdewyn, P.; De Jonghe, S.; Leonczak, P.; Verdonck, S.

The MRGPRX2 (also designated MRGX2), as well as the other MRGPRX receptor subtypes, are expressed in small diameter sensory neurons of the dorsal root ganglia (DRG). MRGPRX2 is further expressed in human mast cells. Therefore, it is assumed that the activation and blockade of the receptor may have an effect on pain sensation and transmission, and may also be involved in itching and allergic reactions. Since no highly potent antagonists for this receptor is known to date, the design and synthesis of such compounds is essential to explore their potential for replacing opioid analgesics, and to treat itching and (pseudo)allergic reactions via this receptor.

We therefore screened our purine-based compound library of more than 20,000 compounds in a β -arrestin enzyme complementation assay to characterize (potential) MRGPRX2 agonists, and, in particular, to identify new antagonists. A tricyclic benzimidazole derivative could be identified as MRGPRX2 antagonist with moderate potency (IC_{50} 11.6 μ M, **A-8**). In order to increase its potency, numerous tricyclic benzimidazole derivatives were synthesized in cooperation with the group of Prof. Herdewijn (Medicinal Chemistry and Director of the Laboratory of Medicinal Chemistry at the Rega Institute for Biomedical Research at the Katholieke Universiteit Leuven, Belgium). Thereby I was largely involved in the development of the regioisomeric derivatives. This led to a dramatic improvement of the potency. The best MRGPRX2 antagonists synthesized in the present thesis, compound **B-40**, displayed an IC_{50} value of 23 nM determined in a β -arrestin assay, and 1 nM in a calcium mobilization assay. Selected MRGPRX2 antagonists were tested for their potential agonistic and antagonistic activity on the other MRGPRX subtypes (MRGPRX1, -X3 and -X4). The newly developed antagonists were exclusively active at MRGPRX2 and inactive at the other MRGPRX subtypes.



In vitro ADME studies were performed by Pharmacelsus for the newly investigated MRGPRX2 antagonists. The antagonists showed poor water solubility, moderate metabolic stability in liver microsomes ($t_{1/2}$ between 5 to 15 min), high clearance (130 to 217 $\mu\text{l}/\text{min}/\text{mg}$ protein), a high plasma protein binding of over 99% and good prospects to cross the blood-brain barrier. The physicochemical properties of the patented compounds could be improved by introducing a nitrogen atom, which is discussed in the unpublished part of this thesis (chapter 8).

Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	<i>Attorney Docket No.</i>	2235.001US
	<i>First Named Inventor</i>	Prof. Dr. Christa Müller
	<i>Title</i>	MRGX Receptor Antagonists
	<i>Priority Mail Express® Label No.</i>	Filed Via EFS @ USPTO.GOV on October 31, 2019

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450
--	--

<p>1. <input type="checkbox"/> Fee Transmittal Form (PTO/SB/17 or equivalent)</p> <p>2. <input type="checkbox"/> Applicant asserts small entity status. See 37 CFR 1.27</p> <p>3. <input type="checkbox"/> Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.</p> <p>4. <input checked="" type="checkbox"/> Specification [Total Pages <u>102</u>] Both the claims and abstract must start on a new page. (See MPEP § 608.01(a) for information on the preferred arrangement)</p> <p>5. <input type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets _____]</p> <p>6. Inventor's Oath or Declaration [Total Pages _____] (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))</p> <p>a. <input type="checkbox"/> Newly executed (original or copy)</p> <p>b. <input type="checkbox"/> A copy from a prior application (37 CFR 1.63(d))</p> <p>7. <input checked="" type="checkbox"/> Application Data Sheet * See note below. See 37 CFR 1.76 (PTO/AIA/14 or equivalent)</p> <p>8. CD-ROM or CD-R in duplicate, large table, or Computer Program (Appendix)</p> <p><input type="checkbox"/> Landscape Table on CD</p> <p>9. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. - c. are required)</p> <p>a. <input type="checkbox"/> Computer Readable Form (CRF)</p> <p>b. <input type="checkbox"/> Specification Sequence Listing on:</p> <p>i. <input type="checkbox"/> CD-ROM or CD-R (2 copies); or</p> <p>ii. <input type="checkbox"/> Paper</p> <p>c. <input type="checkbox"/> Statements verifying identity of above copies</p>	<p style="text-align: center;">ACCOMPANYING APPLICATION PAPERS</p> <p>10. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) Name of Assignee _____</p> <p>11. <input type="checkbox"/> 37 CFR 3.73(c) Statement <input type="checkbox"/> Power of Attorney (when there is an assignee)</p> <p>12. <input type="checkbox"/> English Translation Document (if applicable)</p> <p>13. <input type="checkbox"/> Information Disclosure Statement (PTO/SB/08 or PTO-1449) <input type="checkbox"/> Copies of citations attached</p> <p>14. <input checked="" type="checkbox"/> Preliminary Amendment</p> <p>15. <input type="checkbox"/> Return Receipt Postcard (MPEP § 503) (Should be specifically itemized)</p> <p>16. <input type="checkbox"/> Certified Copy of Priority Document(s) (if foreign priority is claimed)</p> <p>17. <input type="checkbox"/> Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.</p> <p>18. <input checked="" type="checkbox"/> Other: USPTO is hereby authorized to charge any additional fees required under 37 C.F.R. Sec. 1.16; 1.17 and 1.21 to Deposit Acct. 50-1097. _____ _____</p>
---	---

*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 must be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS				
<input checked="" type="checkbox"/> The address associated with Customer Number: <u>25215</u> OR <input type="checkbox"/> Correspondence address below				
Name				
Address				
City	State	Zip Code		
Country	Telephone	Email		

Signature	/Kristen L. Pursley/	Date	2019-10-31
Name (Print/Type)	Kristen L. Pursley	Registration No. (Attorney/Agent)	61161

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:				
Filing Date:				
Title of Invention:	MRGX Receptor Antagonists			
First Named Inventor/Applicant Name:	Dr. Christa Müller			
Filer:	Kristen L. Pursley/Wendy Morgan			
Attorney Docket Number:	2235.001US			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	300	300
UTILITY SEARCH FEE	1111	1	660	660
UTILITY EXAMINATION FEE	1311	1	760	760
Pages:				
Claims:				
Miscellaneous-Filing:				
LATE FILING FEE FOR OATH OR DECLARATION	1051	1	160	160
Petition:	231			

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1880

Electronic Acknowledgement Receipt

EFS ID:	37615086
Application Number:	16670149
International Application Number:	
Confirmation Number:	9307
Title of Invention:	MRGX Receptor Antagonists
First Named Inventor/Applicant Name:	Dr. Christa Müller
Customer Number:	25215
Filer:	Kristen L. Pursley/Wendy Morgan
Filer Authorized By:	Kristen L. Pursley
Attorney Docket Number:	2235.001US
Receipt Date:	31-OCT-2019
Filing Date:	
Time Stamp:	14:45:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$1880
RAM confirmation Number	E20190UE46555930
Deposit Account	501097
Authorized User	Wendy Morgan

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)
 37 CFR 1.20 (Post Issuance fees)
 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Applicaton_2235_001US_AsFiled.pdf	1973163 4a3149d359b0a025473487a6abfcc6c7940acb	yes	103
Multipart Description/PDF files in .zip description					
Document Description			Start	End	
Specification			1	83	
Claims			84	102	
Abstract			103	103	
Warnings:					
Information:					
2	Application Data Sheet	ADS_2235_001US.pdf	1256733 c1566bb6c4a4e106edd4bd09fb0a301347d3341	no	12
Warnings:					
Information:					
3		PreliminaryAmendment_2235_001US.pdf	540571 f58f0a359299d04dbce1105ed1ebe8aa1f2aebf	yes	24
Multipart Description/PDF files in .zip description					
Document Description			Start	End	
Preliminary Amendment			1	1	
Claims			2	23	
Applicant Arguments/Remarks Made in an Amendment			24	24	
Warnings:					
Information:					

4	Transmittal of New Application	Transmittal_2235_001US.pdf	298271	no	2
			5967a49f1792b3ab3aee0dc6277f06f6dea a37d		

Warnings:

Information:

5	Fee Worksheet (SB06)	fee-info.pdf	36589	no	2
			699b3f80de657fe289534d85743a6e0fe14f 9717		

Warnings:

Information:

Total Files Size (in bytes):	4105327
-------------------------------------	---------

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

MrgX2 Antagonists

[0001] The invention relates to MrgX receptor ligands useful for treating, alleviating and/or preventing diseases and disorders related to MrgX receptor function as well as pharmaceutical compositions comprising such compounds and methods for preparing such compounds. The invention is further directed to the use of these compounds, alone or in combination with other therapeutic agents, for alleviating, preventing and/or treating diseases and disorders, especially the use as antinociceptive, anti-inflammatory or antipruritic drugs.

[0002] Mas-related genes (Mrgs) belong to a large family of G protein-coupled receptor genes found in rodents. Human MrgX receptors are G protein-coupled 7-transmembrane receptors sharing 41-52% amino acid identity with each other, but have probably no direct orthologs in rodents. MrgX2 (in the literature also referred to as “mas-related G protein-coupled receptor X2”, MRGX2, MrgprX2, MRGPR-X2, and the like), is a member of the MrgX family (in the literature also referred to as “mas-related G protein-coupled receptor X”, MRGX, MrgprX, Mrgpr-X, and the like). MrgX2 is, for example, expressed in the small diameter neurons of sensory ganglia and mast cells. It can be activated by several compounds, such as substance P, vasoactive intestinal peptide, cortistatin (CST), proadrenomedullin N-terminal peptide (PAMP), LL-37, PMX-53 and β -defensins. MrgX2 activation is related to nociception, adrenal gland secretion and mast cell degranulation.

[0003] One putative physiological agonist for MrgX2 is CST-14. CST-14 showed an EC_{50} value of 25 nM in calcium mobilization assays in recombinant HEK cells (Solinski, H.J.; Gudermann, T.; Breit, A. Pharmacology and signaling of MAS-related G protein-coupled receptors. *Pharmacol. Rev.* **2014**, *3*, 570-97; Robas, N.; Mead, E.; Fidock, M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J. Biol. Chem.* **2003**, *45*, 44400-4).

[0004] MrgX2 can be activated by proadrenomedullin N-terminal 20 peptide (PAMP-20) and its truncated form PAMP-9-20/PAMP-12 (Kamohara, M.; Matsuo, A.; Takasaki, J.; Kohda, M.; Matsumoto, M.; Matsumoto, S.; Soga, T.; Hiyama, H.; Kobori, M.; Katou, M. Identification of MrgX2 as a human G protein-coupled receptor for proadrenomedullin N-terminal peptides. *Biochem. Biophys. Res. Commun.* **2005**, *4*, 1146-52).

[0005] MrgX2 could be activated in calcium assays by morphine (EC_{50} value 4.5 μ M), dextrorphan (EC_{50} 1.4 μ M) as well as 3-methoxymorphinan (EC_{50} 4.7 μ M). (Akuzawa, N.; Obinata, H.; Izumi, T.; Takeda, S. Morphine is an exogenous ligand for MrgX2, a G protein-coupled receptor for cortistatin. *J. Cell Anim. Biol.* **2007**, *12*, 216-221).

[0006] TAN-67, a potent δ -opioid agonist, could be identified as an agonist at the MrgX2 receptor with an EC₅₀ value of about 1 μ M in both β -arrestin and calcium mobilization assays (Southern, C.; Cook, J.M.; Neetoo-Isseljee, Z.; Taylor, D.L.; Kettleborough, C.A.; Merritt, A.; Bassoni, D.L.; Raab, W.J.; Quinn, E.; Wehrman, T.S.; Davenport, A.P.; Brown, A.J.; Green, A.; Wigglesworth, M.J.; Rees, S. Screening β -arrestin recruitment for the identification of natural ligands for orphan G protein-coupled receptors. *J. Biomol. Screen.* **2013**, *18*, 599-609).

[0007] The natural product "Complanadine A" was described as a selective MrgX2 agonist after screening it at 165 G protein-coupled receptors with an EC₅₀ value of 5.5 μ M in calcium assays (Johnson, T.; Siegel, D. Complanadine A, a selective agonist for the Mas-related G protein-coupled receptor X2. *Bioorg. Med. Chem. Lett.* **2014**, *15*, 3512-5).

[0008] Novel synthetic agonists for MrgX2 were proposed by Malik et al. and have a tetracyclic benzimidazole scaffold (Malik, L.; Kelly, N.M.; Ma, J.N.; Currier, E.A.; Burstein, E.S.; Olsson, R. Discovery of non-peptidergic MrgX1 and MrgX2 receptor agonists and exploration of an initial SAR using solid-phase synthesis. *Bioorg. Med. Chem. Lett.* **2009**, *6*, 1729-32).

[0009] MrgX2 receptors are highly expressed on mast cells. Mast cells express, among others, beta-2 adrenergic receptors, adenosine receptors, several chemokine receptors, GPR34, Histamine H4, several nucleotide receptors as well as MrgX1 and MrgX2. The activation of some of these receptors leads to degranulation of the mast cells, which means a potential to treat diseases like asthma and urticaria. MrgX2 is one of these potential target receptors. (Okayama, Y.; Saito, H.; Ra, C. Targeting human mast cells expressing G protein-coupled receptors in allergic diseases. *Allergol. Int.* **2008**, *3*, 197-203).

[0010] Tatemoto *et al.* have found that basic secretagogues could activate MrgX2 receptors with EC₅₀ values between 10⁻⁴ and 10⁻⁷ M in calcium assays using recombinant HEK-293 cells. (Tatemoto, K.; Nozaki, Y.; Tsuda, R.; Komoto, S.; Tomura, K.; Furuno, M.; Ogasawara, H.; Edamura, K.; Takagi, H.; Iwamura, H.; Noguchi, M.; Naito, T. Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem. Biophys. Res. Commun.* **2006**, *4*, 1322-8).

[0011] The C5a receptor antagonist PMX-53, a cyclic hexapeptide based on the terminal amino acid sequence of C5a, behaves as an agonist at MrgX2 receptor. PMX-53 could induce mast cell degranulation and a calcium signal via MrgX2 receptors. It was also found that the C3a agonist, E7, could activate MrgX2 receptors in mast cells and induce degranulation, thus behaving as a dual agonist at MrgX2 and C3a receptors. (Subramanian, H.; Kashem, S.W.; Collington, S.J.; Qu, H.; Lambiris, J.D.; Ali, H. PMX-53 as a dual CD88 antagonist and an agonist for Mas-related gene 2 (MrgX2) in human

mast cells. *Mol. Pharmacol.* **2011**, *6*, 1005-13; Kashem, S.W.; Subramanian, H.; Collington, S.J.; Magotti, P.; Lambris, J.D.; Ali, H. G protein-coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. *Eur. J. Pharmacol.* **2011**, *1-2*, 299-304).

[0012] Another component of the immune system, which was reported to act via MrgX2 receptors, is the antimicrobial peptide LL-37. LL-37 was previously reported to induce chemokine production and mast cell degranulation via unknown mechanisms (Subramanian, H.; Gupta, K.; Guo, Q.; Price, R.; Ali, H. Mas-related gene X2 (MrgX2) is a novel G protein-coupled receptor for the antimicrobial peptide LL-37 in human mast cells: resistance to receptor phosphorylation, desensitization, and internalization. *J. Biol. Chem.* **2011**, *52*, 44739-49).

[0013] Other cationic antimicrobial peptides, human β -defensin 2 and 3 (hBD 2, 3), were found to activate MrgX2 and cause mast cell degranulation (Subramanian, H.; Gupta, K.; Lee, D.; Bayir, A.K.; Ahn, H.; Ali, H. β -Defensins activate human mast cells via Mas-related gene X2. *J. Immunol.* **2013**, *1*, 345-52).

[0014] In a recent study, MrgX2 receptor expression in skin mast cells of patients with chronic urticaria (CU) was compared with that of a nonatopic control. The study showed that the expression levels of MrgX2 mRNA in skin mast cells are much higher than in lung-derived mast cells and that the number of MrgX2⁺ mast cells was significantly greater in skin tissues from CU patients than in nonatopic controls. Hence, the blockade of MrgX2 on human skin mast cells might offer a novel approach to the prevention and treatment of severe CU (Fujisawa, D.; Kashiwakura, J.; Kita, H.; Kikukawa, Y.; Fujitani, Y.; Sasaki-Sakamoto, T.; Kuroda, K.; Nunomura, S.; Hayama, K.; Terui, T.; Ra, C.; Okayama, Y. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J. Allergy. Clin. Immunol.* **2014**, *3*, 622-633).

[0015] Roy *et al.* identified the angiogenic host defense peptide AG-30/5C, which induces angiogenesis and promotes wound healing, as a G protein-biased agonist of MrgX2. AG-30/5C was shown to mediate degranulation of human LAD2 mast cells at a concentration of 0.01 μ M (estimated EC₅₀ value: less than 0.1 μ M) but it was found not to activate β -arrestin-dependent signaling (Roy, S.; Ganguly, A.; Haque, M.; Ali, H. *J. Immunol.* **2019**, *202*(4), 1229-38).

[0016] In 2019, MrgX2 was evaluated as a biomarker for predicting treatment outcomes in allergic asthma in a multicenter cohort study. The G protein-coupled receptor subtype MrgX2 was shown to be overexpressed in the allergic asthma group (in comparison to the non-allergic asthma group).

[0017] Furthermore, high MrgX2 serum levels were significantly associated with allergic asthma requiring moderate-to-high inhaled corticosteroid (ICS) doses. Since high levels suggest the use of higher ICS doses, MrgX2 might be a valuable biomarker for the therapy of patients with uncontrolled severe allergic asthma (An, J.; Lee, J.H.; Won, H.K.; Kang, Y.; Song, W.J.; Kwon, H.S.; Cho, Y.S.; Moon, H.B.; Kim, T.B. *Allergy*, **2019**, doi: 10.1111/all.14084 (Epub ahead of print)) and MrgX2 antagonists may consequently be useful for the treatment of allergic asthma.

[0018] Recently, MrgX2 was demonstrated to be activated by the small chemokine (C-X-C motif) ligand 14 (CXCL14) in a dose-dependent manner using the cellosaurus CHEM1 cell line recombinantly expressing MrgX2. Its determined EC₅₀ value was 0.570 μ M. In order to confirm the specificity for MrgX2, CXCL14 was tested in calcium assays using CHEM1 cells without MrgX2 overexpression and was shown not to mediate calcium release (Golz S. *et al.* European patent, EP 3 011 340 B1, Mrg receptor modulation, filed 16.06.2014, issued 22.11.2017).

[0019] So far, only very weakly potent MrgX2 antagonists have been described in literature:

- **the tripeptide Gln-D-Trp(Formyl)-Phe-benzylester**, abbreviated **QWF**, which is a neurokinin-1 receptor antagonist as well; used concentrations: 1 μ M vs. 500 nM substance P in calcium imaging assay and 100 μ M vs. 1 μ M substance P in human LAD2 mast cell degranulation assay; β -hexosaminidase release of SP alone was: 60% and of SP and QWF together: 20% (Azimi, E.; Reddy, V.B.; Shade, K.C.; Anthony, R.M.; Talbot, S.; Pereira, P.J.; Lerner, E.A. Dual action of neurokinin-1 antagonists on Mas-related GPCRs. *JCI Insight* **2016**, 1(16), c89362);
- **Saikosaponin A**, a triterpenoid glycoside, extracted from the radix of *Bupleurum falcatum*; used concentration: 0.50 μ M vs. 30 μ g/ mL (\approx 31.2 μ M) compound 48/80 in human LAD2 mast cell degranulation assay; β -hexosaminidase release of substance P alone: 100% and of substance P and saikosaponin A together: 30% (Wang, N.; Che, D.; Zhang, T.; Liu, R.; Cao, J.; Wang, J.; Zhao, T.; Ma, P.; Dong, X.; He, L. Saikosaponin A inhibits compound 48/80-induced pseudo allergy via the Mrgprx2 pathway in vitro and in vivo. *Biochem. Pharmacol.* **2018**, 148, 147-54);
- **Isoliquiritigenin**, a chalcone isolated from the roots and stems of *Glycyrrhiza glabra*, *G. uralensis* and *G. inflata*; used concentration: 50 μ M vs. 30 μ g/mL (\approx 31.2 μ M) compound 48/80 in human LAD2 mast cell degranulation assay; β -hexosaminidase release of compound 48/80 alone: 85% and of compound 48/80 and isoliquiritigenin together: 40% (Hou, Y.; Che, D.; Ma, P.; Zhao, T.; Zeng, Y.; Wang, N. Anti-pseudo-allergy effect of isoliquiritigenin is MrgX2-dependent. *Immunol. Lett.* **2018**, 198, 52-59);
- **Compound 1 (1-(5H-10 λ ²-phenazin-5-yl)ethan-1-one) and compound 2 (3-(pyridine-2-ylmethyl)-2-thioxo-2,3-dihydro-4H-1 λ ²-quinazolin-4-one)** were tested versus CST-14 in calcium mobilization assays; their IC₅₀ values were 1.6 and 2.5 μ M, respectively (Ogasawara, H.; Furuno,

M.; Edamura, K.; Noguchi, M. Novel MrgX2 antagonists inhibit IgE-independent activation of human umbilical cord blood-derived mast cells. *J. Leukoc. Biol.*, 2019, 106(5), 1069-77).

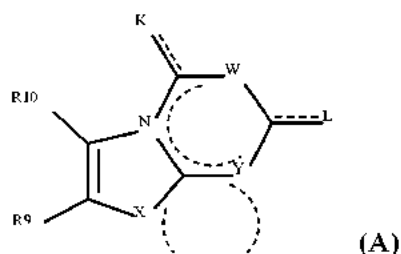
[0020] The human MrgX2 receptor represents a fundamentally new drug target, and the development of potent MrgX2 receptor antagonists to be used for antinociception or neuroprotection or other diseases requires the design of novel drugs targeting the MrgX2 receptor.

[0021] It was an object of the invention to provide compounds that have advantages compared to the compounds of the prior art. The compounds should act as potent and selective MrgX receptor antagonists, in particular with a high ability to block the G_i protein-coupled pathway including biased antagonists (preferably or solely blocking the G-protein-coupled pathway in contrast to β -arrestin recruitment, exhibit bioavailability, and thus may be useful as antinociceptive or antipruritic drugs or for the prevention or treatment of other diseases. Moreover, it was an object of the invention to provide methods for preparing said compounds. It was furthermore an object of the invention to provide compounds and pharmaceutical formulations for the treatment, alleviation and/or prevention of a host of diseases and disorders connected to MrgX2 function. It was a further object of the invention to provide the use of these compounds for alleviating, preventing and/or treating diseases and disorders connected to MrgX function, particularly for, but not limited to the use as antinociceptive or antipruritic agents.

[0022] This object has been solved by the subject-matter of the patent claims.

[0023] The present invention is directed to certain derivatives which act as MrgX2 receptor antagonists and therefore are useful as antinociceptive or antipruritic drugs.

[0024] In a first aspect, the invention is directed to a method for preventing or treating a disease or disorder that is associated with the MrgX2 receptor comprising administering to a subject in need thereof a therapeutically effective amount of an MrgX2 antagonist according to general formula (A)



wherein

K means =O or =S or =NH, **W** means -CR1=, and **L** means -R2; or

K means -R2, **W** means -CR1=, and **L** means =O; or

K means -R1, **W** means -N=, and **L** means -R2; or

K means **-R1**, **W** means **-CH=**, and **L** means **-R2**;

X means **-N=** and **Y** means **-NR3-**; or

X means **-NR4-** and **Y** means **-N=**; or

X means **-NR4-** and **Y** means **-CR0=**; or

X means **-N=** and **Y** means **-S-**; or

X means **-O-** and **Y** means **-N=**; or

X means **-S-** and **Y** means **-N=**; or

X means **-N=** and **Y** means **-N=**;

R0, **R1**, **R2**, **R3**, **R4** independently of one another mean **-H**; **-C₁₋₆-alkyl**; **-C₁₋₆-cycloalkyl**; **-phenyl**; **-C₁₋₆-alkyl-phenyl**; **-heteroaryl** selected from the group consisting of **thienyl**, **furanyl** and **pyrrolyl**; **-C(=O)OH**; **-C(=O)O-C₁₋₆-alkyl**; **-CN**; **-OH**; **-O-C₁₋₆-alkyl**, **-NH₂**, **-S-C₁₋₆-alkyl**; **-F**, **-Cl**, **-Br**, or **-I**; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

R9 and **R10** independently of one another mean **-H**, **-C₁₋₆-alkyl**, **-CN**, **-OH**, **-O-C₁₋₆-alkyl**, **-F**, **-Cl**, **-Br**, **-I**, or **R9** and **R10** together with the carbon atoms to which they are attached form a phenyl ring optionally substituted with **R5**, **R6**, **R7** and **R8**;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean **-H**, **-C₁₋₆-alkyl**, **-CN**, **-OH**, **-O-C₁₋₆-alkyl**, **-F**, **-Cl**, **-Br**, **-I**; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "**C₁₋₆-alkyl**" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from **-CN**, **-C(=O)OH**, **-C(=O)O-C₁₋₆-alkyl**, **-OH**, **-O-C₁₋₆-alkyl**, **-NH₂**, **-NH-C₁₋₆-alkyl**, **-N(C₁₋₆-alkyl)₂**, **-N₃**, **-F**, **-Cl**, **-Br**, **-I**;

wherein in each case the "**C₁₋₆-cycloalkyl**" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from **-CN**, **-C(=O)OH**, **-C(=O)O-C₁₋₆-alkyl**, **-OH**, **-O-C₁₋₆-alkyl**, **-NH₂**, **-NH-C₁₋₆-alkyl**, **-N(C₁₋₆-alkyl)₂**, **-N₃**, **-F**, **-Cl**, **-Br**, **-I**;

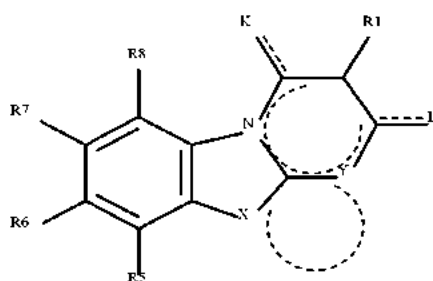
wherein in each case the "**phenyl**" may be unsubstituted, mono- or disubstituted with a substituent independently selected from **-CN**, **-C(=O)OH**, **-C(=O)O-C₁₋₆-alkyl**, **-OH**, **-O-C₁₋₆-alkyl**, **-NH₂**, **-NH-C₁₋₆-alkyl**, **-N(C₁₋₆-alkyl)₂**, **-N₃**, **-F**, **-Cl**, **-Br**, **-I**;

wherein in each case the "**heteroaryl**" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from **-CN**, **-C(=O)OH**, **-C(=O)O-C₁₋₆-alkyl**, **-OH**, **-O-C₁₋₆-alkyl**, **-NH₂**, **-NH-C₁₋₆-alkyl**, **-N(C₁₋₆-alkyl)₂**, **-N₃**, **-F**, **-Cl**, **-Br**, **-I**;

or a physiologically acceptable salt thereof.

[0025] In a preferred embodiment of the method according to the invention, the disease or disorder that is associated to the MrgX2 receptor is selected from the group consisting of pain, especially acute, nociceptive, neuropathic or chronic pain, inflammatory pain or itch. In another preferred embodiment of the method according to the invention, the disease or disorder that is associated to the MrgX2 receptor is selected from the group consisting of anxiety, stress and stress-associated syndromes, depression, epilepsy, Alzheimer's disease, senile dementia, general cognitive dysfunctions, learning and memory disorders (as a nootropic), withdrawal symptoms, alcohol and/or drug and/or medicament abuse and/or dependency, sexual dysfunctions, cardiovascular diseases, hypotension, hypertension, tinnitus, pruritus, migraine, impaired hearing, deficient intestinal motility, impaired food intake, anorexia, obesity, locomotor disorders, diarrhoea, cachexia, urinary incontinence or as a muscle relaxant, anticonvulsive or anaesthetic or for co-administration in the case of treatment with an opioid analgesic or with an anaesthetic, for diuresis or antinatriuresis, anxiolysis, for modulation of motor activity, for modulation of neurotransmitter secretion and treatment of neurodegenerative diseases associated therewith, for the treatment of withdrawal symptoms and/or for reducing the addictive potential of opioids. In yet another preferred embodiment of the method according to the invention, the disease or disorder that is associated to the MrgX2 receptor is selected from the group consisting of asthma, urticaria, skin inflammation, dry skin, atopic eczema, psoriasis, urticaria, scabies, non-allergic hypersensitivity reactions, fibrosis and itch.

[0026] In a preferred embodiment of the method according to the invention, the MrgX2 antagonist is according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -R2; or

K means -R2 and **L** means =O; and

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -NR4- and **Y** means -CR0=; or

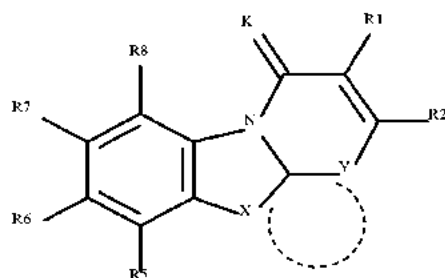
X means -N= and **Y** means S; or

X means -O- and Y means -N=; or

X means -S- and Y means -N=; or

X means -N= and Y means -N=.

[0027] In another preferred embodiment of the method according to the invention, the MrgX2 antagonist is according to general formula (C)



(C)

wherein

K means =O or =S; and

X means -N= and Y means -NR3-; or

X means -NR4- and Y means -N=; or

X means -NR4- and Y means -CR0=; or

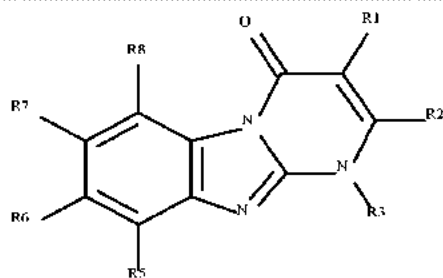
X means -N= and Y means S; or

X means -O- and Y means -N=; or

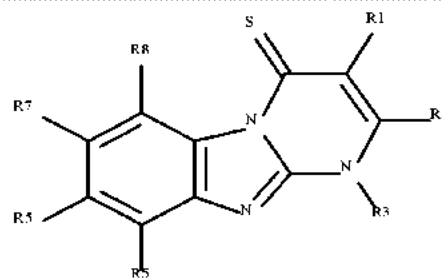
X means -S- and Y means -N=; or

X means -N= and Y means -N=.

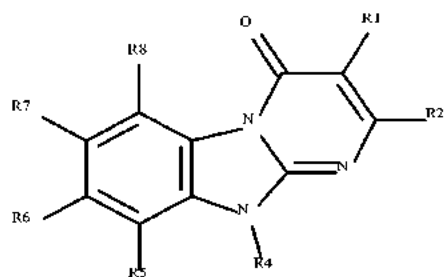
[0028] In a preferred embodiment of the method according to the invention, the MrgX2 antagonist is selected from the group consisting of compounds according to general formula (I), (II), (III), (IV), (V), (VI), (VII), (VIII), (IX), (X), (XI), and (XII):



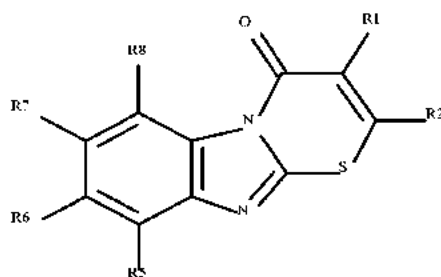
(I)



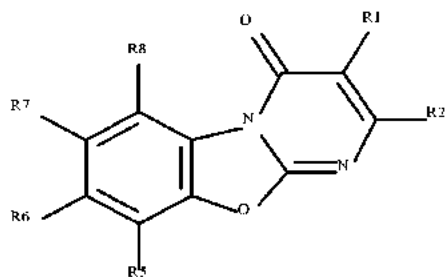
(II)



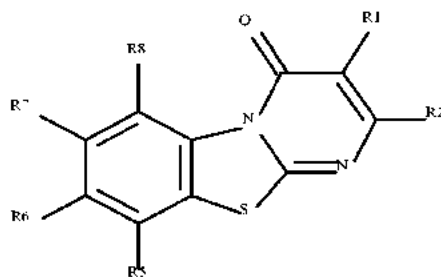
(III)



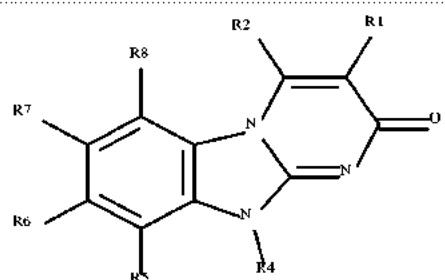
(IV)



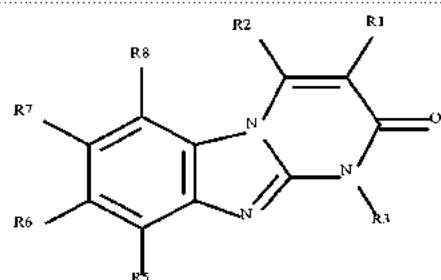
(V)



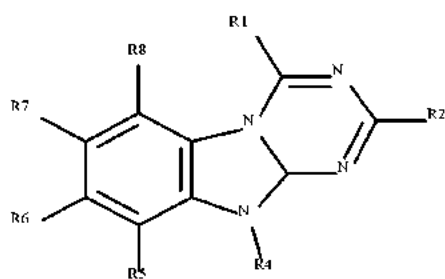
(VI)



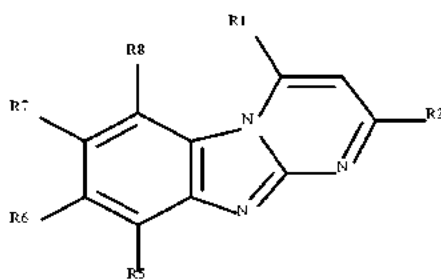
(VII)



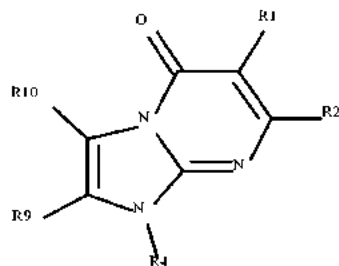
(VIII)



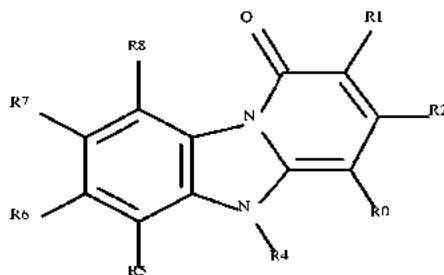
(IX)



(X)



(XI)



(XII)

[0029] According to a preferred embodiment of the method according to the invention, in general formula (A),

R0 means -H or -CN;

R1 means -H, -CH₃, -CH₂CH₃, -CH₂CH₂CH₃, -CH₂CH₂CH₂CH₃, -CH₂CH₂CH(CH₃)₂, -CH₂CH₂C(=O)OCH₂CH₃, -CH₂CH₂-CN, -CH₂CH₂-Cl, -CH₂CH₂-OH, -CH₂CH₂-OCH₃, -CH₂CH₂-N₃, -CH₂CH₂-NHCH₃, -C(=O)OH, -C(=O)OCH₃, -C(=O)OCH₂CH₃, -CH₂-phenyl with phenyl being unsubstituted, -CH₂CH₂-phenyl with phenyl being unsubstituted, -S-CH₃ or -NH₂;

and/or

R2 means -H, -CH₃, -CH₂CH₂CH₃, -CH(CH₃)₂, -CH₂CH(CH₃)₂, -C(CH₃)₃, -cyclopropyl, -phenyl with phenyl being unsubstituted, -para-methoxyphenyl, -2-thienyl, -CH₂C(=O)OH, -C(=O)OCH₃, -CH₂C(=O)OCH₃, -CH₂C(=O)OCH₂CH₃, -C(CH₃)₂(OH), -CH₂OCH₃, -SCH₃, -NH₂ or -OH;

or

R1 and **R2** together with the atoms to which they are attached form a cyclohexyl ring and mean -CH₂CH₂CH₂CH₂-;

and/or

R3 means -H, -CH₃, -CH₂CH₂CH₃, -CH₂-phenyl, -CH₂-*p*-chlorophenyl or -CH₂-CN;

and/or

R4 means -H, -CH₃ or -CH₂-phenyl with phenyl being unsubstituted;

and/or

R9 means -H; and/or **R10** means -H; or **R9** and **R10** together with the carbon atoms to which they are attached form a phenyl ring optionally substituted with **R5**, **R6**, **R7** and **R8**;

wherein

R5, **R6**, **R7** and **R8** independently of one another mean -H, -CH₃, -OH, -OCH₃, -F, -Cl, -Br or -CN, and preferably

R5 means -H or -CH₃; and/or

R6 means -H, -CH₃, -OH, -OCH₃, -F, -Cl, -Br or -CN; and/or

R7 means -H, -CH₃, -F, -Cl, -Br, -CN, -OCH₃ or -OH; and/or

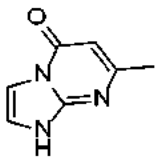
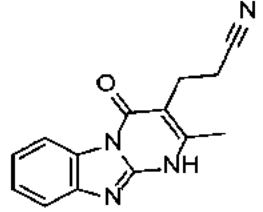
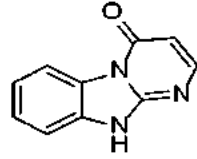
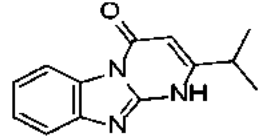
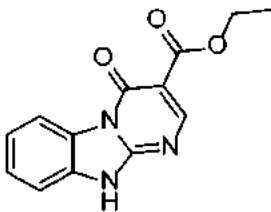
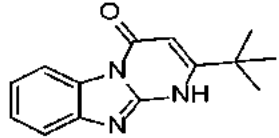
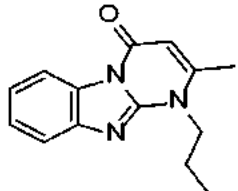
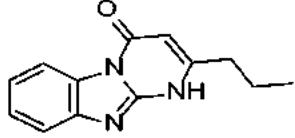
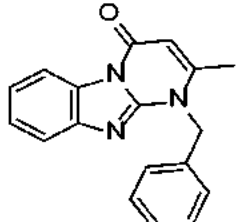
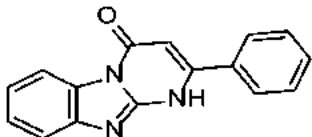
R8 means -H;

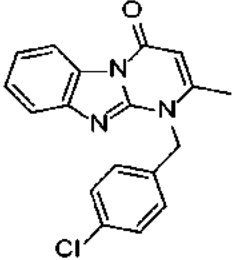
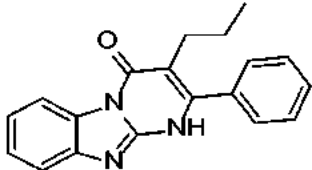
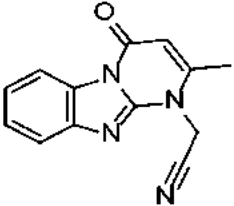
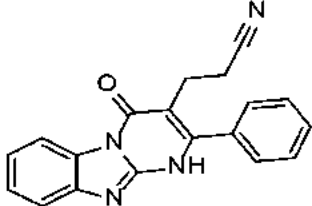
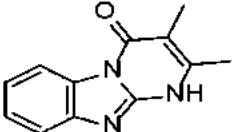
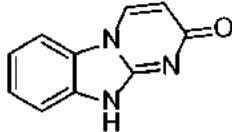
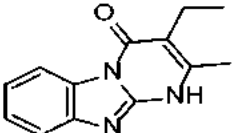
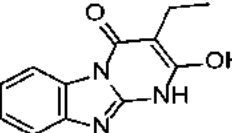
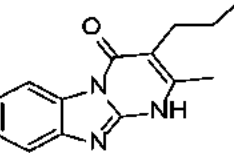
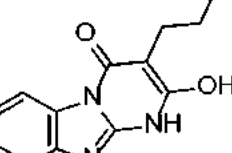
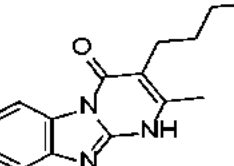
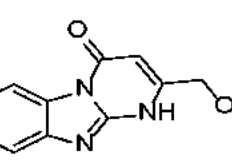
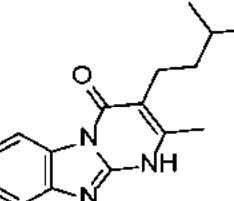
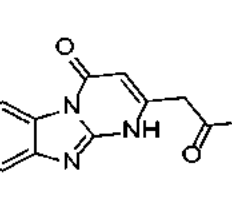
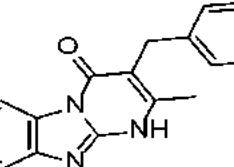
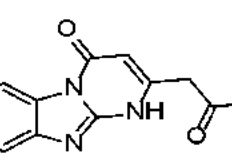
or

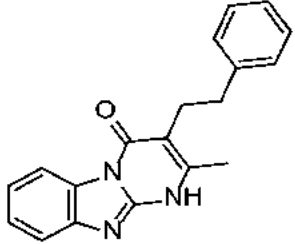
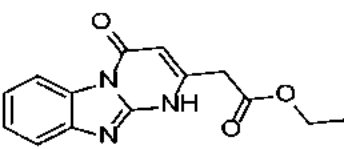
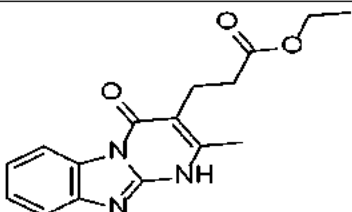
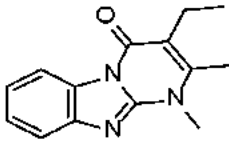
R6 and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring.

[0030] In a preferred embodiment of the method according to the invention, the MrgX2 antagonist is selected from compounds

(I) A-1 to A-30 and the physiologically acceptable salts thereof:

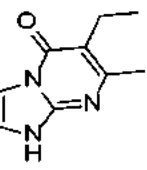
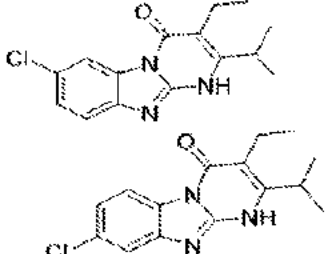
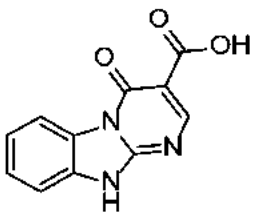
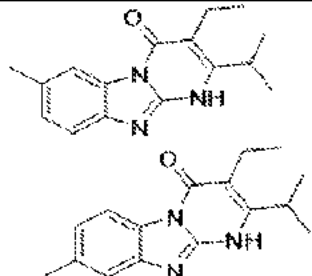
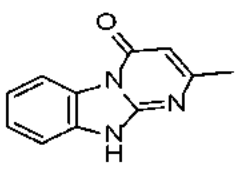
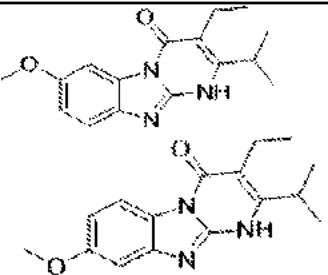
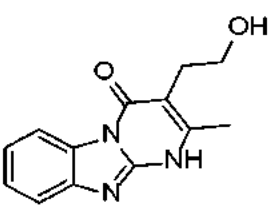
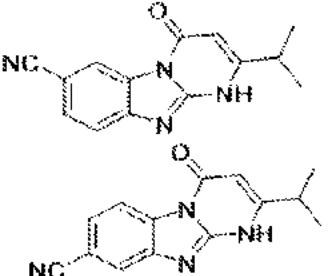
A-1		A-16	
A-2		A-17	
A-3		A-18	
A-4		A-19	
A-5		A-20	

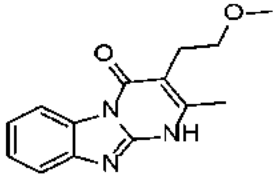
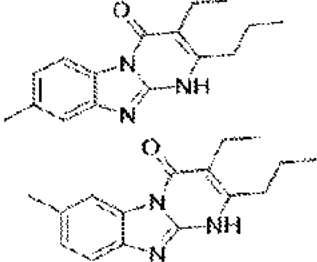
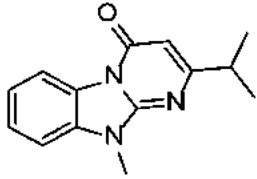
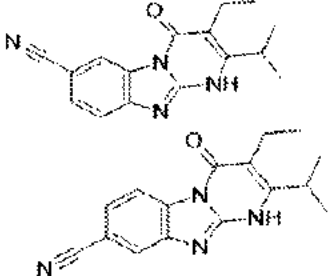
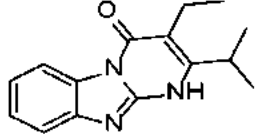
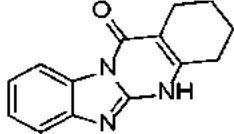
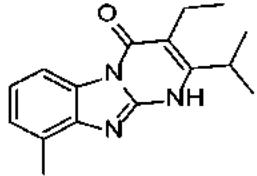
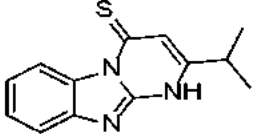
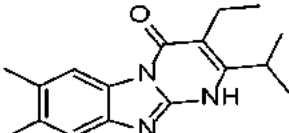
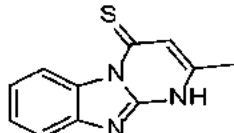
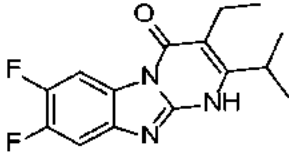
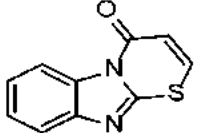
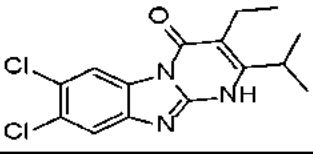
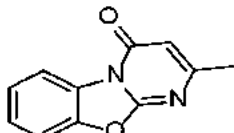
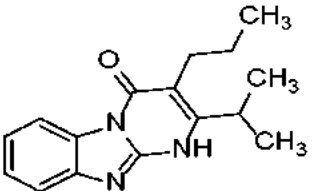
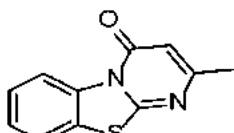
A-6		A-21	
A-7		A-22	
A-8		A-23	
A-9		A-24	
A-10		A-25	
A-11		A-26	
A-12		A-27	
A-13		A-28	

A-14		A-29	
A-15		A-30	

and

(II) B-31 to B-66 and the physiologically acceptable salts thereof:

B-31		B-49 preferably ca. 1:1	
B-32		B-50	
B-33		B-51 preferably ca. 1:1.5	
B-34		B-52	

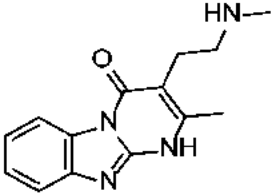
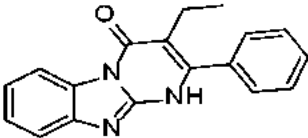
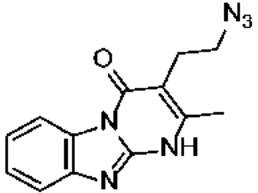
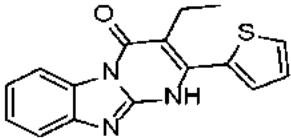
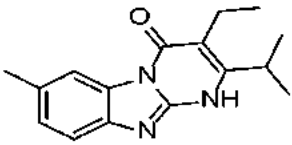
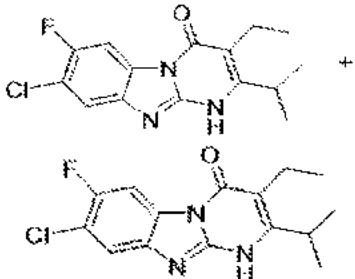
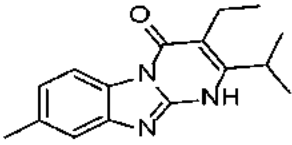
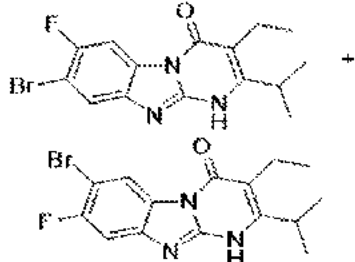
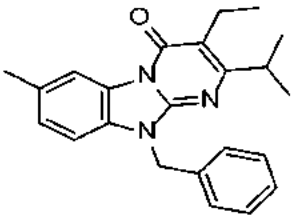
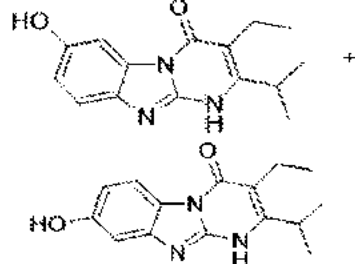
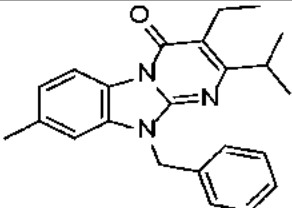
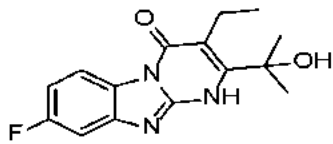
B-35		B-53	
B-36		B-54 preferably ca. 1.5: 1	
B-37		B-55	
B-38		B-56	
B-39		B-57	
B-40		B-58	
B-41		B-59	
B-42		B-60	

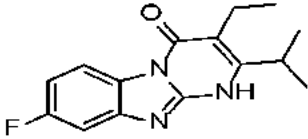
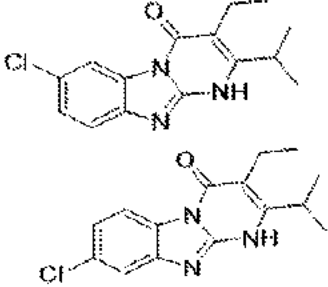
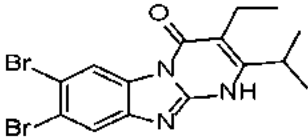
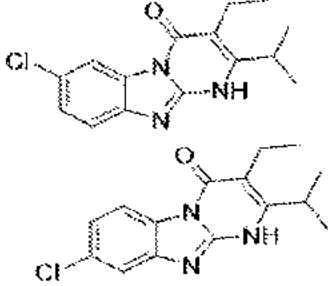
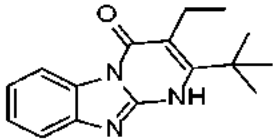
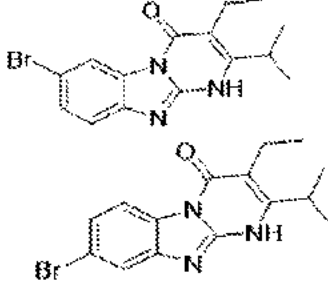
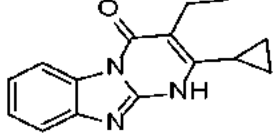
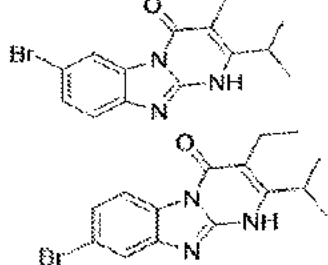
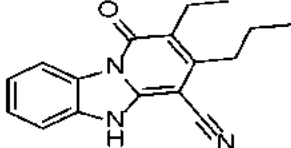
B-43		B-61	
B-44		B-62	
B-45		B-63	
B-46		B-64	
B-47		B-65	
B-48 preferably ca. 1: 2		B-66	

and

(III) C-67 to C-87 and the physiologically acceptable salts thereof:

C-67		C-78	
------	--	------	--

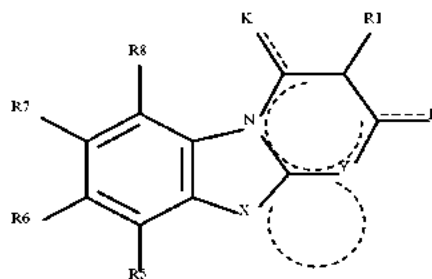
C-68		C-79	
C-69		C-80	
C-70		C-81	
C-71		C-82	
C-72		C-83	
C-73		C-84	

C-74		C-84*	
C-75		C-85	
C-76		C-86	
C-77		C-87	
		C-88	

[0031] Preferably, the MrgX2 antagonist is administered orally.

[0032] Preferably, the MrgX2 antagonist is administered once daily, twice daily or thrice daily.

[0033] Another aspect of the invention relates to MrgX2 antagonist according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -R2; or

K means -R2 and **L** means =O; and

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -N= and **Y** means S; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

R1, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I, or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

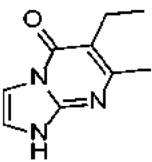
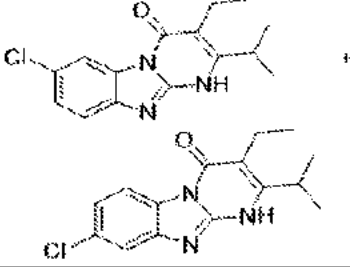
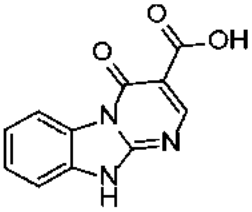
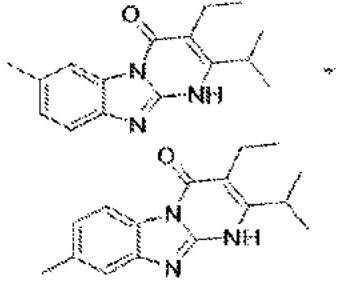
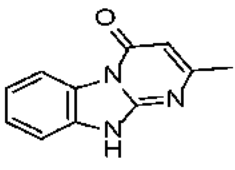
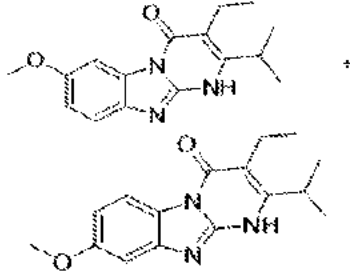
with the proviso that

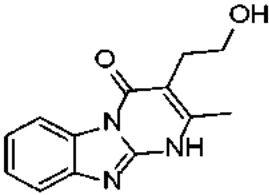
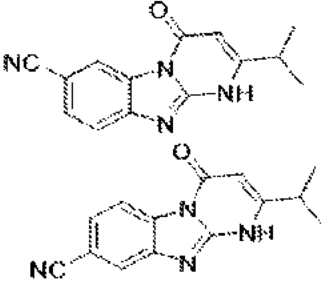
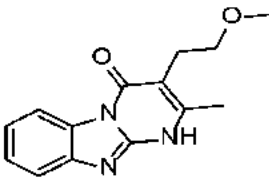
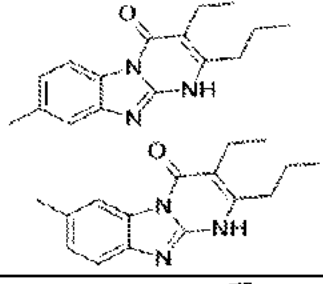
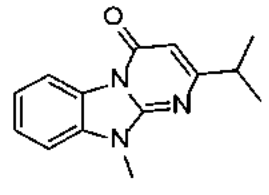
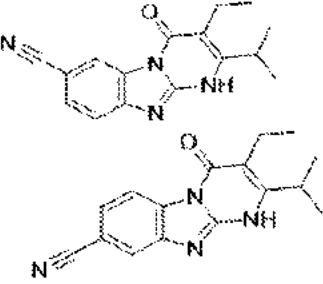
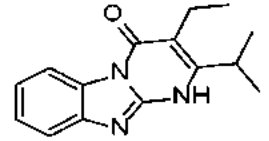
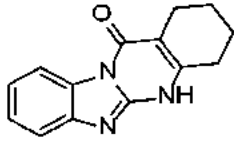
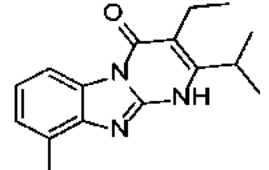
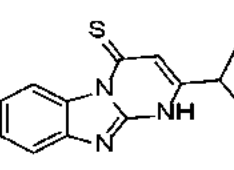
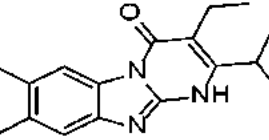
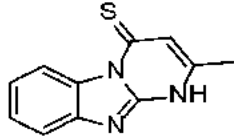
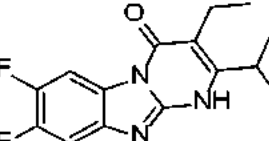
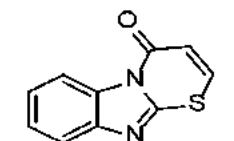
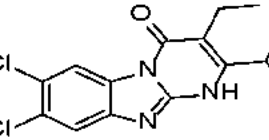
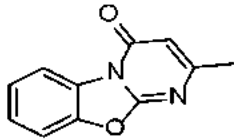
- at least one of **R5, R6, R7** and **R8** does not mean -H; and/or
- at least three of **R1, R2, R3, R4, R5, R6, R7** and **R8** do not mean -H; and/or
- A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29 and/or A-30 are not included,

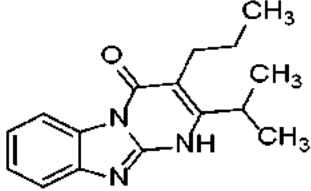
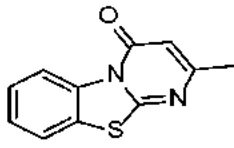
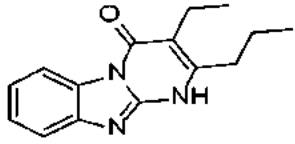
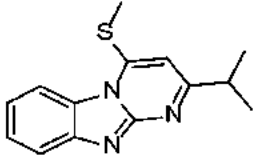
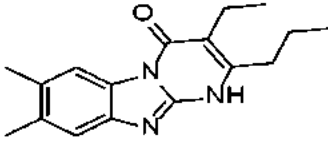
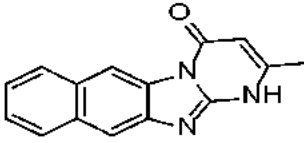
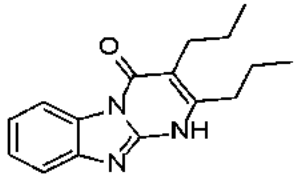
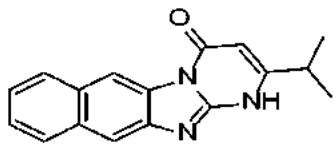
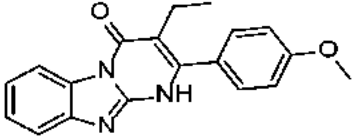
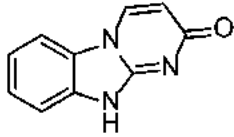
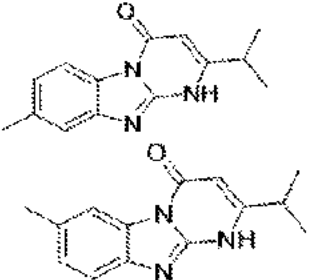
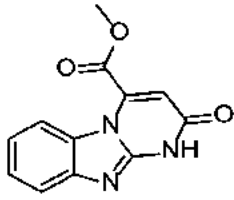
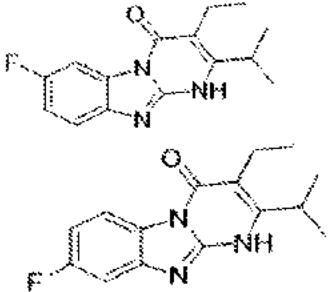
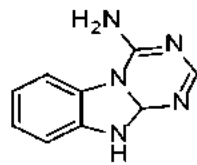
or a physiologically acceptable salt thereof.

[0034] A further aspect of the invention relates to an MrgX2 antagonist selected from compounds

(II) B-31 to B-66 and the physiologically acceptable salts thereof:

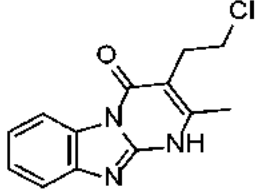
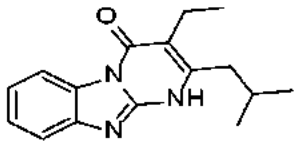
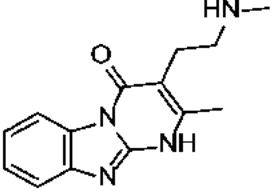
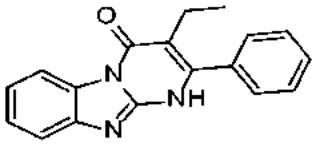
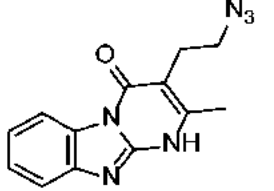
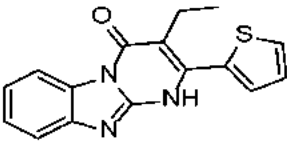
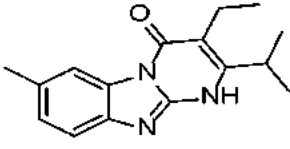
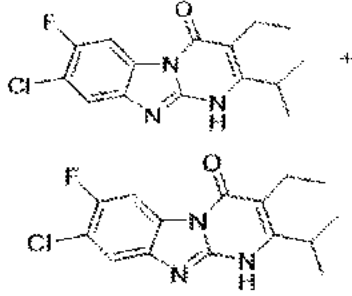
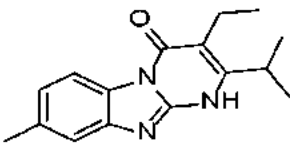
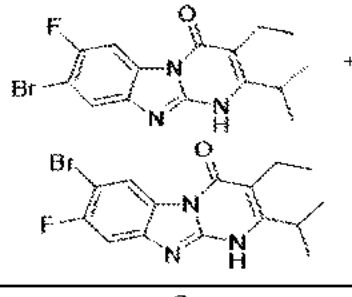
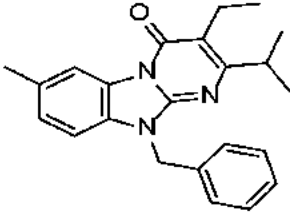
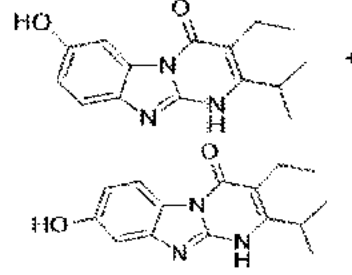
<p>B-31</p>		<p>B-49 preferably ca. 1: 1</p>	
<p>B-32</p>		<p>B-50</p>	
<p>B-33</p>		<p>B-51 preferably ca. 1: 1.5</p>	

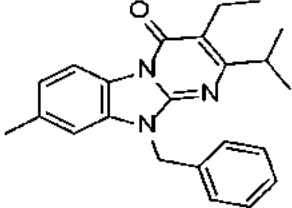
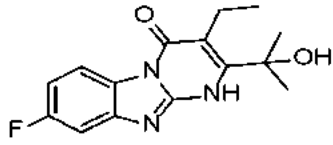
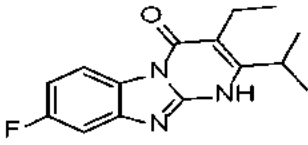
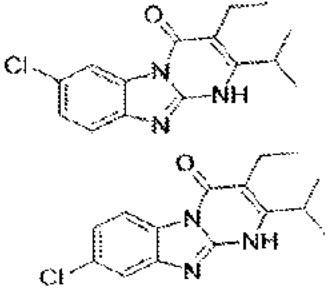
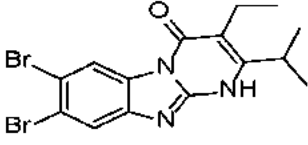
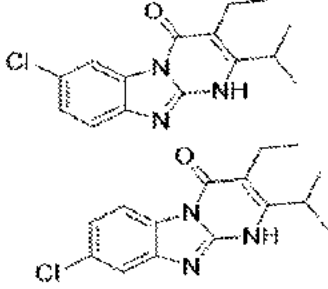
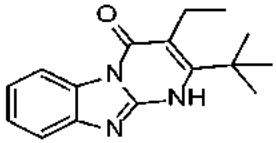
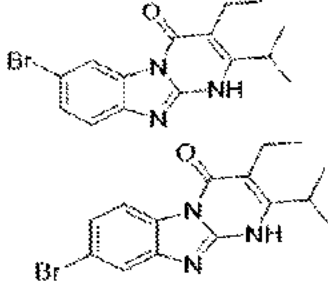
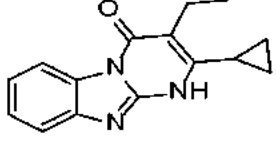
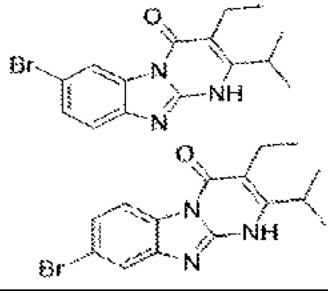
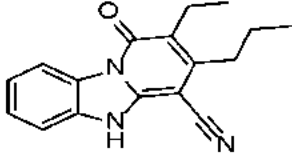
B-34		B-52	
B-35		B-53	
B-36		B-54 preferably ca. 1.5: 1	
B-37		B-55	
B-38		B-56	
B-39		B-57	
B-40		B-58	
B-41		B-59	

<p>B-42</p>		<p>B-60</p>	
<p>B-43</p>		<p>B-61</p>	
<p>B-44</p>		<p>B-62</p>	
<p>B-45</p>		<p>B-63</p>	
<p>B-46</p>		<p>B-64</p>	
<p>B-47</p>		<p>B-65</p>	
<p>B-48 preferably ca. 1: 2</p>		<p>B-66</p>	

and

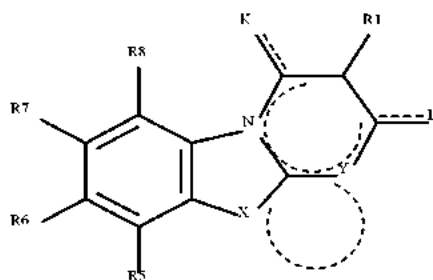
(III) C-67 to C-87 and the physiologically acceptable salts thereof.

C-67		C-78	
C-68		C-79	
C-69		C-80	
C-70		C-81	
C-71		C-82	
C-72		C-83	

C-73		C-84	
C-74		C-84*	
C-75		C-85	
C-76		C-86	
C-77		C-87	
		C-88	

[0035] Another aspect of the invention relates to a pharmaceutical composition comprising

(i) an MrgX2 antagonist according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -R2; or

K means -R2 and **L** means =O; and

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -N= and **Y** means S; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

R1, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

with the proviso that

- at least one of **R5**, **R6**, **R7** and **R8** does not mean -H; and/or
- at least three of **R1**, **R2**, **R3**, **R4**, **R5**, **R6**, **R7** and **R8** do not mean -H; and/or
- A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29 and/or A-30 are not included;

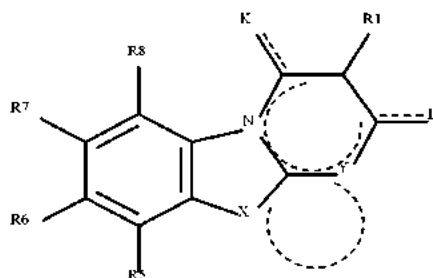
or a physiologically acceptable salt thereof; or

- (ii) an MrgX2 antagonist which is
 - selected from any one of compounds (II) B-31 to B-66 and the physiologically acceptable salts thereof; or
 - selected from any one of compounds (III) C-67 to C-87 and the physiologically acceptable salts thereof;

and a physiologically acceptable excipient.

[0036] Yet another aspect of the invention relates to a pharmaceutical dosage form comprising

- (i) an MrgX2 antagonist according to general formula (B)



(B)

wherein

- K** means =O or =S, and **L** means -R₂; or
- K** means -R₂ and **L** means =O; and
- X** means -N= and **Y** means -NR₃-; or
- X** means -NR₄- and **Y** means -N=; or
- X** means -N= and **Y** means S; or
- X** means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

R1, R2, R3, R4 independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

wherein **R5, R6, R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

with the proviso that

- at least one of **R5, R6, R7** and **R8** does not mean -H; and/or

- at least three of **R1, R2, R3, R4, R5, R6, R7** and **R8** do not mean -H; and/or

- A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29 and/or A-30 are not included;

or a physiologically acceptable salt thereof; or

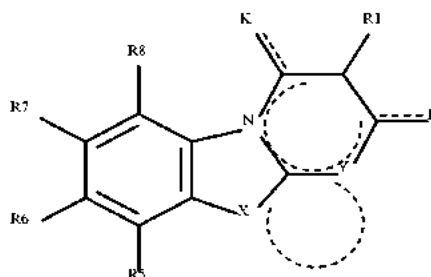
(ii) an MrgX2 antagonist which is

- selected from any one of compounds (II) B-31 to B-66 and the physiologically acceptable salts thereof; or

- selected from any one of compounds (III) C-67 to C-87 and the physiologically acceptable salts thereof;

or

- (iii) an MrgX2 antagonist according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -R2; or

K means -R2 and **L** means =O; and

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -N= and **Y** means S; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

R1, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

with the proviso that

- at least one of **R5, R6, R7** and **R8** does not mean -H; and/or
- at least three of **R1, R2, R3, R4, R5, R6, R7** and **R8** do not mean -H; and/or
- A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29 and/or A-30 are not included;

or a physiologically acceptable salt thereof and further comprising a physiologically acceptable excipient; or

(iv) an MrgX2 antagonist which is

- selected from any one of compounds (II) B-31 to B-66 and the physiologically acceptable salts thereof; or
- selected from any one of compounds (III) C-67 to C-87 and the physiologically acceptable salts thereof;

and further comprising a physiologically acceptable excipient.

[0037] Preferably, the pharmaceutical dosage form is selected from **tablets and capsules**.

[0038] As used herein, the terms "physiologically acceptable salt" refer to those salts which retain the biological effectiveness and properties of the compound according to general formula (A), (B) and (C). Such salts include, but are not restricted to: (1) an acid addition salt which is obtained by reaction of the free base of the compound according to general formula (A), (B) and (C) with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perchloric acid and the like, or with organic acids such as acetic acid, oxalic acid, (D)- or (L)- malic acid, maleic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, tartaric acid, citric acid, succinic acid or malonic acid and the like, preferably hydrochloric acid or (L)-malic acid; or (2) salts

formed when an acidic proton present in the compound according to general formula (A), (B) and (C) either is replaced by a metal ion, e. g., an alkali metal ion, such as sodium or potassium, an alkaline earth ion, such as magnesium or calcium, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

[0039] The compound of general formula (A), (B) and (C) may also act as a prodrug. A "prodrug" preferably refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial. A prodrug may be converted into the parent drug by various mechanisms, including enzymatic processes and metabolic hydrolysis.

[0040] A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2-10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule. The prodrugs of compounds of general formula (A), (B) and (C) are within the scope of this invention.

[0041] Additionally, it is contemplated that compounds of general formula (A), (B) and (C) would be metabolized by enzymes in the body of the organism such as a human being to generate a metabolite that can modulate the activity of the MrgX2 receptor. Such metabolites are within the scope of the present invention.

[0042] Preferably, unless otherwise stated, the following terms used in the specification and claims have the following meanings:

[0043] Unless expressly stated otherwise, "alkyl" preferably refers to an aliphatic hydrocarbon including straight chain, or branched chain groups. Preferably, the alkyl group has 1 to 10 carbon atoms (C₁-C₁₀ alkyl), more preferably 1 to 6 carbon atoms (C₁-C₆ alkyl) and most preferably 1 to 4 carbon atoms (C₁-C₄ alkyl), e. g., methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and the like. The aliphatic hydrocarbon may be saturated or unsaturated. When it is unsaturated, it may contain one or more unsaturations, i. e., -C=C-double and/or -C≡C-triple bonds. If there is more than one unsaturation, the unsaturations may be conjugated or isolated. Thus, for the purpose of the specification the term "alkyl" encompasses saturated hydrocarbons as well as alkenyl, alkynyl and alkenynyl residues.

"Alkenyl" preferably refers to an alkyl group, as defined above, consisting of at least two carbon atoms and at least one carbon-carbon double bond e.g., ethenyl, propenyl, butenyl or pentenyl and their structural isomeric forms such as 1- or 2-propenyl, 1-, 2-, or 3-butenyl and the like. "Alkynyl" preferably refers to an alkyl group, as defined above, consisting of at least two carbon atoms and at least one carbon-carbon triple bond e. g., acetylene, ethynyl, propynyl, butynyl, or pentynyl and their structural isomeric forms as described above. Alkyl may be substituted or unsubstituted. When substituted, the substituent group(s) is one or more, for example one or two groups, individually selected from the group consisting of -C₃-C₈ cycloalkyl; -C₆-C₁₄ aryl; a 5-10 membered -heteroaryl wherein 1 to 4 ring atoms are independently selected from N, O or S; a 5-10 membered heterocycloalkyl wherein 1 to 3 ring atoms are independently selected from N, O or S; -OH; -O-C₁-C₁₀ alkyl (= C₁-C₁₀ alkoxy); -O-C₃-C₈ cycloalkyl (= C₃-C₈ cycloalkoxy); -O-C₆-C₁₄ aryl (= C₆-C₁₄ aryloxy); -SH; -S-C₁-C₁₀ alkyl (= alkylthio); -S-C₆-C₁₄ aryl (= C₆-C₁₄ arylthio); -CN; -halo; -carbonyl; -thiocarbonyl; -O-carbamyl; -N-carbamyl; -O-thiocarbamyl; -N-thiocarbamyl; -C-amido; -N-amido; -C-carboxy; -O-carboxy; -NO₂; -silyl; -sulfinyl; -sulfonyl; and -NR^bR^c where R^b and R^c are independently selected from the group consisting of -H, -C₁-C₄ alkyl, -C₃-C₈ cycloalkyl, -C₆-C₁₄ aryl, -carbonyl, -acetyl, -sulfonyl, -amino, and trifluoromethanesulfonyl; or R^b and R^c, together with the nitrogen atom to which they are attached, combine to form a five- or six-membered heterocyclo-alkyl ring. Preferably, the substituent(s) is/are independently selected from -chloro, -fluoro, -bromo, -hydroxy, -methoxy, -nitro, -carboxy, -methoxycarbonyl, -sulfonyl, or -amino.

[0044] Unless expressly stated otherwise, "cycloalkyl" preferably refers to cyclic hydrocarbon residue that contains no heteroatoms as ring members and that is not aromatic. "Cyclo-alkyl" may encompass a single cycle or more than one cycle. Preferably, cycloalkyl has 3 to 8 carbon atoms (-C₃-C₈ cycloalkyl). Cycloalkyl may be saturated, e.g., cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, adamantane; or unsaturated (e.g., cycloalkenyl, cycloalkynyl), e.g., cyclobutenyl, cyclopentenyl, cyclohexenyl, cyclo-hexadiene, cycloheptatriene and the like. Cycloalkyl may be substituted or unsubstituted. When substituted, the substituent group(s) is one or more, for example one or two groups, individually selected from -C₁-C₁₀ alkyl; -C₃-C₈ cycloalkyl; -C₆-C₁₄ aryl; 5-10 membered -heteroaryl wherein 1 to 4 ring atoms are independently selected from N, O or S; 5-10 membered -heterocycloalkyl wherein 1 to 3 ring atoms are independently selected from N, O or S; -OH; -O-C₁-C₁₀ alkyl; -O-C₃-C₈ cycloalkyl; -O-C₆-C₁₄ aryl; -SH; -S-C₁-C₁₀ alkyl; -S-C₆-C₁₄ aryl; -CN; -halo; -carbonyl; -thiocarbonyl; -O-carbamyl; -N-carbamyl; -O-thiocarbamyl; -N-thiocarbamyl; -C-amido; -N-amido; -C-carboxy; -O-carboxy; -NO₂; -silyl; -sulfinyl; -sulfonyl; and -NR^bR^c where R^b and R^c are independently selected from the group consisting of -H, -C₁-C₄ alkyl, -C₃-C₈ cycloalkyl, -C₆-C₁₄ aryl, -carbonyl, -acetyl, -sulfonyl, -amino, and trifluoromethanesulfonyl; or R^b and R^c, together with the nitrogen atom to which they are attached, combine to form a five- or six-membered heterocycloalkyl ring. Preferably,

the substituent(s) is/are independently selected from -chloro, -fluoro, -bromo, -methyl, -ethyl, -hydroxy, -methoxy, -nitro, -carboxy, -methoxycarbonyl, -sulfonyl, or -amino.

[0045] Unless expressly stated otherwise, "heterocycloalkyl" preferably refers to a monocyclic or fused ring of 5 to 10 ring atoms containing one, two, or three heteroatoms in the ring which are selected from the group consisting of N, O and -S(O)_n where n is 0-2, the remaining ring atoms being carbon. The rings may be saturated or unsaturated, i.e. the rings may have one or more double bonds. However, the rings are not aromatic (heterocycloalkyl ≠ heteroaryl). Examples, without limitation, of heterocycloalkyl groups are pyrrolidine, piperidine, piperazine, morpholine, imidazolidine, tetrahydropyridazine, tetrahydrofuran, thiomorpholine, tetrahydropyridine, and the like. Heterocycloalkyl may be substituted or unsubstituted. When substituted, the substituted group(s) is one or more, for example one, two, or three substituents, independently selected from the group consisting of -C₁-C₁₀ alkyl; -C₃-C₈ cycloalkyl; -C₆-C₁₄ aryl; 5-10 membered -heteroaryl wherein 1 to 4 ring atoms are independently selected from N, O or S; 5-10 membered -heterocycloalkyl wherein 1 to 3 ring atoms are independently selected from N, O or S; -OH; -O-C₁-C₁₀ alkyl; -O-C₃-C₈ cycloalkyl; -O-C₆-C₁₄ aryl; -SH; -S-C₁-C₁₀ alkyl; -S-C₆-C₁₄ aryl; -CN; -halo; -carbonyl; -thiocarbonyl; -O-carbamyl; -N-carbamyl; -O-thiocarbamyl; -N-thiocarbamyl; -C-amido; -N-amido; -C-carboxy; -O-carboxy; -NO₂; -silyl; -sulfinyl; -sulfonyl; and -NR^bR^c where R^b and R^c are independently selected from the group consisting of -H, -C₁-C₄ alkyl, -C₃-C₈ cycloalkyl, -C₆-C₁₄ aryl, -carbonyl, -acetyl, -sulfonyl, -amino, and trifluoromethanesulfonyl; or R^b and R^c, together with the nitrogen atom to which they are attached, combine to form a five- or six-membered heterocycloalkyl ring. Preferably, the substituent(s) is/are independently selected from -chloro, -fluoro, -bromo, -methyl, -ethyl, -hydroxy, -methoxy, -nitro, -carboxy, -methoxycarbonyl, -sulfonyl, or -amino.

[0046] Unless expressly stated otherwise, "aryl" preferably refers to an aromatic all-carbon monocyclic or fused-ring polycyclic group (i.e., rings which share adjacent pairs of carbon atoms) of 6 to 14 ring atoms and having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is one or more, for example one, two, or three substituents, independently selected from the group consisting of -C₁-C₁₀ alkyl; -C₃-C₈ cycloalkyl; -C₆-C₁₄ aryl; 5-10 membered -heteroaryl wherein 1 to 4 ring atoms are independently selected from N, O or S; 5-10 membered -heterocycloalkyl wherein 1 to 3 ring atoms are independently selected from N, O or S; -OH; -O-C₁-C₁₀ alkyl; -O-C₃-C₈ cycloalkyl; -O-C₆-C₁₄ aryl; -SH; -S-C₁-C₁₀ alkyl; -S-C₆-C₁₄ aryl; -CN; -halo; -carbonyl; -thiocarbonyl; -O-carbamyl; -N-carbamyl; -O-thiocarbamyl; -N-thiocarbamyl; -C-amido; -N-amido; -C-carboxy; -O-carboxy; -NO₂; -silyl; -sulfinyl; -sulfonyl; and -NR^bR^c where R^b and R^c are independently selected from the group consisting of -H, -C₁-C₄ alkyl, -C₃-C₈ cycloalkyl, -C₆-C₁₄ aryl, -carbonyl, -acetyl, -sulfonyl, -amino, and trifluoromethanesulfonyl; or R^b and R^c, together with the

nitrogen atom to which they are attached, combine to form a five- or six-membered heterocycloalkyl ring.

[0047] Preferably the substituent(s) is/are independently selected from -chloro, -fluoro, -bromo, -methyl, -ethyl, -hydroxy, -methoxy, -nitro, -carboxy, -methoxycarbonyl, -sulfonyl, or -amino.

[0048] Unless expressly stated otherwise, "heteroaryl" preferably refers to a monocyclic or fused aromatic ring (i.e., rings which share an adjacent pair of atoms) of 5 to 10 ring atoms in which one, two, three or four ring atoms are selected from the group consisting of N, O and S and the rest being carbon. Examples, without limitation, of heteroaryl groups are pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, quinoliziny, quinazolinyl, pthalazinyl, quinoxaliny, cinnolinyl, naphthyridinyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5, 6, 7, 8-tetrahydroisoquinolyl, purinyl, pteridinyl, pyridinyl, pyrimidinyl, carbazolyl, xanthenyl or benzoquinolyl. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is one or more, for example one or two substituents, independently selected from the group consisting of -C₁-C₁₀ alkyl; -C₃-C₈ cycloalkyl; -C₆-C₁₄ aryl; 5-10 membered -heteroaryl wherein 1 to 4 ring atoms are independently selected from N, O or S; 5-10 membered -heterocycloalkyl wherein 1 to 3 ring atoms are independently selected from N, O or S; -OH; -O-C₁-C₁₀ alkyl; -O-C₃-C₈ cycloalkyl; -O-C₆-C₁₄ aryl; -SH; -S-C₁-C₁₀ alkyl; -S-C₆-C₁₄ aryl; -CN; -halo; -carbonyl; -thiocarbonyl; -O-carbamyl; -N-carbamyl; -O-thiocarbamyl; -N-thiocarbamyl; -C-amido; -N-amido; -C-carboxy; -O-carboxy; -NO₂; -silyl; -sulfinyl; -sulfonyl; and -NR^bR^c where R^b and R^c are independently selected from the group consisting of -H, -C₁-C₄ alkyl, -C₃-C₈ cycloalkyl, -C₆-C₁₄ aryl, -carbonyl, -acetyl, -sulfonyl, -amino, and trifluoromethanesulfonyl; or R^b and R^c, together with the nitrogen atom to which they are attached, combine to form a five- or six-membered heterocycloalkyl ring. Preferably the substituent(s) is/are independently selected from -chloro, -fluoro, -bromo, -methyl, -ethyl, -hydroxy, -methoxy, -nitro, -carboxy, -methoxy-carbonyl, -sulfonyl, or -amino.

[0049] "Hydroxy" preferably refers to an -OH group.

[0050] "Alkoxy" preferably refers to an -O-unsubstituted alkyl and -O-substituted alkyl group, as defined herein. Examples include and are not limited to -methoxy, -ethoxy, -propoxy, -butoxy, and the like.

[0051] "Cycloalkoxy" preferably refers to an O cycloalkyl group, as defined herein. One example is -cyclopropyloxy.

[0052] "Aryloxy" preferably refers to both an -O-aryl and an -O-heteroaryl group, as defined herein. Examples include and are not limited to -phenoxy, -naphthyloxy, -pyridyloxy, -furanlyoxy, and the like.

[0053] "Mercapto" preferably refers to an -SH group.

[0054] "Alkylthio" preferably refers to both an -S-alkyl and an -S-cycloalkyl group, as defined herein. Examples include and are not limited to -methylthio, -ethylthio, and the like.

[0055] "Arylthio" preferably refers to both an -S-aryl and an -S-heteroaryl group, as defined herein. Examples include and are not limited to -phenylthio, -naphthylthio, -pyridylthio, -furanlythio, and the like.

[0056] "Sulfinyl" preferably refers to a -S(=O)-R^a group, wherein, R^a is selected from the group consisting of -H, -OH, -alkyl, -cycloalkyl, -aryl, -heteroaryl (bonded through a ring carbon) and -heterocycloalkyl (bonded through a ring carbon), as defined herein.

[0057] "Sulfonyl" preferably refers to a -S(=O)₂-R^a group wherein, R^a is selected from the group consisting of -H, -OH, -alkyl, -cycloalkyl, -aryl, -heteroaryl (bonded through a ring carbon) and -heterocycloalkyl (bonded through a ring carbon), as defined herein.

[0058] "Trihalomethyl" preferably refers to a -CX₃ group wherein X is a halo group as defined herein e. g., -trifluoromethyl, -trichloromethyl, -tribromomethyl, -dichlorofluoromethyl, and the like.

[0059] "Carbonyl" preferably refers to a -C(=O)-R^a group, where R^a is selected from the group consisting of -H, -alkyl, -cycloalkyl, -aryl, -heteroaryl (bonded through a ring carbon) and -heterocycloalkyl (bonded through a ring carbon), as defined herein. Representative examples include and are not limited to -acetyl, -propionyl, -benzoyl, -formyl, -cyclopropylcarbonyl, -pyridinylcarbonyl, -pyrrolidin-1-ylcarbonyl, and the like.

[0060] "Thiocarbonyl" preferably refers to a -C(=S)-R^a group, with R^a as defined herein.

[0061] "C-carboxy" and "carboxy" which are used interchangeably herein preferably refer to a -C(=O)O-R^a group, with R^a as defined herein, e. g. COOH, -methoxycarbonyl, -ethoxy-carbonyl, -benzyloxycarbonyl, and the like.

[0062] "O-carboxy" preferably refers to a $-OC(=O)R^a$ group, with R^a as defined herein, e.g. -methylcarboxyloxy, -phenylcarboxyloxy, -benzylcarboxyloxy, and the like.

[0063] "Acetyl" preferably refers to a $-C(=O)CH_3$ group.

[0064] "Carboxylic acid" preferably refers to a $-C(=O)OR^a$ group in which R^a is -H.

[0065] "Halo" or "halogen" preferably refers to -fluorine, -chlorine, -bromine or -iodine.

[0066] "Cyano" preferably refers to a -CN group.

[0067] "Nitro" preferably refers to a $-NO_2$ group.

[0068] "O-carbamyl" preferably refers to a $-OC(=O)NR^bR^c$ group, with R^b and R^c as defined herein.

[0069] "N-carbamyl" preferably refers to a $R^cOC(=O)NR^b$ - group, with R^b and R^c as defined herein.

[0070] "O-thiocarbamyl" preferably refers to a $-OC(=S)NR^bR^c$ group, with R^b and R^c as defined herein.

[0071] "N-thiocarbamyl" preferably refers to a $R^cOC(=S)NR^b$ - group, with R^b and R^c as defined herein.

[0072] "Amino" preferably refers to an $-NR^bR^c$ group, wherein R^b and R^c are independently -H or unsubstituted C_1 - C_6 alkyl, e.g. $-NH_2$, -dimethylamino, -diethylamino, -ethylamino, -methyl-amino, and the like.

[0073] "C-amido" preferably refers to a $-C(=O)NR^bR^c$ group, with R^b and R^c as defined herein. For example, R^b is -H or unsubstituted $-C_1$ - C_4 alkyl and R^c is -H, $-C_1$ - C_4 alkyl optionally substituted with -heterocycloalkyl, -hydroxy, or -amino. For example, $-C(=O)NR^bR^c$ may be -aminocarbonyl, -dimethylaminocarbonyl, -diethylaminocarbonyl, -diethylamino-ethylaminocarbonyl, -ethylaminoethylaminocarbonyl, and the like.

[0074] "N-amido" preferably refers to a $R^cC(=O)NR^b$ - group, with R^b and R^c as defined herein, e.g. -acetylamino, and the like.

[0075] If not expressly stated otherwise, any residue, group or moiety defined herein that can be substituted is preferably substituted with one or more substituents independently selected from the group

consisting of -C₁-C₁₀ alkyl; -C₃-C₈ cycloalkyl; -C₆-C₁₄ aryl; 5-10 membered -heteroaryl wherein 1 to 4 ring atoms are independently selected from N, O or S; 5-10 membered -heterocycloalkyl wherein 1 to 3 ring atoms are independently selected from N, O or S; -OH; -O-C₁-C₁₀ alkyl; -O-C₃-C₈ cycloalkyl; -O-C₆-C₁₄ aryl; -SH; -S-C₁-C₁₀ alkyl; -S-C₆-C₁₄ aryl; -CN; -halo; -carbonyl; -thiocarbonyl; -O-carbamyl; -N-carbamyl; -O-thio-carbamyl; -N-thiocarbamyl; -C-amido; -N-amido; -C-carboxy; -O-carboxy; -NO₂; -silyl; -sulfinyl; -sulfonyl; and -NR^bR^c where R^b and R^c are independently selected from the group consisting of -H, -C₁-C₄ alkyl, -C₃-C₈ cycloalkyl, -C₆-C₁₄ aryl, -carbonyl, -acetyl, -sulfonyl, -amino, and trifluoromethanesulfonyl; or R^b and R^c, together with the nitrogen atom to which they are attached, combine to form a five- or six-membered heterocyclo-alkyl ring. Preferably the substituent(s) is/are independently selected from -chloro, -fluoro, -bromo, -methyl, -ethyl, -hydroxy, -methoxy, -nitro, -carboxy, -methoxycarbonyl, -sulfonyl, or -amino.

[0076] The invention also relates to the stereoisomers of the compounds according to general formula (A), (B) and (C) e.g. the enantiomers or diastereomers in racemic, enriched or substantially pure form.

[0077] In another aspect, the present invention relates to a pharmaceutical composition comprising any of the compounds or salts of the present invention and, optionally, a pharmaceutically acceptable carrier or excipient. This composition may additionally comprise further compounds or medicaments, such as, for example, neuroprotective or antinociceptive agents besides the compounds according to general formula (A), (B) and (C).

[0078] "Pharmaceutical composition" preferably refers to a mixture of one or more of the compounds described herein, or physiologically/pharmaceutically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically/ pharmaceutically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[0079] As used herein, a "physiologically/pharmaceutically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

[0080] A "pharmaceutically acceptable excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[0081] Physiologically or pharmaceutically acceptable carriers and excipients are known to the skilled person. In this regard it can be referred to, e.g., H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende technische Gebiete*, Editio Cantor Aulendorf, 2001.

[0082] The pharmaceutical composition according to the invention can be, e.g., solid, liquid or pasty.

[0083] A further aspect of the invention relates to a pharmaceutical dosage form comprising the pharmaceutical composition according to the invention.

[0084] The pharmaceutical dosage form according to the invention may be adapted for various routes of administration (e.g. systemic, parenteral, topic, local), such as oral administration, infusion, injection and the like.

[0085] Pharmaceutical dosage forms that are adapted for oral administration include tablets, pellets, capsules, powders, granules and the like.

[0086] The pharmaceutical dosage form is preferably adapted for administration once daily, twice daily or thrice daily. The pharmaceutical dosage form may release the compound according to general formula (A), (B) and (C) immediately (immediate release formulation) or over an extended period of time (retarded release, delayed release, prolonged release, sustained release, and the like).

[0087] The compounds according to the invention show agonistic or antagonistic effects at the MrgX2 receptors. They may among other indications be used as antinociceptive and/or antipruritic drugs.

[0088] In another aspect, the invention relates to the use of the compounds according to general formula (A), (B) and (C) for activating or antagonizing MrgX2 receptor function. Thus, in one embodiment, the compounds of the invention may be used as antinociceptive drugs. In a further aspect, the compounds according to general formula (A), (B) and (C) may thus also be used for the prevention, alleviation and/or treatment of a disease or disorder related to MrgX2 receptor activity.

[0089] The terms "diseases and disorders related to MrgX2 receptor function", "diseases and disorders connected to MrgX2 receptor function" and "disease or disorder related to MrgX2 receptor activity" are used interchangeably herein to refer to a condition involving MrgX2 receptor activity. Examples for such diseases and disorders are neurodegenerative diseases, nociceptive pain, and neuropathic pain. Since the adenine receptor is also expressed in brain, e.g. in the cortex, also included are CNS disorders, such as neuroinflammatory conditions and neurodegenerative disorders (e.g. Alzheimer's and Parkinson's disease).

[0090] "Treat", "treating" and "treatment" preferably refer to a method of alleviating or abrogating an MrgX2 receptor related disease or disorder and/or its attendant symptoms.

[0091] "Prevent", "preventing" and "prevention" preferably refer to a method of hindering an MrgX2 receptor related disease or disorder from occurring, i.e. a prophylactic method.

[0092] "Organism" preferably refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukaryotic cell or as complex as a mammal, including a human being.

[0093] "Therapeutically effective amount" preferably refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated.

[0094] Preferably, the subject afflicted by a disease treated, alleviated or prevented according to the invented use is a human.

[0095] A further aspect of the invention relates to the use of a compound according to general formula (A), (B) and (C) for the manufacture of a pharmaceutical composition according to the invention or of a pharmaceutical dosage form according to the invention for preventing, ameliorating or treating pain or a pruitus.

[0096] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the term "includes" shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0097] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any

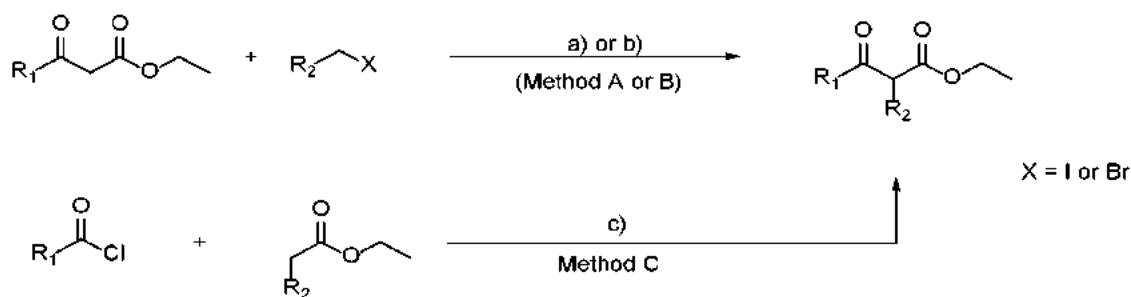
subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0098] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0099] The present inventions will be explained in more detail in the following examples. However, the examples are only used for illustration and do not limit the scope of the present invention.

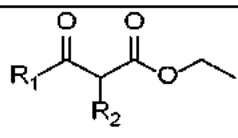
[0100] For all reactions, analytical grade solvents were used. ^1H , ^{13}C and ^{19}F NMR spectra were recorded using spectrometers: Bruker Avance 300 (^1H NMR: 300 MHz, ^{13}C NMR: 75 MHz), Bruker Avance 500 (^1H NMR: 500 MHz, ^{13}C NMR: 125 MHz, ^{19}F NMR: 470 MHz) or Bruker Avance 600 (^1H NMR: 600 MHz, ^{13}C NMR: 150 MHz). Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) or residual solvent signal for ^1H , residual solvent signal for ^{13}C and trifluoroacetic acid (-77.0 ppm) for ^{19}F NMR. Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, dt = doublet of triplets, dq = doublet of quartets, td = triplet of doublets, ddd = doublet of doublet of doublets, m = multiplet, br = broad. Coupling constants are expressed in Hz. High resolution mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at $3 \mu\text{L}\cdot\text{min}^{-1}$ and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC. Flash column chromatography was performed on ICN silica gel 63–200 mesh, 60 Å.

Synthesis of 2-alkyl-3-oxoesters:



Scheme. Synthesis of 2-alkyl-3-oxoesters. Reagents and conditions: a) NaH, THF, rt; b) K_2CO_3 , DMF, rt; c) BuLi, DIPEA, THF, -78 °C to rt.

Table. Overview of the synthesized 3-oxoesters.

Entry		
	R ₁	R ₂
1	isopropyl (methylethyl)	ethyl
2	<i>tert</i> -butyl (1,1-dimethylethyl)	ethyl
3	phenyl	ethyl
4	cyclopropyl	ethyl
5	isobutyl (2-methylpropyl)	ethyl
6	propyl	ethyl
7	isopropyl (methylethyl)	propyl
8	propyl	propyl
9	methyl	2-methoxyethyl
10	<i>p</i> -methoxyphenyl	ethyl

Method A:

[0101] Appropriate 3-oxoester (1.0 equiv.) was added to a suspension of NaH (60% in mineral oil, 1.0 mol. equiv.) in THF (1 ml/1 mmol of 3-oxoester) at room temperature and resulting mixture was stirred for 30 min at room temperature. Then alkyl halide (1 mol. equiv.) was added and mixture was refluxed for defined period of time (TLC control). After cooling to room temperature reaction was quenched with saturated aqueous NH₄Cl (1.2 ml/1 mmol of NaH). The aqueous layer was separated and extracted with AcOEt (3x). Organic layers were combined, dried over MgSO₄, filtered and concentrated *in vacuo* (at 30 °C). The crude product was purified by flash column chromatography on silica gel giving colorless liquid which was pure enough to be used in the next step without additional purification and fraction contaminated with starting material which was not further purified.

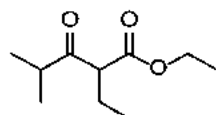
Method B:

[0102] A mixture of 3-oxoester (1 mol. equiv.), alkyl halide (1.1 mol. equiv.) and K₂CO₃ (1.5 mol. equiv.) in DMF (2.5 ml/1 ml of 3-oxoester) was stirred at room temperature overnight. Next, H₂O (10 vol of DMF) was added and mixture was extracted with AcOEt (3x). Organic layers were combined, dried over MgSO₄, filtered and concentrated *in vacuo* (at 30 °C). The crude product was purified by flash column chromatography on silica gel giving colorless liquid which was pure enough to be used in the next step without additional purification and fraction contaminated with starting material which was not further purified.

Method C:

[0103] To a solution of butyl lithium (2.3 mol equiv.) in THF at -78°C , was added diisopropylamine (DIPEA; 2.4 mol equiv.) dropwise. The mixture was stirred at -78°C for half an hour. Ethyl ester (2 mol equiv.) was added dropwise via cannula over 30 minutes and the mixture was allowed to stir further at -78°C . Lastly acid chloride (1 mol equiv.) was added dropwise to the reaction mixture and after addition the reaction was heated to room temperature and allowed to stir for another 2 hours. After reaction completion the reaction was quenched using saturated NH_4Cl solution and the mixture was extracted three times with AcOEt. The combined organic layers were dried using Na_2SO_4 , filtered and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel yielding a colorless to light yellow oil.

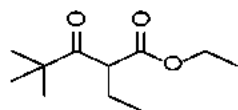
Ethyl 2-ethyl-4-methyl-3-oxopentanoate:



Chemical Formula: $\text{C}_{10}\text{H}_{18}\text{O}_3$

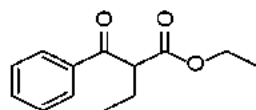
[0104] The title compound was obtained from ethyl isobutyrylacetate (2.50 g; 15.80 mmol) and ethyl iodide according to Method A in 90% yield (2.64 g, 14.17 mmol). ^1H NMR (CDCl_3 , 300 MHz): δ (ppm) 0.92 (t, $J = 7.4$ Hz, 3H), 1.10 (d, $J = 6.9$ Hz, 3H), 1.12 (d, $J = 6.9$ Hz, 3H), 1.26 (t, $J = 7.1$ Hz, 3H), 1.87 (dq, $J = 7.4, 7.3$ Hz, 2H), 2.80 (sept, $J = 6.9$ Hz, 1H), 3.53 (t, $J = 7.3$ Hz, 3H), 4.18 (q, $J = 7.1$ Hz, 2H). ^{13}C NMR (CDCl_3 , 75MHz): δ (ppm) 12.18, 14.22, 18.16, 18.45, 21.84, 40.72, 58.77, 61.26, 169.90, 209.26.

Ethyl 2-ethyl-4,4-dimethyl-3-oxopentanoate:



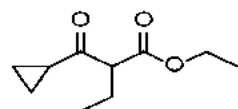
[0105] The title compound was obtained from trimethylacetyl chloride (0.50 g; 4.15 mmol) and ethyl butanoate according to Method C in 38% yield (320 mg, 1.60 mmol). ^1H NMR (CDCl_3 , 300MHz): δ (ppm) 0.91 (t, $J = 7.4$ Hz, 3H), 1.17 (s, 9H), 1.24 (t, $J = 7.5$ Hz, 3H), 1.80 (m, 2H), 3.80 (t, $J = 7.1$ Hz, 1H), 4.13 (q, $J = 7.1$ Hz, 2H).

Ethyl 2-benzoylbutanoate:



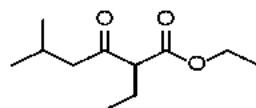
[0106] The title compound was obtained from benzoyl chloride (0.50 g; 3.56 mmol) and ethyl butanoate according to Method C and the crude was used in the next reaction without further purification.

Ethyl 2-(cyclopropanecarbonyl)butanoate:



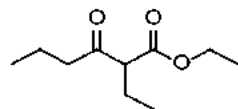
[0107] The title compound was obtained from cyclopropanecarbonyl chloride (0.50 g; 4.78 mmol) and ethyl butanoate according to Method C in 26% yield (320 mg, 1.25 mmol). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 0.95 (t, *J* = 7.4 Hz, 3H), 1.09 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.94 (q, *J* = 7.4 Hz, 2H), 2.07 (m, 1H), 3.47 (t, *J* = 7.4 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H).

Ethyl 2-ethyl-5-methyl-3-oxohexanoate:



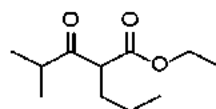
[0108] The title compound was obtained from 3-methylbutyryl chloride (0.50 g; 4.15 mmol) and ethyl butanoate according to Method C in 58% yield (490 mg, 2.45 mmol). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 0.89 (m, 9H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.55 (m, 3H), 1.68–1.94 (m, 2H), 2.39–2.55 (m, 1H), 4.18 (dd, *J* = 8.7, 5.7 Hz, 2H).

Ethyl 2-ethyl-3-oxohexanoate:



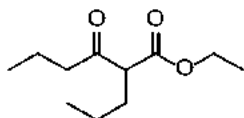
[0109] The title compound was obtained from ethyl butyrylacetate (1.496 ml; 1.48 g; 9.353 mmol) and ethyl iodide according to Method A in 76% yield (1.320 g, 7.087 mmol) as a colorless liquid. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 0.91 (t, *J* = 7.5 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.62 (sext, *J* = 7.5 Hz, 2H), 1.83–1.92 (m, 2H), 2.44 (m, 1H), 2.54 (m, 1H), 3.35 (t, *J* = 7.2 Hz, 1H), 4.19 (q, *J* = 7.2 Hz, 2H). ¹³C NMR (CDCl₃, 75MHz): δ (ppm) 12.06, 13.68, 14.22, 17.02, 21.70, 43.87, 60.84, 61.28, 169.98, 205.50.

Ethyl 4-methyl-3-oxo-2-propylpentanoate:



[0110] The title compound was obtained from ethyl isobutyrylacetate (2 mmol) and propyl iodide according to Method A in 68% (273 mg, 1.363 mmol) yield as a colorless liquid. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 0.93 (t, *J* = 7.3 Hz, 3H), 1.10 (d, *J* = 6.9 Hz, 3H), 1.11 (d, *J* = 6.9 Hz, 3H), 1.23–1.34 (m, 5H), 1.78–1.86 (m, 2H), 2.77 (sept, *J* = 6.9 Hz, 1H), 3.61 (t, *J* = 7.3 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H). ¹³C NMR (CDCl₃, 75MHz): δ (ppm) 12.98, 13.22, 17.22, 17.49, 29.52, 39.67, 56.05, 60.29, 169.02, 208.34.

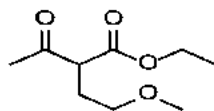
Ethyl 3-oxo-2-propylhexanoate:



Chemical Formula: C₁₁H₂₀O₃
Exact Mass: 200.14

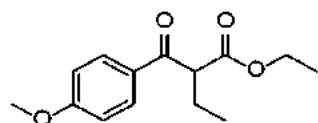
[0111] To a mixture of ethyl 3-oxohexanoate (1 mmol) in THF (5 ml) was added *t*-BuOK (1.1 mmol) and the mixture was stirred for 15 min at room temperature. Then alkyl halide (1 mmol) was added dropwise and mixture was stirred at room temperature overnight. After that time TLC control showed presence of mainly starting material. Mixture was brought to reflux and kept refluxing for 20 h. Then it was poured into saturated aqueous NH₄Cl (50 ml) and extracted with AcOEt (3x). Organic layers were combined, dried over MgSO₄, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (CH₂Cl₂) affording 99 mg of colorless liquid (0.494 mmol, 49%) which was pure enough to be used in the next step. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 0.91 (t, *J* = 7.5 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H), 1.24–1.37 (m, 5H), 1.51–1.66 (m, 2H), 1.70–1.75 (m, 2H), 3.46 (t, *J* = 7.5 Hz, 1H), 4.18 (q, *J* = 7.2 Hz, 2H). ¹³C NMR (CDCl₃, 75MHz): δ (ppm) 12.68, 12.93, 13.22, 16.04, 19.83, 29.35, 42.79, 58.11, 60.28, 169.10, 204.54.

Ethyl 2-acetyl-4-methoxybutanoate:



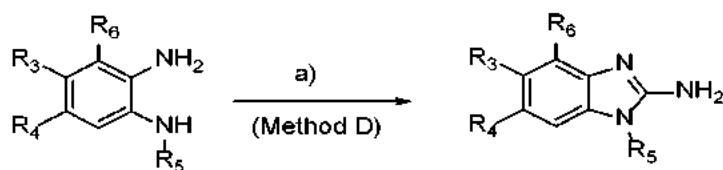
[0112] The title compound was obtained from ethyl acetoacetate (1.25 mmol) and 1-bromo-2-methoxyethane according to Method A in 74% yield (174 mg, 0.925 mmol) as a yellow liquid. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 1.28 (t, *J* = 7.2 Hz, 3H), 2.08–2.16 (m, 2H), 2.26 (s, 3H), 3.29 (s, 3H), 3.37–3.41 (m, 2H), 3.64 (t, *J* = 6.9 Hz, 1H), 4.20 (q, *J* = 7.2 Hz, 2H). ¹³C NMR (CDCl₃, 75MHz): δ (ppm) 14.19, 28.21, 29.38, 56.34, 58.69, 61.49, 70.05, 169.79, 203.15.

Ethyl 2-(4-methoxybenzoyl)butanoate:



[0113] The title compound was obtained from 2.0 mmol of ethyl 3-(4-methoxyphenyl)-3-oxopropanoate and ethyl iodide according to Method A in 65% yield (325 mg, 1.298 mmol) as a colorless liquid. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 0.98 (t, *J* = 7.5 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 3H), 1.98–2.08 (m, 2H), 3.87 (s, 3H), 4.11–4.20 (m, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 9.0 Hz, 2H). ¹³C NMR (CDCl₃, 75MHz): δ (ppm) 12.25, 14.17, 22.58, 24.88, 55.62, 55.77, 114.01, 129.57, 131.06, 163.92, 170.32, 193.76.

Synthesis of 2-aminoimidazole derivatives:



Scheme. Synthesis of 2-aminoimidazole derivatives. Reagents and conditions: a) BrCN, EtOH, 50 °C.

Table. Overview of the synthesized 2-aminobenzimidazole derivatives.

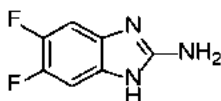
Entry				
	R ₃	R ₄	R ₅	R ₆
1	F	F	H	H
2	Cl	Cl	H	H
3	Br	Br	H	H
4*	Mc	Mc	H	H
5	H	H	H	Me
6	Me (H)	H (Me)	H	H
7	CN (H)	H (CN)	H	H
8	Cl (H)	H (Cl)	H	H
9	F (H)	H (F)	H	H
10	OMe (H)	H (OMe)	H	H
11	Mc	H	Bn	H
12	H	Mc	Bn	H

* Product isolated as hydrobromide salt.

Method D:

[0114] To an appropriate aromatic diamino compound (1 mol. equiv.) in EtOH (4.5 ml/1 mmol starting material) was added cyanogen bromide (1.2 mol. equiv.) and resulting mixture was stirred at 50 °C till disappearance of starting material (TLC control). Then volatiles were removed *in vacuo* and the solid residue was re-dissolved (or suspended) in H₂O. Water mixture was extracted with EtOAc (3x). Organic layer was discarded and water layer was brought to pH 9–10 (according to universal indicator paper) using 25% NH₄OH or 1 M aqueous NaOH and it was extracted with EtOAc (3x). Combined organic layers were dried over MgSO₄. Drying agent was removed, residue was concentrated to dryness and purified on silica gel column if needed.

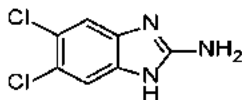
5,6-Difluoro-1H-benzo[d]imidazol-2-amine:



Chemical Formula: C₇H₆F₂N₃
Exact Mass: 169,05

[0115] The title compound was synthesized from 1,2-diamino-4,5-difluorobenzene (500 mg, 3.47 mmol) according to the Method D. Purification of crude product on silica gel column (4–6% of MeOH in CH₂Cl₂ with 2% (v/v) addition of 7 N ammonia in MeOH) afforded 459 mg (2.71 mmol; 78%) of product as a pale brown solid. ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 6.31 (br, 2H), 7.05–7.08 (m, 2H). ¹³C NMR (DMSO-d₆, 150MHz): δ (ppm) 99.63, 134.3 (br), 144.6 (dd, *J* = 232.4 Hz, *J* = 15.1 Hz), 156.99. ¹⁹F NMR (DMSO-d₆, 470 MHz): δ (ppm) –150.2 ¹H NMR (CD₃OD, 600 MHz): δ (ppm) 4.94 (br, 2H), 7.01 (t, *J* = 9.0 Hz, 2H). ¹³C NMR (CD₃OD, 150MHz): δ (ppm) 100.1 (d, *J* = 15.1 Hz), 134.6 (br), 147.3 (dd, *J* = 236.9 Hz, *J* = 16.6 Hz), 158.14. ¹⁹F NMR (CD₃OD, 470 MHz): δ (ppm) –150.7 (t, *J* = 8.7 Hz). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₇H₆F₂N₃: 170.0524, found 170.0531.

5,6-Dichloro-1H-benzo[d]imidazol-2-amine:

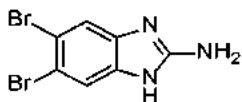


Chemical Formula: C₇H₅Cl₂N₃
Exact Mass: 200,99

[0116] The title compound was synthesized from 1,2-diamino-4,5-dichlorobenzene (500 mg, 2.82 mmol) according to the Method D affording 568 mg (quant.) of product as a pale yellow solid (without column purification). ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 6.58 (s, 2H), 7.27 (s, 2H). ¹³C NMR

(DMSO- d_6 , 75MHz): δ (ppm) 112.35, 120.76, 138.78, 157.12. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_7H_6Cl_2N_3$: 201.9933, found 201.9934.

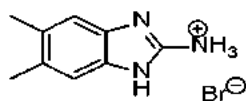
5,6-Dibromo-1H-benzo[d]imidazol-2-amine:



Chemical Formula: $C_7H_5Br_2N_3$
Exact Mass: 288.89

[0117] The title product was synthesized from 4,5-dibromo-1,2-diaminobenzene (100 mg, 0.376 mmol) according to the Method D. Purification by silica gel column chromatography (3–10% of MeOH in CH_2Cl_2) afforded 100 mg (0.343 mmol, 91%) of product as a white solid. 1H NMR (DMSO- d_6 , 300 MHz) δ (ppm) 6.93 (br, 2H), 7.46 (s, 2H).

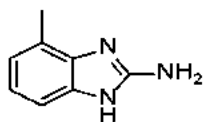
5,6-Dimethyl-1H-benzo[d]imidazol-2-amine hydrobromide:



Chemical Formula: $C_9H_{12}BrN_3$
Exact Mass: 241.02

[0118] Product was synthesized from 1,2-diamino-4,5-dimethylbenzene based on the Method D. After addition of concentrated aqueous NH_3 product was still present in H_2O . Water layer was concentrated and solid residue was purified on silica gel column (20% of MeOH in CH_2Cl_2 with 2% vol. addition of 7 N ammonia in MeOH) affording 676 mg (2.792 mmol; 76%) of product as a pale yellow solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.26 (s, 6H), 7.11 (s, 2H), 7.97 (br, 2H). ^{13}C NMR (DMSO- d_6 , 75MHz): δ (ppm) 19.61, 111.90, 129.07, 130.57, 150.66. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_{12}N_3$: 162.1026 found 162.1033.

4-Methyl-1H-benzo[d]imidazol-2-amine:

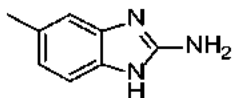


Chemical Formula: $C_8H_9N_3$
Exact Mass: 147.08

[0119] The title compound was synthesized from 1,2-diamino-3-methylbenzene (500 mg, 4.09 mmol) according to the Method D. Purification of crude product on silica gel column (4–5% of MeOH in CH_2Cl_2 with 2% vol. addition of 7 N ammonia in MeOH) afforded 327 mg (2.22 mmol; 54%) of product as a pale orange solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.34 (s, 3H), 6.08 (br, 2H), 6.67 (d, J = 7.2 Hz, 1H), 6.75 (t, J = 7.5 Hz, 1H), 6.93 (d, J = 7.5 Hz, 1H). ^{13}C NMR (DMSO- d_6 , 75MHz): δ (ppm)

16.61, 108.77, 118.84, 120.07, 121.15, 137.17, 138.37, 154.76. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_8H_{10}N_3$: 148.0869, found 148.0874.

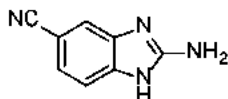
5-Methyl-1H-benzo[d]imidazol-2-amine:



Chemical Formula: $C_8H_9N_3$
Exact Mass: 147.08

[0120] The title product was synthesized from 1,2-diamino-4-methylbenzene (500 mg, 4.093 mmol) according to the Method D affording 596 mg (4.049 mmol, 96%) of product as red-brown solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.30 (s, 3H), 6.08 (br, 2H), 6.67 (d, $J = 7.8$ Hz, 1H), 6.91 (s, 1H), 6.96 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (DMSO- d_6 , 75MHz): δ (ppm) 21.19, 111.03, 111.89, 119.92, 127.67, 136.34, 138.77, 155.01. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_8H_{10}N_3$: 148.0869, found: 148.0861.

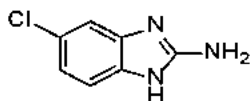
2-Amino-1H-benzo[d]imidazole-5-carbonitrile:



Chemical Formula: $C_8H_6N_4$
Exact Mass: 158.06

[0121] The title product was obtained from 3,4-diaminobenzonitrile (500 mg, 3.76 mmol) with slightly modified Method D. After removal of volatiles, solid residue was redissolved in H_2O and pH was adjusted to 8–9 using 5 N aqueous NaOH. Precipitate that formed was filtered-off, washed with H_2O and dried under vacuum. Filtrate and washings were combined and extracted with EtOAc (3x). Combined organic layers were dried over $MgSO_4$. After removal of drying agent and solvent residue was put on silica gel column (8% of MeOH in CH_2Cl_2 with 2% vol. addition of 7 N ammonia MeOH) affording additional 77 mg of product. Total yield: 590 mg (3.73 mmol, 99%) of product as a grey solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 6.67 (bs, 2H), 7.20 (d, $J = 7.8$ Hz, 1H), 7.27 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 100.01, 112.43, 114.34, 120.88, 123.86, 138.16, 143.67, 157.67. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_8H_7N_4$: 159.0665, found: 159.0669.

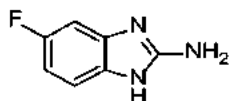
5-Chloro-1H-benzo[d]imidazol-2-amine:



Chemical Formula: $C_7H_6ClN_3$
Exact Mass: 167.03

[0122] The title compound was synthesized from 4-chloro-1,2-diaminobenzene (500 mg, 3.51 mmol) according to the Method D. Purification of crude product by silica gel column chromatography (5–10% of MeOH in CH₂Cl₂ with 2% vol. addition of 7 N ammonia in MeOH) afforded 512 mg (3.05 mmol; 87%) of product as a brown solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 6.36 (bs, 2H), 6.85 (dd, 1H, *J* = 8.1 Hz, *J* = 1.8 Hz), 7.07 (t, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 1.8 Hz, 1H). ¹³C NMR (DMSO-d₆, 75 MHz): δ (ppm) 111.63, 111.75, 118.46, 123.26, 136.80, 140.86, 156.37. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₇H₇ClN₃: 168.0323, found 168.0329.

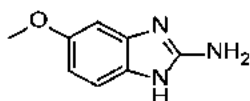
5-Fluoro-1*H*-benzo[*d*]imidazol-2-amine:



Chemical Formula: C₇H₆FN₃
Exact Mass: 151.05

[0123] The title compound was synthesized from 1,2-diamino-4-fluorobenzene (500 mg, 3.96 mmol) according to the Method D. Crude product was suspended in a mixture of CH₂Cl₂ and cyclohexane, precipitate was filtered-off, washed with cyclohexane and dried affording 502 mg (3.32 mmol; 84%) of product as a brown solid. ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 6.26 (s, 2H), 6.62–6.66 (m, 1H), 6.88 (dd, dd, *J* = 9.6, 1.6 Hz, 1H), 7.03 (dd, *J* = 6.4, 5.4 Hz, 1H). ¹³C NMR (DMSO-d₆, 150MHz): δ (ppm) 99.3 (d, *J* = 25.6 Hz), 105.4 (d, *J* = 24.1 Hz), 110.57, 133.71 (br), 141.00 (br), 156.68, 157.6 (d, *J* = 229.4 Hz). ¹⁹F NMR (DMSO-d₆, 470 MHz): δ (ppm) –124.5 (br). ¹H NMR (CD₃OD, 600 MHz): δ (ppm) 4.92 (bs, 2H), 6.70 (ddd, *J* = 10.2, 8.4, 2.4 Hz, 1H), 6.89 (dd, *J* = 9.6, 2.4 Hz, 1H), 7.07 (dd, *J* = 8.4, 4.2 Hz, 1H). ¹³C NMR (CD₃OD, 150MHz): δ (ppm) 100.2 (d, *J* = 27.2 Hz), 107.8 (d, *J* = 25.6 Hz), 112.1 (d, *J* = 10.6 Hz), 134.26, 140.64, 158.4 (d, *J* = 226.4 Hz), 160.7. ¹⁹F NMR (CD₃OD, 470 MHz): δ (ppm) –(125.64–125.59) (td, *J* = 9.6 Hz, *J* = 4.6 Hz). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₇H₇FN₃: 152.0618, found 152.0621.

5-Methoxy-1*H*-benzo[*d*]imidazol-2-amine:

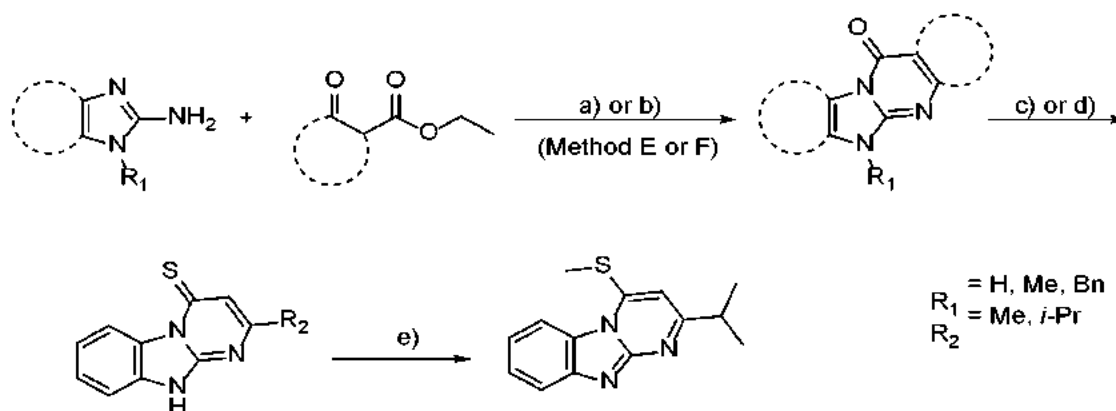


Chemical Formula: C₈H₉N₃O
Exact Mass: 163.07

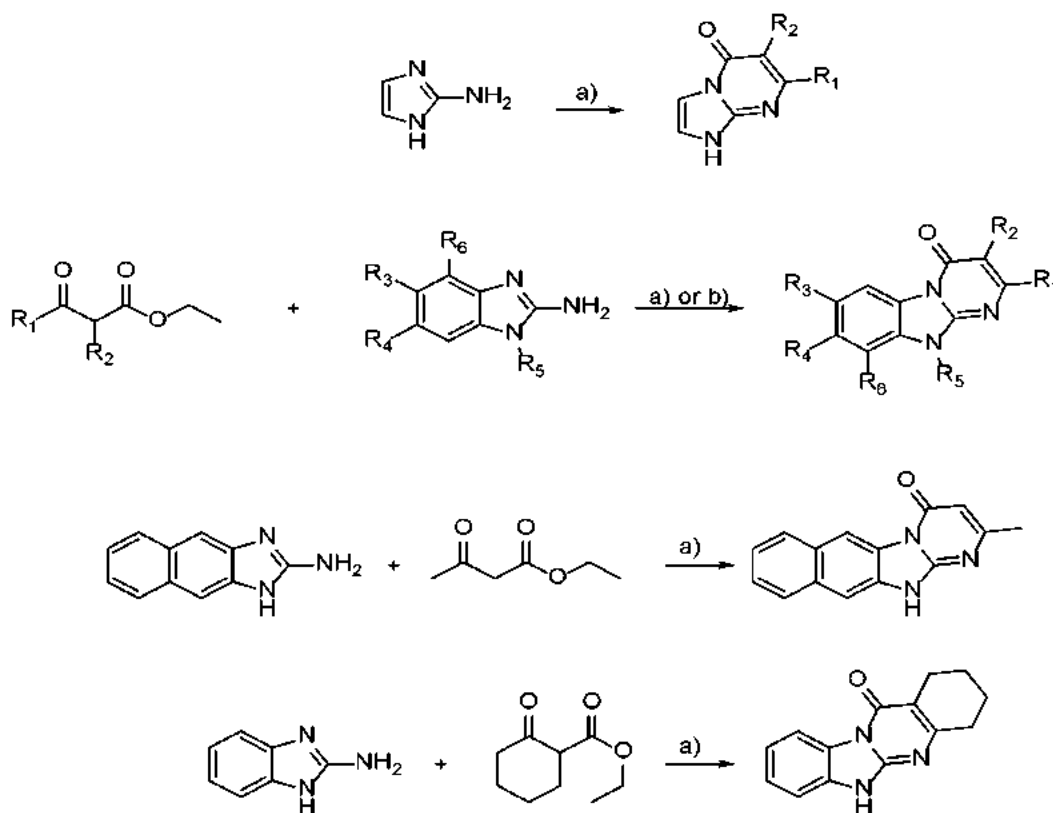
[0124] The title compound was synthesized from 1,2-diamino-4-methoxybenzene (500 mg, 3.62 mmol) according to the general procedure. Purification of crude product on FC (6–10% of CH₃OH in CH₂Cl₂ with 1% vol. addition of 7N NH₃/CH₃OH) afforded 451 mg (2.77 mmol; 76%) of product as a pale brown solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 3.70 (s, 3H), 6.10 (bs, 2H), 6.48 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.70 (d, *J* = 2.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H). ¹³C NMR (DMSO-d₆, 75 MHz): δ (ppm)

55.38, 97.58, 106.07, 110.91, 131.81, 140.03, 153.91, 155.25. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_8H_{10}N_3O$: 164.0818, found 164.0827.

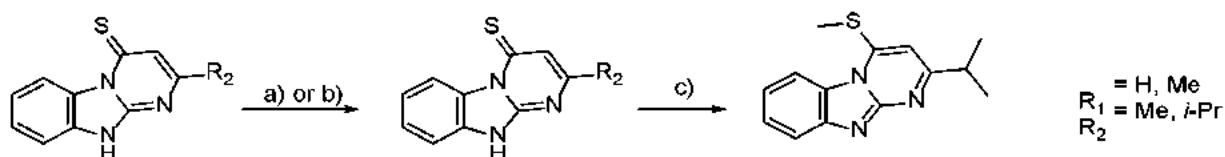
Synthesis of substituted imidazo[1,2-*a*]pyrimidin-5(1*H*)-ones, benzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one derivatives and some 4(10*H*)-thio analogues:



Scheme. Synthesis of imidazo[1,2-*a*]pyrimidin-5(1*H*)-one and benzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one derivatives. Reagents and conditions: a) NaOEt, EtOH, reflux; b) DMF, reflux; c) P_2S_5 , pyridine, reflux; d) Lawesson's reagent, toluene, reflux; e) Me_2SO_4 , $NaHCO_3$, acetone, reflux.



Scheme. Synthesis of substituted imidazo[1,2-*a*]pyrimidin-5(1*H*)-one and benzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one derivatives. Reagents and conditions: a) NaOEt, EtOH, reflux; b) DMF, reflux;



Scheme. Synthesis of 2-substituted benzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-thione derivatives and 2-isopropyl-4-(methylthio)benzo[4,5]imidazo[1,2-*a*]pyrimidine. Reagents and conditions: a) P_2S_5 , pyridine, reflux; b) Lawesson's reagent, toluene, reflux; c) Me_2SO_4 , NaHCO_3 , acetone, reflux.

General procedures:

Method E:

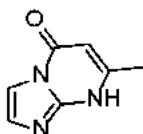
[0125] To a mixture of 2-aminoimidazole derivative (1 mol. equiv.) and 3-oxoester (1.0–1.1 mol. equiv.) in absolute EtOH (3 ml/1 mmol of starting imidazole derivative) was added sodium ethoxide (21% in EtOH; 2 mol. equiv.). Resulting mixture was refluxed for several hours (TLC control). Upon disappearance of starting materials or no further progress of the reaction mixture was cooled to room temperature. Volatiles were removed under reduced and a crude product was purified using flash silica gel column chromatography or preparative TLC. In case of not sufficient purity of product after chromatographic purification, solid was suspended in MeOH, filtered and dried affording target compound.

Method F:

[0126] To an appropriate 2-aminoimidazole derivative (1 mol. equiv.) in DMF (1–1.5 ml/1 mmol of starting material) was added 3-oxoester (1–1.64 mol. equiv.) and the mixture was refluxed for several hours (TLC control). Upon disappearance of starting materials or no further progress of the reaction mixture was cooled to room temperature. If precipitation occurred MeOH or EtOH was added, solid was filtered off, washed with solvent indicated and dried under vacuum. In other case volatiles were removed and residue was purified on silica gel column chromatography.

[0127] Purity of all products was determined by HPLC analysis and was at least 95%.

*7-Methylimidazo[1,2-*a*]pyrimidin-5(8*H*)-one*

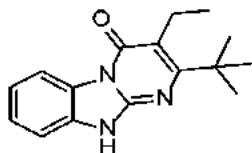


Chemical Formula: $\text{C}_7\text{H}_7\text{N}_3\text{O}$

Exact Mass: 149,06

[0128] The title compound was synthesized according to Method E starting from 2-aminoimidazole hemisulfate (100 mg, 0.758 mmol) and ethyl acetoacetate (1.1 mol. equiv). Purification by silica gel column chromatography (0–6% gradient of MeOH in CH₂Cl₂) afforded 90 mg (0.603 mmol, 80%) as a white solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 2.61 (s, 3H), 5.68 (s, 1H), 7.36 (d, *J* = 2.2 Hz, 1H), 7.52 (d, *J* = 2.2 Hz, 1H), 12.60 (br, 1H). ¹³C NMR (DMSO-d₆, 75MHz): δ (ppm) 22.08, 95.88, 107.00, 121.06, 145.80, 156.91, 159.27. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₇H₈N₃O: 150.0662, found: 150.0665.

2-(tert-Butyl)-3-ethylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

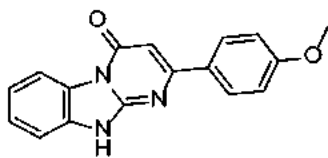


Chemical Formula: C₁₆H₁₉N₃O

Exact Mass: 269,15

[0129] The title compound was synthesized according to Method F starting from 2-aminobenzimidazole (200 mg, 1.50 mmol) and ethyl 2-ethyl-4,4-dimethyl-3-oxopentanoate (1.1 mol. equiv). Reaction time: 16 h. After removal of volatiles, crude product was purified on silica gel column (30% of EtOAc in heptane) affording 30.2 mg (0.112 mmol; 7%) of product as an off-white solid. ¹H NMR (DMSO, 300 MHz): δ 1.15 (t, *J* = 7.2 Hz, 3H), 1.46 (s, 9H), 2.76 (q, *J* = 7.1 Hz, 2H), 7.29 (ddd, *J* = 8.4, 5.6, 3.1 Hz, 1H), 7.44 (m, 2H), 8.45 (d, *J* = 8.0 Hz, 1H), 12.78 (s, 1H). ¹³C NMR (DMSO, 75 MHz): δ 13.91, 19.89, 30.48, 69.91, 110.80, 113.15, 121.40, 125.50, 126.20, 145.85, 160.54 HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₆H₂₀N₃O: 270.16007, found: 270.1595.

2-(4-Methoxyphenyl)benzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



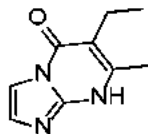
Chemical Formula: C₁₇H₁₃N₃O₂

Exact Mass: 291,10

[0130] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (100 mg, 0.751 mmol) and ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (1 mol. equiv). Reaction time: 20 h. Column chromatography of crude product (16–20% AcOEt in CH₂Cl₂ as an eluent) afforded 63 mg (0.216 mmol; 29%) of product as an off-white solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 3.83 (s, 3H), 6.56 (s, 1H), 7.05 (d, *J* = 9.0 Hz, 1H), 7.29–7.38 (m, 1H), 7.48–7.49 (m, 2H), 8.09 (d, *J* = 9.0 Hz, 1H), 8.46 (d, *J* = 8.1 Hz, 1H), 13.01 (br, 1H). ¹³C NMR (DMSO-d₆, 75 MHz): δ (ppm) 55.29, 95.73, 110.97, 114.01, 115.59, 121.77, 125.84, 126.04, 128.58, 129.16, 130.64,

149.46, 159.77, 160.32, 161.10. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{14}N_3O$: 292.1080, found: 292.1081.

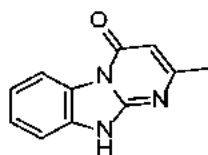
6-Ethyl-7-methylimidazo[1,2-a]pyrimidin-5(8H)-one



Chemical Formula: $C_9H_{11}N_3O$
Exact Mass: 177.09

[0131] The title compound was synthesized according to Method E starting from 2-aminoimidazole hemisulfate (50 mg, 0.379 mmol) and ethyl 2-ethyl-3-oxobutanoate (1 mol. equiv). Purification on preparative TLC (6% of MeOH in CH_2Cl_2) afforded 38 mg (0.214 mmol, 56%) as a white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.02 (t, $J = 7.5$ Hz, 3H), 2.32 (s, 3H), 2.48 (q, $J = 7.5$ Hz, 2H; overlapped with residual solvent signal), 7.36 (d, $J = 2.1$ Hz, 1H), 7.48 (d, $J = 2.1$ Hz, 1H), 12.40 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75MHz): δ (ppm) 13.50, 18.30, 19.56, 106.68, 108.15, 122.10, 143.98, 153.73, 156.92. 1H NMR (CD_3OD , 300 MHz): δ (ppm) 1.11 (t, $J = 7.5$ Hz, 3H), 2.41 (s, 3H), 2.61 (q, $J = 7.5$ Hz, 2H), 4.87 (br, 2H), 7.25 (d, $J = 2.4$ Hz, 1H), 7.52 (d, $J = 2.4$ Hz, 1H). ^{13}C NMR (CD_3OD , 75MHz): δ (ppm) 13.68, 19.68, 20.21, 108.12, 111.14, 122.10, 145.68, 157.67, 159.52. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_{12}N_3O$: 178.0975, found: 178.0975.

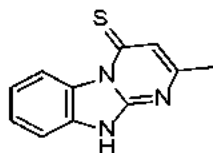
2-Methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{11}H_9N_3O$
Exact Mass: 199.07

[0132] The title compound was synthesized according to Method F starting from 2-aminobenzimidazole (100 mg, 0.751 mmol) and ethyl acetoacetate (1 mol. equiv). Reaction time: 1.5 h. Filtration, washing with H_2O and drying afforded product as an off-white solid (98 mg, 0.492 mmol, 66%). 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.31 (s, 3H), 5.84 (s, 1H), 7.29 (t, $J = 7.5$ Hz, 1H), 7.43 (t, $J = 7.5$ Hz, 1H), 7.53 (d, 1H, $J = 7.5$ Hz), 8.37 (d, $J = 7.5$ Hz, 1H), 12.73 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75MHz): δ (ppm) 21.78, 98.53, 113.76, 115.21, 121.41, 125.56, 126.94, 135.42, 148.40, 159.28. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{11}H_{10}N_3O$: 200.0818, found: 200.0822.

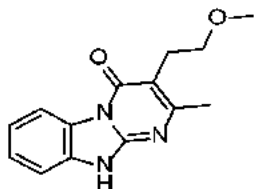
2-Methylbenzo[4,5]imidazo[1,2-a]pyrimidine-4(10H)-thione



Chemical Formula: $C_{11}H_9N_3S$
Exact Mass: 215,05

[0133] Mixture of 2-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one (**1**); 460 mg, 2.309 mmol) and P_2S_5 (513 mg, 2.309 mmol) in pyridine (2.5 ml) was refluxed for 20 h. Then it was cooled to room temperature, 8 ml of H_2O was added and resulting mixture was extracted with CH_2Cl_2 (3 x). Organic layers were combined, dried over $MgSO_4$, filtered and solvents were removed *in vacuo*. Residue was co-evaporated with toluene (3 x). Solid residue was purified by flash column chromatography on silica gel (40% of heptane in AcOEt) affording 12 mg (0.056 mmol, 2.5%) of product as a pale brown solid. 1H NMR ($DMSO-d_6$, 300 MHz): δ (ppm) 2.34 (s, 3H), 7.00 (s, 1H), 7.34–7.58 (m, 1H), 7.58–7.59 (m, 2H), 9.73 (d, $J = 8.4$ Hz, 1H), 13.50 (br, 1H). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{11}H_{10}N_3S$: 216.0590, found: 216.059.

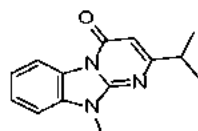
*3-(2-Methoxyethyl)-2-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one*



Chemical Formula: $C_{14}H_{15}N_3O_2$
Exact Mass: 257,12

[0134] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (123 mg, 0.924 mmol) and ethyl 2-acetyl-4-methoxybutanoate (1 mol. equiv). Reaction time: overnight. After column chromatography (2–3% gradient of MeOH in CH_2Cl_2 as an eluent) fractions containing product were combined and concentrated to dryness. Solid was suspended in EtOH, filtered-off and dried affording 58 mg of off-white solid. Filtrate containing small amount of product was concentrated and the residue was put on preparative TLC (3% of MeOH in CH_2Cl_2). Elution of appropriate band afforded additional 14 mg of product. Total yield: 82 mg (0.319 mmol, 35%). 1H NMR ($DMSO-d_6$, 300 MHz): δ (ppm) 2.37 (s, 3H), 2.76 (t, $J = 7.2$ Hz, 2H), 3.26 (s, 3H), 3.44 (t, $J = 7.2$ Hz, 2H), 7.28 (td, $J = 7.8, 1.2$ Hz, 1H), 7.42 (td, $J = 7.8, 1.2$ Hz, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 8.39 (d, $J = 7.8$ Hz, 1H), 12.60 (br, 1H). ^{13}C NMR ($DMSO-d_6$, 75MHz): δ (ppm) 19.87, 25.33, 57.85, 70.60, 105.77, 113.57, 115.05, 121.05, 125.47, 126.81, 135.51, 146.75, 155.27, 159.46. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{14}H_{16}N_3O_2$: 258.1237, found: 258.1235.

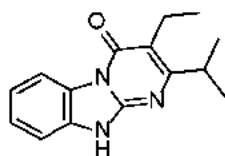
*2-Isopropyl-10-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one*



Chemical Formula:
 $C_{14}H_{15}N_3O$
Exact Mass: 241.12

[0135] The title compound was synthesized according to Method F starting from 1-methyl-1*H*-benzo[*d*]imidazol-2-amine (50 mg, 0.340 mmol) and ethyl 4-methyl-3-oxopentanoate (2.0 mol. equiv). Reaction time: overnight. DMF was removed under reduced pressure. Crude product was purified by column chromatography on silica gel using (0–4% gradient of MeOH in CH_2Cl_2) affording 10 mg (0.041 mmol, 12%) of product as an off-white solid. 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.30 (d, $J = 6.9$ Hz, 6H), 2.86 (sept, $J = 6.9$ Hz, 1H), 3.80 (s, 3H), 7.29–7.36 (m, 2H), 7.46 (td, $J = 7.8, 0.9$ Hz, 1H), 7.63 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 75MHz): δ (ppm) 21.82, 28.32, 36.29, 99.02, 108.28, 116.91, 122.53, 125.68, 126.07, 131.71, 149.03, 161.09, 173.73. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{14}H_{16}N_3O$: 242.1288, found: 242.1290.

3-Ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{15}H_{17}N_3O$
Exact Mass: 255.14

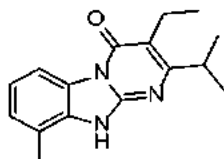
[0136] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (117 mg, 0.875 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1 mol. equiv). Reaction time: 5.5 h. Crude product was purified on column chromatography (2% MeOH in CH_2Cl_2). Fractions containing product were combined and concentrated to dryness. The residue was suspended in EtOH, filtered-off and dried affording 39 mg (0.153 mmol; 17%) of product as an off-white solid.

[0137] The title compound was also synthesized according to Method F starting from 2-aminobenzimidazole (358 mg, 2.684 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1 mol. equiv). Reaction time: 22 h. Filtration of reaction mixture afforded 410 mg (1.688 mmol, 67%) product as an off-white solid.

[0138] 1H NMR (pyridine- d_5 , 500 MHz): δ (ppm) 1.20 (t, $J = 7.5$ Hz, 3H), 1.27 (d, $J = 6.5$ Hz, 6H), 2.77 (q, $J = 7.5$ Hz, 2H), 3.13 (sept, $J = 6.5$ Hz, 1H), 7.21 (t, $J = 7.5$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 7.47 (d, $J = 8.0$ Hz, 1H), 8.85 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (pyridine- d_5 , 125 MHz): δ (ppm) 15.17, 18.82, 21.96, 31.52, 111.76, 112.37, 116.67, 121.64, 126.21, 127.56, 148.83, 160.99, 166.61. 1H NMR

(DMSO- d_6 , 300 MHz): δ (ppm) 1.08 (t, $J = 7.2$ Hz, 3H), 1.23 (d, $J = 6.6$ Hz, 6H), 2.55 (q, $J = 7.2$ Hz, 2H), 3.24 (sept, $J = 6.6$ Hz, 1H), 7.29–7.31 (m, 1H), 7.42–7.44 (m, 2H), 8.44 (d, $J = 8.1$ Hz, 1H), 12.71 (br, 1H). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{18}N_3O$: 256.1444, found: 256.1442.

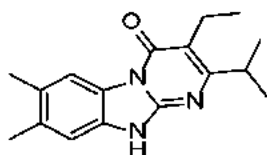
3-Ethyl-9-methyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{16}H_{19}N_3O$
Exact Mass: 269.15

[0139] The title compound was synthesized according to Method F starting from 4-methyl-1*H*-benzo[*d*]imidazol-2-amine (150 mg, 1.02 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 40 h. Volatiles were removed under reduced pressure and the residue was suspended in MeOH. Solid was filtered-off and dried. Filtrate was concentrated to dryness and the procedure was repeated. Combined solids were subjected to silica gel column separation (25% of EtOAc in cyclohexane) affording 89 mg (0.330 mmol, 32%) of product (as a single isomer) as an off-white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.08 (t, $J = 7.5$ Hz, 3H), 1.24 (d, $J = 6.6$ Hz, 6H), 2.48 (s, 3H), 2.60 (q, $J = 7.5$ Hz, 2H), 3.19–3.26 (m, 1H), 7.17 (t, $J = 7.8$ Hz, 1H), 7.23 (br d, $J = 6.9$ Hz, 1H), 8.26 (d, $J = 7.5$ Hz, 1H), 12.78 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 14.54, 16.31, 17.74, 21.56, 30.60, 111.26 (br), 112.82, 120.73 (br), 121.18, 125.51, 126.46, 129.56, 147.64, 159.62, 167.05 (br). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{20}N_3O$: 270.1601, found: 270.1594.

7,8-Dimethyl-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

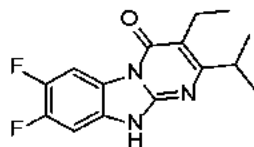


Chemical Formula: $C_{17}H_{21}N_3O$
Exact Mass: 283.17

[0140] The title compound was synthesized according to Method F starting from 5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-amine hydrobromide (333 mg, 1.375 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.64 mol. equiv). Reaction time: 24 h. MeOH was added and precipitate was filtered-off from reaction mixture, washed with MeOH and dried affording 141 mg (0.497 mmol, 36%) of product as a beige solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.07 (t, $J = 7.5$ Hz, 3H), 1.21 (d, $J = 6.6$ Hz, 6H), 2.33 (s, 6H), 2.59 (q, $J = 7.5$ Hz, 2H), 3.21 (sept, $J = 6.6$ Hz, 1H), 7.18 (s, 1H), 8.22 (s, 1H), 12.52 (br, 1H). 1H NMR (pyridine- d_5 , 300 MHz): δ (ppm) 1.33 (t, $J = 7.5$ Hz, 3H; overlapped with 1.38 (d, $J = 6.9$ Hz, 6H), 2.26 (s, 6H), 2.91 (q, $J = 7.5$ Hz, 2H), 3.25 (sept, $J = 6.9$ Hz, 1H), 7.35 (s, 1H),

8.81 (s, 1H). ^{13}C NMR (pyridine- d_5 , 75 MHz): δ (ppm) 15.44, 19.11, 20.29, 20.58, 22.32, 31.82, 112.23, 112.52, 117.49, 126.06, 130.33, 131.08 (br), 135.11, 149.24, 161.12, 166.89. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{22}\text{N}_3\text{O}$: 284.1757, found: 284.1753.

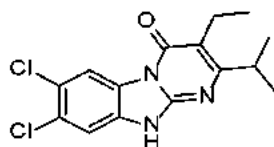
7,8-Difluoro-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula:
 $\text{C}_{15}\text{H}_{15}\text{F}_2\text{N}_3\text{O}$
Exact Mass: 291.12

[0141] The title compound was synthesized according to Method F starting from 5,6-difluoro-1*H*-benzo[*d*]imidazol-2-amine (150 mg, 0.886 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.1 mol. equiv). Reaction time: 22 h. MeOH was added, precipitate was filtered-off, washed with MeOH and dried affording 147 mg (0.505 mmol, 57%) of product as a pale yellow solid. ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 1.05 (t, $J = 7.5$ Hz, 3H), 1.25 (br, 6H), 2.56 (q, $J = 7.5$ Hz, 2H), 3.23 (br, 1H), 7.54 (br, 1H), 8.26 (br, 1H), 12.93 and 14.36 (2 x br, 1H). ^{19}F NMR (DMSO- d_6 , 470 MHz): δ (ppm) -140.67, -145.54, -145.12, -138.93. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{16}\text{F}_2\text{N}_3\text{O}$: 292.1256, found: 292.1253.

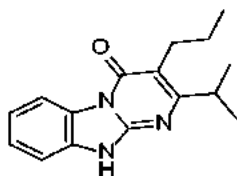
7,8-Dichloro-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula:
 $\text{C}_{15}\text{H}_{15}\text{Cl}_2\text{N}_3\text{O}$
Exact Mass: 323.06

[0142] The title compound was synthesized according to Method F starting from 5,6-dichloro-1*H*-benzo[*d*]imidazol-2-amine (150 mg, 0.742 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 24 h. Volatiles were removed under reduced pressure and crude product was purified on silica gel column (0–25% gradient of EtOAc in cyclohexane) affording 47 mg (0.145 mmol, 20%) of product as a beige solid. ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.07 (t, $J = 7.2$ Hz, 3H), 1.27 (d, $J = 6.6$ Hz, 6H), 2.57 (q, $J = 7.2$ Hz, 2H), 3.25 (sept, $J = 6.6$ Hz, 1H), 7.70 (s, 1H), 8.45 (s, 1H), 12.71 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 14.40, 17.59, 20.78, 29.79, 115.91, 122.57, 126.45, 127.71, 148.24, 159.31. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}$: 324.0665, found: 324.0668, 326.0638.

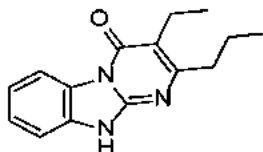
2-Isopropyl-3-propylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: C₁₆H₁₉N₃O
Exact Mass: 269.15

[0143] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (168 mg, 1.258 mmol) and ethyl 4-methyl-3-oxo-2-propylpentanoate (1 mol. equiv). Reaction time: 20 h. After column chromatography (10–20% AcOEt in CH₂Cl₂ as an eluent) fractions containing product were combined and concentrated to dryness. Solid was suspended in MeOH, filtered-off and dried affording 86 mg (0.320 mmol; 25%) of product as an off-white solid. ¹H NMR (pyridine-d₅, 300 MHz): δ (ppm) 1.06 (t, *J* = 7.5 Hz, 3H), 1.36 (d, *J* = 6.6 Hz, 6H), 1.75 (scxt, *J* = 7.5 Hz, 2H), 2.81–2.86 (m, 2H), 3.27 (scpt, *J* = 6.6 Hz, 1H), 7.29 (td, *J* = 8.1, 0.9 Hz, 1H), 7.42 (td, *J* = 7.8, 0.9 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 8.93 (d, *J* = 7.8 Hz, 1H). ¹³C NMR (pyridine-d₅, 75 MHz): δ (ppm) 14.70, 22.07, 24.03, 27.65, 31.73, 110.97, 111.88, 116.80, 121.76, 126.32, 127.69, 133.30, 149.00, 161.33, 166.87. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₆H₂₀N₃O: 270.1601, found: 270.1601.

Ethyl-2-propylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: C₁₅H₁₇N₃O
Exact Mass: 255.14

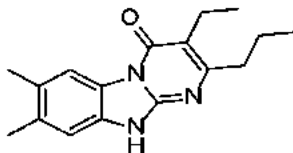
[0144] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (46 mg, 0.342 mmol) and ethyl 3-oxo-2-ethylhexanoate (1.1 mol. equiv). Reaction time: overnight. Purification on column chromatography using 2–3% gradient of MeOH in CH₂Cl₂ as an eluent afforded 38 mg (0.149 mmol, 44%) of product as a white solid.

[0145] The title compound was also synthesized according to Method F starting from 2-aminobenzimidazole (358 mg, 2.684 mmol) and ethyl 3-oxo-2-propylhexanoate (1 mol. equiv). Reaction time: 24 h. Filtration of reaction mixture, washing precipitate with EtOH and drying afforded 320 mg (1.253 mmol, 47%) of product as an off-white solid.

[0146] ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 0.96 (t, *J* = 7.2 Hz, 3H), 1.08 (t, *J* = 7.5 Hz, 3H), 1.69 (sext, *J* = 7.8 Hz, 2H), 2.54 (q, *J* = 7.2 Hz, 2H), 2.59–2.61 (m, 2H), 7.26 (td, *J* = 8.4, 1.2 Hz, 1H), 7.41 (td, *J* = 7.8, 1.2 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 8.40 (d, *J* = 7.8 Hz, 1H), 12.55 (br, 1H). ¹³C NMR

(DMSO- d_6 , 150MHz): δ (ppm) 13.87, 14.24, 18.14, 21.90, 34.58, 111.14 (br), 113.32, 115.20, 121.07, 125.63, 126.81, 147.01, 159.69. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{18}N_3O$: 256.1444, found: 256.1445.

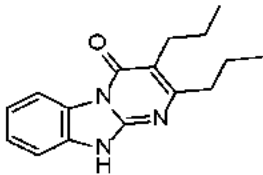
7,8-Dimethyl-3-ethyl-2-propylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{17}H_{21}N_3O$
Exact Mass: 283.17

[0147] The title compound was synthesized according to Method F starting from 5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-amine hydrobromide (305 mg, 1.260 mmol) and ethyl 2-ethyl-3-oxohexanoate (1.5 mol. equiv). Reaction time: 24 h. Water was added and precipitate was filtered-off and dried. Purification on silica gel column (2–3% gradient of MeOH in CH_2Cl_2) afforded 65 mg of pure product as a yellow-orange solid. Second fraction containing contaminated product was concentrated to dryness and put on preparative TLC (2% of MeOH in CH_2Cl_2 with 1% vol. of 7 N ammonia in MeOH) affording additional 60 mg of product. Total yield: 125 mg (0.441 mmol, 23%). 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 0.97 (t, $J = 7.5$ Hz, 3H), 1.08 (t, $J = 7.5$ Hz, 3H), 1.69 (sext, $J = 7.5$ Hz, 2H), 2.33 (s, 6H), 2.51–2.61 (m, 4H; overlapped with residual solvent signal), 7.24 (s, 1H), 8.20 (s, 1H), 12.44 (br, 1H). 1H NMR (pyridine- d_5 , 300 MHz): δ (ppm) 0.99 (t, $J = 7.2$ Hz, 3H), 1.33 (t, $J = 7.2$ Hz, 3H), 1.85 (sext, $J = 7.5$ Hz, 2H), 2.27 and 2.28 (2 x s, 6H), 2.71 (t, $J = 7.5$ Hz, 2H), 2.84 (q, $J = 7.2$ Hz, 2H), 7.44 (s, 1H), 8.79 (s, 1H). ^{13}C NMR (pyridine- d_5 , 75 MHz): δ (ppm) 14.55, 14.99, 19.46, 20.35, 20.63, 22.95, 36.18, 112.66, 113.83, 117.21, 126.65, 130.24, 133.88, 134.89, 148.54, 159.59, 161.01. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{21}N_3O$: 284.1757, found: 284.1758.

2,3-Dipropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

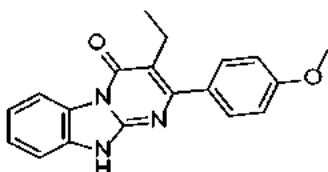


Chemical Formula: $C_{16}H_{19}N_3O$
Exact Mass: 269.15

[0148] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (66 mg, 0.494 mmol) and ethyl 3-oxo-2-propylhexanoate (1 mol. equiv). Reaction time: overnight. Purification by column chromatography using 2–3% gradient of MeOH in CH_2Cl_2 as an eluent afforded 40 mg (0.148 mmol, 30%) of product as an off-white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 0.93–1.00 (m, 6H), 1.50 (sext, $J = 7.5$ Hz, 2H), 1.70 (sext, $J = 7.5$ Hz, 2H), 2.48–2.52

(m, 2H; overlapped with residual solvent signal), 2.59–2.64 (m, 2H), 7.24–7.30 (m, 1H), 7.39–7.45 (m, 1H), 7.48 (d, $J = 7.8$ Hz, 1H), 8.40 (d, $J = 8.1$ Hz, 1H), 12.48 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75MHz): δ (ppm) 13.76, 13.96, 21.74, 22.43, 26.71, 34.53, 109.53, 113.09, 115.11, 120.97, 125.52, 126.70, 134.99, 146.90, 157.90, 159.75. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}$: 270.1601, found: 270.1599.

3-Ethyl-2-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

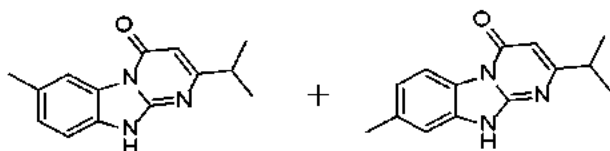


Chemical Formula: $\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}_2$
Exact Mass: 319.13

[0149] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (140 mg, 1.051 mmol) and ethyl 2-(4-methoxybenzoyl)butanoate (1 mol. equiv). Reaction time: overnight. After column chromatography (2–3% gradient of MeOH in CH_2Cl_2 as an eluent) fractions containing product were combined and concentrated to dryness. Solid was suspended in MeOH, filtered-off and dried affording 50 mg (0.156 mmol; 15%) of product as an off-white solid. ^1H NMR (pyridine- d_5 , 300 MHz): δ (ppm) 1.37 (t, $J = 7.2$ Hz, 3H), 2.80 (q, $J = 7.2$ Hz, 2H), 3.76 (s, 3H), 7.14 (d, $J = 8.7$ Hz, 2H), 7.36 (t, $J = 7.8$ Hz, 1H), 7.49 (t, $J = 7.8$ Hz, 1H), 7.68–7.74 (m, 3H), 8.97 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (pyridine- d_5 , 75 MHz): δ (ppm) 14.86, 20.70, 55.67, 113.00, 113.74, 114.48, 116.67, 121.85, 122.35, 126.33, 128.22, 130.77, 131.12, 131.23, 148.28, 156.53, 160.88, 161.34. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{O}$: 320.1393, found: 320.1388.

Mixture of 2-isopropyl-7-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one and 2-isopropyl-8-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

Chemical Formula: $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}$
Exact Mass: 241.12



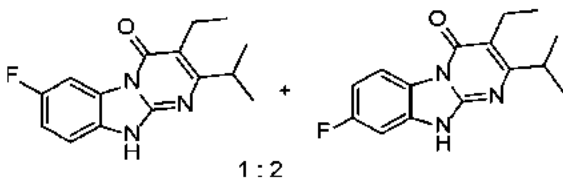
ca. 1.1 : 1

[0150] The title mixture of compounds was obtained according to Method E starting from 2-amino-5-methyl-1H-benzo[d]imidazole (212 mg, 1.440 mmol) and ethyl 4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: overnight. Column chromatography (0–35% gradient of AcOEt in CH_2Cl_2 as an eluent) afforded 191 mg (0.792 mmol; 55%) of product as a mixture of 2 isomers (ca. 1.1:1; an off-white solid). Two isomers were not separable on chromatographic conditions tested. ^1H NMR (DMSO- d_6 , 300

MHz): δ (ppm) 1.23 (d, $J = 6.6$ Hz, 6H), 2.44 (s, 3H), 2.81 (sept, $J = 6.6$ Hz, 1H), 5.85 and 5.86 (2 x s, 1H), 7.10 (d, $J = 8.1$ Hz, 0.56H), 7.24–7.26 (m, 1H), 7.34 (d, $J = 8.4$ Hz, 0.44H), 8.23–8.26 (m, 1H), 12.78 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 21.10, 21.27, 21.38, 21.41, 34.47, 34.58, 96.31, 96.64, 111.99 (br), 114.95, 115.39, 122.49, 124.09, 126.39, 126.65, 130.79, 135.46, 148.98, 149.09, 159.49, 159.67. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{16}\text{N}_3\text{O}$: 242.1288, found: 242.1284.

Mixture of 3-ethyl-7-fluoro-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one and 3-ethyl-8-fluoro-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

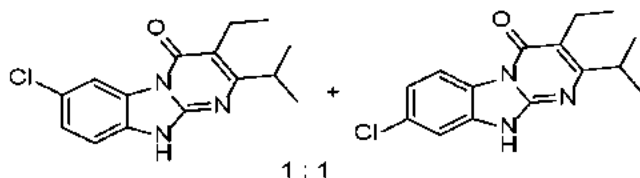
Chemical Formula: $\text{C}_{15}\text{H}_{16}\text{FN}_3\text{O}$
Exact Mass: 273.13



[0151] The title mixture of compounds was obtained according to Method F starting from 2-amino-5-fluoro-1H-benzo[d]imidazole (150 mg, 0.992 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 24 h. MeOH was added and precipitate was filtered-off, washed with MeOH and dried affording 135 mg (0.494 mmol, 50%) of mixture of 2 isomers (in a ratio ca. 1:2) as a pale brown flakes. ^1H NMR (pyridine- d_5 , 500 MHz): δ (ppm) 1.29 and 1.30 (2 x t, $J = 7.5$ Hz and $J = 7.5$ Hz, 3H), 1.37 (d, $J = 6.5$ Hz, 6H), 2.82–2.88 (m, 2H), 3.25 (sept, $J = 6.5$ Hz, 1H), 7.12–8.84 [3H; 7.12 (td, $J = 9.5$ Hz, $J = 2.5$ Hz), 7.28 (td, $J = 9.5$ Hz, $J = 2.5$ Hz), 7.41 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz), 7.49 (dd, $J = 8.5$ Hz, $J = 4.5$ Hz), 8.84 (dd, $J = 9.0$ Hz, $J = 5.0$ Hz)]. ^{19}F NMR (pyridine- d_5 , 470 MHz): δ (ppm) –120.58, –115.31. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{17}\text{FN}_3\text{O}$: 274.1350, found: 274.1351.

Mixture of 7-chloro-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one and 8-chloro-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

Chemical Formula: $\text{C}_{15}\text{H}_{16}\text{ClN}_3\text{O}$
Exact Mass: 289.10

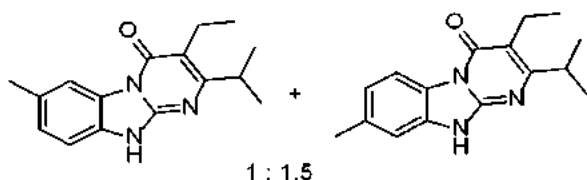


[0152] The title mixture of compounds was obtained according to Method F starting from 2-amino-5-chloro-1H-benzo[d]imidazole (140 mg, 0.835 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 24 h. EtOH was added and precipitate was filtered-off, washed with EtOH and dried affording 86 mg (0.297 mmol, 36%) of mixture of 2 isomers (in a ratio ca. 1:1) as a beige solid. ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.07 (t, $J = 7.2$ Hz, 3H), 1.25 (d, $J = 6.6$ Hz, 6H), 2.59

(q, $J = 7.2$ Hz, 2H), 3.23 (sept, $J = 6.6$ Hz, 1H; overlapped with H₂O signal), 7.29–8.39 [3H; 7.29 (dd, $J = 8.7$ Hz, $J = 1.8$ Hz), 7.45–7.48 (m), 8.35–8.39 (m)]. ¹H NMR (pyridine-d₅, 300 MHz): δ (ppm) 1.29 (m, 3H), 1.37 (d, $J = 6.6$ Hz, 6H), 2.83 (q, $J = 7.5$ Hz, 2H), 3.23 (sept, $J = 6.6$ Hz, 1H), 7.35–8.98 [3H; 7.35 (dd, $J = 8.4$ Hz, $J = 1.8$ Hz); 7.46–7.53 (m); 7.68 (d, $J = 1.8$ Hz); 8.81 (d, $J = 8.4$ Hz); 8.98 (d, $J = 1.2$ Hz)]. HRMS (ESI): m/z [M+H]⁺ calcd for C₁₅H₁₇ClN₃O: 290.1054, found: 290.1061, 292.1031.

Mixture of 3-ethyl-2-isopropyl-7-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one and 3-ethyl-2-isopropyl-8-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

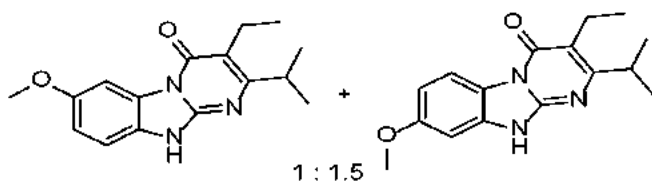
Chemical Formula: C₁₆H₁₉N₃O
Exact Mass: 269.15



[0153] The title mixture of compounds was obtained according to Method F starting from 2-amino-5-methyl-1H-benzo[d]imidazole (395 mg, 2.684 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 22 h. Product was filtered-off from reaction mixture, washed with EtOH and dried affording 402 mg (1.492 mmol, 56%) mixture of 2 isomers (in a ratio ca. 1:1.5) as pale brown solid. Two isomers were not separable under chromatographic conditions tested. ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 1.06 (t, $J = 7.2$ Hz, 3H), 1.21 (d, $J = 6.0$ Hz, 6H), 2.43 and 2.44 (2 x s, 3H), 2.58 (q, $J = 7.5$ Hz, 2H), 3.18 (m, 1H), 7.08 (d, $J = 8.4$ Hz, 0.6H), 7.20–7.29 (m, 1.4H), 8.27–8.29 (m, 1H), 12.63 (br, 1H). ¹³C NMR (DMSO-d₆, 150 MHz): δ (ppm) 14.64, 17.82, 21.23, 21.38, 21.71, 30.74, 110.17 (br), 110.60 (br), 111.51 (br), 115.18, 115.70, 122.27, 123.73, 125.98, 126.80, 128.21, 128.49 (br), 130.56, 135.74, 147.70, 159.55, 159.71, 167.11 (br). HRMS (ESI): m/z [M+H]⁺ calcd for C₁₆H₂₀N₃O: 270.1601, found: 270.1599.

Mixture of 3-ethyl-2-isopropyl-7-methoxybenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one and 3-ethyl-2-isopropyl-8-methoxybenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one

Chemical Formula: C₁₆H₁₉N₃O₂
Exact Mass: 285.15

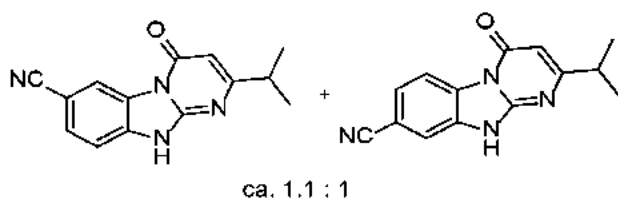


[0154] The title mixture of compounds was obtained according to Method F starting from 2-amino-5-methoxy-1H-benzo[d]imidazole (150 mg, 0.919 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.1 mol. equiv). Reaction time: 24 h. MeOH was added and precipitate was filtered-off, washed with

MeOH and dried affording 138 mg (0.484 mmol, 53%) of product as a mixture of 2 isomers (in a ratio ca. 1:1.5) as a brown solid. ¹H NMR (pyridine-d₅, 300 MHz): δ (ppm) 1.32 (t, *J* = 7.5 Hz, 3H), 1.36 and 1.38 (2 x d, *J* = 6.6 Hz, 6H), 2.85–2.93 (m, 2H), 3.24 (br sept, *J* = 6.6 Hz, 1H), 6.99–8.87 [3H; 6.99 (dd, *J* = 8.7 Hz, *J* = 2.4 Hz), 7.18–7.22 (m), 7.47 (d, *J* = 9.0 Hz), 8.66 (d, *J* = 2.7 Hz), 8.87 (d, *J* = 8.7 Hz)]. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₆H₂₀N₃O₂: 286.1550, found: 286.1549.

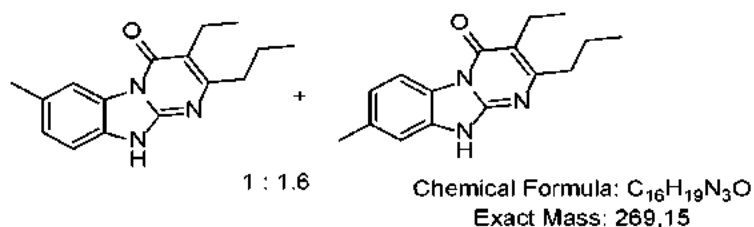
Mixture of 2-isopropyl-4-oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidine-7-carbonitrile and 2-isopropyl-4-oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidine-8-carbonitrile

Chemical Formula: C₁₄H₁₂N₄O
Exact Mass: 252,10



[0155] The title mixture of compounds was obtained according to Method E starting from 2-amino-1*H*-benzo[*d*]imidazole-5-carbonitrile (100 mg, 0.632 mmol) and ethyl 4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 8 h. Column chromatography of crude product (50% of AcOEt in cyclohexane as an eluent) afforded 34 mg (0.135 mmol; 21%) of product as a mixture of 2 isomers (in a ratio 1.1:1) as an off-white solid. Two isomers were not separable under chromatographic conditions tested. ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 1.24–1.25 (2 x d, *J* = 7.2 Hz and *J* = 6.6 Hz, 6H), 2.87 (sept, *J* = 6.9 Hz, 1H), 5.84 and 5.89 (2 x s, 1H), 7.65–7.66 (dd, *J* = 8.4, 1.2 Hz and d, *J* = 8.4 Hz; 1H), 7.80 (dd, *J* = 8.4, 1.2 Hz, 0.45H), 8.01 (s, 0.4H), 8.41 (d, *J* = 7.8 Hz, 0.4H), 8.58 (d, *J* = 1.2 Hz, 0.45H). ¹³C NMR (DMSO-d₆, 150 MHz): δ (ppm) 21.29, 21.34, 95.49, 96.31, 102.88, 107.65, 116.03, 118.83, 119.59, 119.76, 125.29, 127.37, 129.68, 130.83, 149.55, 150.05, 159.72, 159.88. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₄H₁₃N₄O: 253.1084, found: 253.1084.

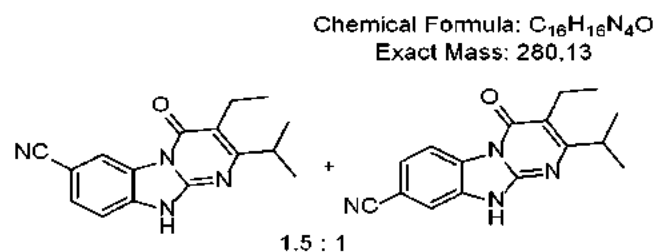
Mixture of 3-ethyl-7-methyl-2-propylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10*H*)-one and 3-ethyl-8-methyl-2-propylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10*H*)-one



[0156] The title mixture of compounds was obtained according to Method F starting from 2-amino-5-methyl-1*H*-benzo[*d*]imidazole (189 mg, 1.284 mmol) and ethyl 2-ethyl-3-oxohexanoate (1.2 mol. equiv). Reaction time: 20 h. Product was filtered-off from reaction mixture, washed with EtOH and

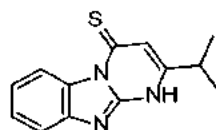
dried affording 149 mg (0.553 mmol, 43%) of product (mixture of 2 isomers in a ratio 1:1.6) as grey solid. Two isomers were not separable on chromatographic column. ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 0.96 (t, *J* = 7.2 Hz, 3H), 1.07 (t, *J* = 7.2 Hz, 3H), 1.69 (sext, *J* = 7.2 Hz, 2H), 2.43 and 2.44 (2 x s, 3H), 2.53 (q, *J* = 7.2 Hz, 2H), 2.55–2.60 (m, 2H), 7.07 (d, *J* = 8.4 Hz, 0.6H), 7.23 (d, *J* = 7.8 Hz, 0.36H), 7.26 (s, 0.6H), 7.34 (d, *J* = 7.8 Hz, 0.36H), 8.24–8.26 (m, 1H), 12.47 (br, 1H). ¹³C NMR (DMSO-d₆, 150 MHz): δ (ppm) 13.93, 14.26, 18.18, 21.28, 21.44, 21.87, 21.90, 34.88, (br), 111.05 (br), 111.41 (br), 114.90, 115.39, 122.18, 124.56, 126.62, 126.87, 130.38, 135.34, 147.01, 147.11, 159.53, 159.71. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₆H₂₀N₃O: 270.1601, found: 270.1602.

Mixture of 7-cyano-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one and 8-cyano-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



[0157] The title mixture of compounds was obtained according to Method F starting from 2-amino-5-cyano-1*H*-benzo[*d*]imidazole (150 mg, 0.948 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.0 mol. equiv). Reaction time: 24 h. Volatiles were removed under reduced pressure. Solid residue was suspended in EtOH, solid was filtered-off, washed with EtOH and dried affording 61 mg (0.218 mmol, 23%) of product (mixture of 2 isomers in a ratio ca. 1.5:1) as a pale yellow solid. ¹H NMR (pyridine-d₅, 300 MHz): δ (ppm) 1.28 (t, *J* = 7.5 Hz, 3H), 1.38 and 1.39 (2 x d, *J* = 6.9 Hz, 6H), 2.81 (br q, *J* = 7.2 Hz, 2H), 3.26 (sept, *J* = 6.9 Hz, 1H), 7.63–9.20 [3H; 7.63 (d, *J* = 9.0 Hz), 7.76 (dd, *J* = 8.1 Hz, *J* = 1.5 Hz), 8.01 (d, *J* = 1.5 Hz), 8.85 (d, *J* = 8.1 Hz), 9.20 (d, *J* = 1.5 Hz)]. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₆H₁₇N₄O: 281.1397, found: 281.1390.

2-Isopropylbenzo[4,5]imidazo[1,2-*a*]pyrimidine-4(10*H*)-thione

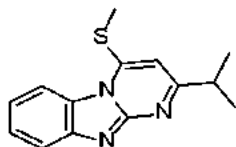


Chemical Formula:
C₁₃H₁₃N₃S
Exact Mass: 243.08

[0158] Mixture of 2-isopropylbenzo[4,5]imidazo[1,2-*a*]pyrimidine-4(10*H*)-one (); 300 mg, 1.320 mmol) and Lawesson's reagent (1.068 g, 2.640 mmol) in toluene (15 ml) was refluxed overnight. The mixture was cooled to room temperature, precipitate was filtered-off, washed with toluene and dried. Purification using flash column chromatography on silica gel (0–3% gradient of MeOH in CH₂Cl₂)

afforded 226 mg of pale yellow solid. Additional amount of product (30 mg) was obtained from purification (column chromatography) of filtrate from the reaction mixture. Total yield: 256 mg (1.052 mmol, 80%). ¹H NMR (DMSO-d₆, 600 MHz): δ (ppm) 1.25 (d, *J* = 7.2 Hz, 6H), 2.88 (sept, *J* = 7.2 Hz, 1H), 6.99 (s, 1H), 7.35 (ddd, *J* = 8.4, 7.2, 1.8 Hz, 1H), 7.54–7.59 (m, 2H), 9.72 (d, *J* = 8.4 Hz, 1H). ¹³C NMR (DMSO-d₆, 150 MHz): δ (ppm) 21.59, 34.73, 111.40, 115.78, 118.29, 121.02, 127.48, 128.10, 131.46, 148.39, 166.87, 177.86. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₃H₁₄N₃S: 244.0903, found: 244.0889.

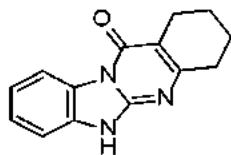
2-Isopropyl-4-(methylthio)benzo[4,5]imidazo[1,2-a]pyrimidine



Chemical Formula:
C₁₄H₁₅N₃S
Exact Mass: 257.10

[0159] To a mixture of 2-isopropylbenzo[4,5]imidazo[1,2-*a*]pyrimidine-4(10*H*)-thione (100 mg, 0.411 mmol) and NaHCO₃ (43 mg (0.512 mmol) in acetone (2 ml) was added Me₂SO₄ (78 μl, 0.822 mmol) and resulting mixture was refluxed for 20 h. Volatiles were removed *in vacuo* and crude product was purified first on silica gel column chromatography (0–10% gradient of MeOH in CH₂Cl₂) and second on preparative TLC (4% of MeOH in CH₂Cl₂) affording 17 mg (0.066 mmol, 16%) of product. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 1.39 (d, *J* = 6.9 Hz, 6H), 2.74 (s, 3H), 3.11 (sept, *J* = 6.9 Hz, 1H), 6.46 (s, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) 14.93, 21.77, 37.25, 100.53, 115.50, 119.88, 121.00, 125.77, 128.57, 144.82, 151.72, 152.88, 171.54. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₄H₁₆N₃S: 258.1059, found: 258.1064.

1,3,4,6-Tetrahydrobenzo[4,5]imidazo[2,1-*b*]quinazolin-12(2*H*)-one

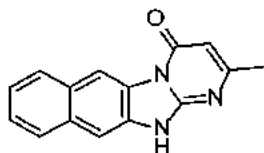


Chemical Formula: C₁₄H₁₃N₃O
Exact Mass: 239.11

[0160] The title compound was synthesized according to Method E starting from 2-aminobenzimidazole (100 mg, 0.751 mmol) and ethyl 2-oxocyclohexane-1-carboxylate (1 mol. equiv). Reaction time: 5 h. Volatiles were removed under reduced pressure. Column chromatography (2% MeOH in CH₂Cl₂ as an eluent) of crude reaction mixture afforded 151 mg (0.631 mmol; 84%) of product as a white solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 1.71–1.79 (m, 4H), 2.45–2.48 (m, 2H), 2.61–

2.65 (m, 2H), 7.26 (td, $J = 8.1, 1.2$ Hz, 1H), 7.40 (td, $J = 8.1, 0.9$ Hz, 1H), 7.49 (d, $J = 7.8$ Hz, 1H), 7.38 (d, $J = 7.8$ Hz, 1H), 12.49 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 21.41, 21.64, 21.71, 29.44, 106.25, 113.47, 114.95, 120.88, 125.34, 126.85, 135.65, 146.84, 154.80, 159.46. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{14}\text{N}_3\text{O}$: 240.1132, found: 240.1132.

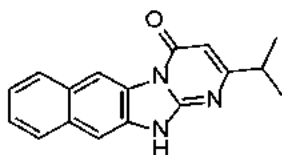
2-Methylnaphtho[2',3':4,5]imidazo[1,2-a]pyrimidin-4(12H)-one



Chemical Formula: $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}$
Exact Mass: 249.09

[0161] The title compound was synthesized according to Method E starting from 2-amino-1*H*-naphtho[2,3-*d*]imidazole (100 mg, 0.546 mmol) and ethyl acetoacetate (1 mol. equiv). Reaction time: 6 h. Volatiles were removed under reduced pressure. After column chromatography (50–55% gradient of AcOEt in CH_2Cl_2 and next 2% of MeOH in CH_2Cl_2) fractions containing product were combined and concentrated to dryness. Solid was suspended in MeOH, filtered-off and dried affording 47 mg (0.188 mmol; 34%) of product as a pale brown solid. ^1H NMR (pyridine- d_5): δ (ppm) 2.40 (s, 3H), 6.18 (s, 1H), 7.52–7.65 (m, 2H), 8.06–8.15 (m, 3H), 9.44 (s, 1H). HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{12}\text{N}_3\text{O}$: 250.0975, found: 250.0974.

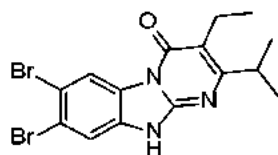
2-Isopropyl-naphtho[2',3':4,5]imidazo[1,2-a]pyrimidin-4(12H)-one



Chemical Formula: $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}$
Exact Mass: 277.12

[0162] The title compound was synthesized according to Method E starting from 2-amino-1*H*-naphtho[2,3-*d*]imidazole (100 mg, 0.546 mmol) and ethyl acetoacetate (1.3 mol. equiv). Reaction time: 22 h. Column chromatography of crude product (2% of MeOH in CH_2Cl_2) afforded 85 mg (0.306 mmol; 56%) of product as an off-white solid. ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.27 (d, $J = 6.9$ Hz, 6H), 2.84 (sept, $J = 6.9$ Hz, 1H), 5.95 (s, 1H), 7.44–7.54 (m, 2H), 7.87 (s, 1H), 8.04 (d, $J = 8.1$ Hz, 1H), 8.12 (d, $J = 7.8$ Hz, 1H), 8.91 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 21.32, 34.76, 97.81, 107.02, 112.66, 124.12, 125.55, 126.80, 127.11, 128.35, 128.67, 131.38, 150.93, 159.82, 171.58. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}$: 278.1288, found: 278.1293.

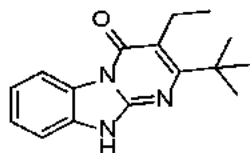
7,8-Dibromo-3-ethyl-2-isopropylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{15}H_{15}Br_2N_3O$
Exact Mass: 410,96

[0163] To a solution of 5,6-dibromo-1H-benzimidazol-2-amine (100 mg, 0.343 mmol) in pyridine was added ethyl 2-ethyl-4-methyl-3-oxopentanoate (1 mol. Equiv.). The reaction mixture was refluxed for 48 hours. The solvent was evaporated and the crude was purified using column chromatography (heptane:EtOAc – 5:1) yielding 31.2 mg of the product as a beige solid (0.075 mmol, 22%). 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.19 (t, $J = 7.4$ Hz, 3H), 1.35 (d, $J = 6.9$ Hz, 6H), 2.68 (q, $J = 7.4$ Hz, 2H) 3.34 (m, 1H), 7.85 (s, 1H), 8.84 (s, 1H). ^{13}C NMR (pyridine- d_5 , 75 MHz): δ (ppm) 14.73, 18.37, 21.18, 29.78, 54.90, 61.47, 111.23, 115.14, 118.21, 119.99, 120.59, 137.11, 160.10, 162.36. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{16}N_3OBr_2$: 411.96556, found: 411.9651.

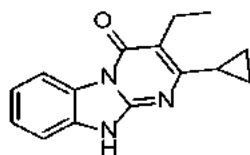
2-(tert-Butyl)-3-ethylbenzof[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{18}H_{19}N_3O$
Exact Mass: 269,15

[0164] The title compound was synthesized according to Method F starting from 2-Aminobenzimidazole (200 mg, 1.50 mmol) and ethyl 2-ethyl-4,4-dimethyl-3-oxopentanoate (1.1 mol. equiv). Reaction time: 16 h. After removal of volatiles crude product was purified by silica gel column chromatography (30% of EtOAc in heptane) affording 30.2 mg (0.112 mmol; 7%) of product as an off-white solid. 1H NMR (DMSO, 300 MHz): δ (ppm) 1.15 (t, $J = 7.2$ Hz, 3H), 1.46 (s, 9H), 2.76 (q, $J = 7.1$ Hz, 2H), 7.29 (ddd, $J = 8.4, 5.6, 3.1$ Hz, 1H), 7.44 (m, 2H), 8.45 (d, $J = 8.0$ Hz, 1H), 12.78 (s, 1H). ^{13}C NMR (DMSO, 75 MHz): δ (ppm) 13.91, 19.89, 30.48, 69.91, 110.80, 113.15, 121.40, 125.50, 126.20, 145.85, 160.54. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{20}N_3O$: 270.16007, found: 270.1595.

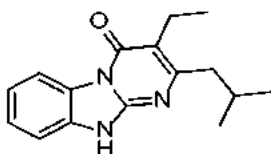
2-Cyclopropyl-3-ethylbenzof[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{15}H_{15}N_3O$
Exact Mass: 253,12

[0165] The title compound was synthesized according to Method F starting from 2-aminobenzimidazole (146 mg, 1.10 mmol) and ethyl 2-(cyclopropanecarbonyl)butanoate (1.1 mol. equiv). Reaction time: 60 h. After removal of volatiles, crude product was purified by column chromatography (30% of EtOAc in heptane) affording 34 mg (0.134 mmol; 12%) of product as a white solid. ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) 1.02 (m, 4H), 1.12 (t, *J* = 7.4 Hz, 3H), 2.17 (m, 1H), 2.72 (q, *J* = 7.4 Hz, 2H), 7.28 (m, 1H), 7.41 (m, 2H), 8.44 (d, *J* = 7.9 Hz, 1H), 12.54 (br, 1H). ¹³C NMR (pyridine-*d*₅, 75 MHz): δ (ppm) 10.17, 14.78, 19.55, 30.92, 71.79, 111.73, 114.31, 117.46, 122.46, 126.85, 132.65, 149.85, 160.95, 164.22. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₅H₁₆N₃O; 254.12878, found: 254.1292.

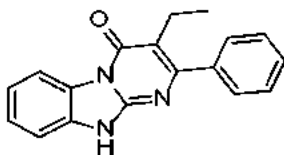
3-Ethyl-2-isobutylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



Chemical Formula: C₁₈H₁₉N₃O
Exact Mass: 269.15

[0166] The title compound was synthesized according to Method F starting from 2-aminobenzimidazole (300 mg, 2.25 mmol) and ethyl 2-ethyl-5-methyl-3-oxohexanoate (1.1 mol. equiv). Reaction time: 60 h. After removal of volatiles crude product was purified by column chromatography (50% of EtOAc in heptane). The fractions contained product were combined and concentrated to dryness. Resulting solid was suspended in a minimal amount of MeOH, the solid was collected by filtration and purified by column chromatography (heptane:EtOAc – 7:3) yielding 79 mg (0.293 mmol; 13%) of product as a white solid. ¹H NMR (pyridine-*d*₅, 300 MHz): δ (ppm) 0.98 (d, *J* = 6.6 Hz, 6H), 1.31 (t, *J* = 7.4 Hz, 3H), 2.29 (sept, *J* = 6.9 Hz, 1H), 2.62 (d, *J* = 7.3 Hz, 2H), 2.80 (q, *J* = 7.3 Hz, 2H), 7.31 (t, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 8.93 (d, 1H, *J* = 7.9 Hz). ¹³C NMR (pyridine-*d*₅, 75 MHz): δ (ppm) 13.87, 18.68, 22.04, 28.06, 29.40, 41.53, 112.24, 113.06, 115.71, 120.76, 125.22, 136.01, 147.54, 156.38, 160.12. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₆H₂₀N₃O; 270.16007, found: 270.1606.

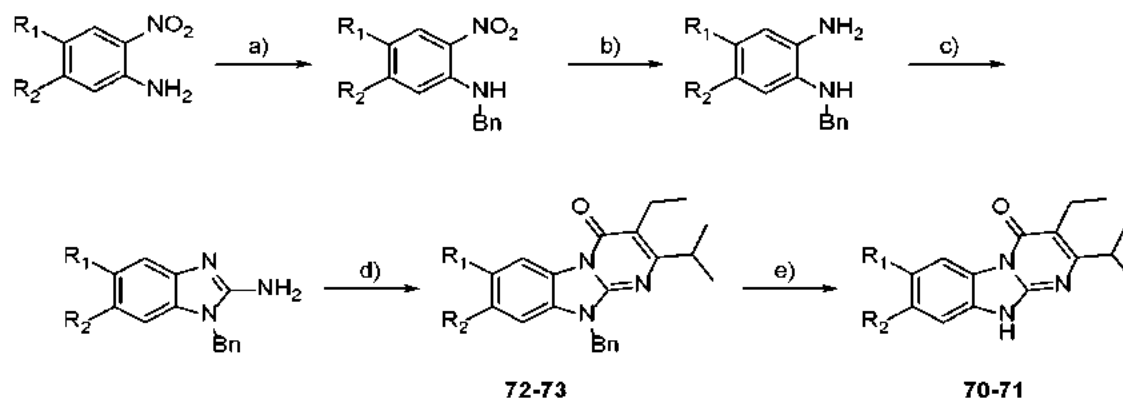
3-Ethyl-2-phenylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



Chemical Formula: C₁₈H₁₅N₃O
Exact Mass: 289.12

[0167] The title compound was synthesized according to Method F starting from 2-aminobenzimidazole (232 mg, 1.75 mmol) and ethyl 2-benzoylbutanoate (1.1 mol. equiv). Reaction time: 60 h. After removal of volatiles crude product was purified on silica gel column (30% of EtOAc in heptane). Fractions containing product were combined and concentrated till dryness. The residue was suspended in a mixture heptane and EtOAc (7:3, v/v) and the precipitate was filtered off. Solid was suspended in 10 ml of MeOH, filtered and dried yielding 35 mg (0.121 mmol; 7%) of product as a white solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 1.09 (t, *J* = 7.1 Hz, 3H), 2.45 (q, *J* = 6.9 Hz, 2H), 7.34 (m, 1H), 7.51 (m, 7H), 8.51 (d, *J* = 8.1 Hz, 1H). ¹³C NMR (pyridine-d₅, 75 MHz): δ (ppm) 14.12, 19.69, 99.66, 115.69, 121.52, 126.18, 128.27, 128.33, 128.69. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₈H₁₆N₃O: 290.1288, found: 290.1292.

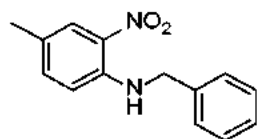
Regioselective synthesis of 3-ethyl-2-isopropyl-7-methyl- and 3-ethyl-2-isopropyl-8-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one :



R₁ = Me, R₂ = H
R₁ = H, R₂ = Me

Scheme. Regioselective synthesis of 3-ethyl-2-isopropyl-7-methyl- and 3-ethyl-2-isopropyl-8-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one. Reagents and conditions: a) BnBr, H₂O, reflux; b) Raney nickel, H₂, MeOH, rt; c) BrCN, EtOH, 50 °C; d) DMF, reflux; e) H₂, Pd/C, CH₂Cl₂, MeOH, rt.

N-Benzyl-4-methyl-2-nitroaniline:

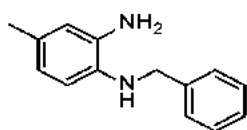


Chemical Formula: C₁₄H₁₄N₂O₂
Exact Mass: 242.11

[0168] Mixture of 4-methyl-2-nitroaniline (1 g, 6.57 mmol) and benzyl bromide (940 μl, 7.89 mmol, 1.2 mol. Equiv.) in H₂O was stirred at reflux for 1.5 h. Then additional 0.6 mol. equiv. (3.95 mmol) of benzyl bromide was added and stirring was continued at reflux for 2 days. After cooling to room

temperature, saturated NaHCO₃ was added and mixture was extracted with EtOAc (3x). Organic layers were combined, washed with H₂O (1 x) and dried over Na₂SO₄. After removal of drying agent and solvent, residue was subjected to silica gel column chromatography (heptane:EtOAc – 9:1) affording 1.29 g (5.33 mmol; 81%) of product as an orange solid. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 2.25 (s, 3H), 4.53 (d, *J* = 5.0 Hz, 2H), 6.73 (d, *J* = 7.5 Hz, 1H), 7.20 (dd, *J* = 7.5, 2.5 Hz, 1H), 7.25–7.38 (m, 5H), 7.99 (d, *J* = 2.5 Hz, 1H), 8.33 (br, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) 19.09, 46.25, 113.34, 124.42, 125.23, 126.13, 126.74, 128.01, 131.04, 136.75, 136.83, 142.63. HRMS (ESI): *m/z* [M+Na]⁺ calcd for C₁₄H₁₄N₂O₂Na: 265.0948, found 265.0950.

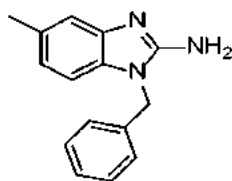
***N*¹-Benzyl-4-methylbenzene-1,2-diamine:**



Chemical Formula: C₁₄H₁₆N₂
Exact Mass: 212,13

[0169] To a solution of *N*-benzyl-4-methyl-2-nitroaniline (420 mg, 1.71 mmol) in methanol was added a slurry of Raney nickel (30 mg) and the mixture was stirred vigorously. The flask was flushed with H₂ gas 3 times. The reaction mixture was allowed to stir for 3 h. The catalyst was removed by filtration through Celite and the reaction was concentrated *in vacuo*, yielding the title compound (1.68 mmol, 98%) as a colorless oil which was immediately used in the next reaction. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 2.19 (s, 3H), 3.36 (br, 3H), 4.23 (s, 2H), 6.50–6.58 (m, 3H), 7.22–7.34 (m, 5H).

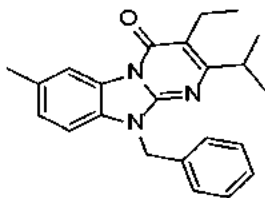
1-Benzyl-5-methyl-1*H*-benzo[d]imidazol-2-amine:



Chemical Formula: C₁₅H₁₅N₃
Exact Mass: 237,13

[0170] The title product was synthesized from *N*¹-benzyl-4-methylbenzene-1,2-diamine (500 mg, 2.36 mmol) according to the Method D affording 294 mg (1.24 mmol, 52%) of product as a white solid. ¹H NMR (DMSO, 300 MHz): δ (ppm) 2.29 (s, 3H), 5.22 (s, 2H), 6.46 (s, 2H), 6.63 (d, *J* = 7.8 Hz, 1H), 6.92 (m, 2H), 7.22 (m, 5H).

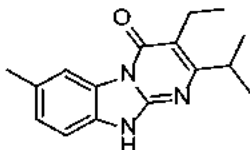
10-Benzyl-3-ethyl-2-isopropyl-7-methylbenzo[4,5*f*]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



Chemical Formula: $C_{23}H_{25}N_3O$
Exact Mass: 359,20

[0171] The title compound was synthesized according to Method F starting from 1-benzyl-5-methyl-1*H*-benzo[*d*]imidazol-2-amine (200 mg, 0.842 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.1 mol. equiv). Reaction time: 16 h. After removal of volatiles crude product was purified on silica gel column (heptane:EtOAc – 9:1) affording 96 mg (0.267 mmol; 32%) of product as an off-white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.08 (t, $J = 7.4$ Hz, 3H), 1.24 (d, $J = 6.6$ Hz, 6H), 2.62 (m, 2H), 3.23 (m, 1H), 5.46 (s, 2H), 7.17 (t, $J = 9.1$ Hz, 1H), 7.31 (m, 3H), 7.50 (m, 2H), 7.69 (d, $J = 9.3$ Hz, 1H), 8.45 (m, 1H).

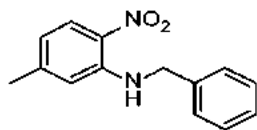
3-Ethyl-2-isopropyl-7-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



Chemical Formula: $C_{16}H_{19}N_3O$
Exact Mass: 269,15

[0172] To a solution of 10-benzyl-3-ethyl-2-isopropyl-7-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one (; 50 mg, 0.139 mmol) in a mixture of CH_2Cl_2 and MeOH (1:1, v/v) was added Pd/C catalyst (10 mol%). The reaction was flushed with H_2 gas and stirred for 5 hours. The mixture was filtered through Celite and the solvent was evaporated. The crude product was purified on silica gel column (heptane:EtOAc – 7:3) yielding 22 mg of the title compound (0.082 mmol, 58%) as a white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.07 (t, $J = 7.4$ Hz, 3H), 1.22 (d, $J = 6.7$ Hz, 6H), 2.45 (s, 3H), 2.60 (m, 2H), 3.21 (m, 1H), 7.28 (m, 2H), , 8.28 (s, 1H), 12.57 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 14.68, 17.89, 21.28, 21.65, 30.68, 110.95, 115.72, 126.25, 126.85, 130.61, 147.77, 159.78. HRMS (ESI): m/z [$M+H$] $^+$ calcd for $C_{16}H_{20}N_3O$: 270.1601, found: 270.1606.

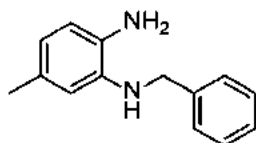
***N*-Benzyl-5-methyl-2-nitroaniline:**



[0173] Mixture of 5-methyl-2-nitroaniline (1.0 g, 6.57 mmol) and benzyl bromide (940 μ l, 7.89 mmol, 1.2 mol. equiv) in H_2O was stirred at reflux for 1.5 h. Then additional 0.6 eq (3.95 mmol) of benzyl bromide was added and stirring was continued at reflux for 2 days. After cooling to room temperature,

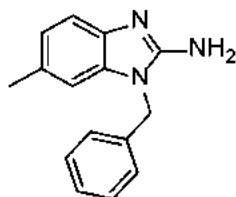
saturated NaHCO_3 was added and mixture was extracted with EtOAc (3x). Organic layers were combined, washed with H_2O (1 x) and dried over Na_2SO_4 . After removal of drying agent and solvent, residue subjected to silica gel column separation (heptane:EtOAc – 9:1) affording 1.08 g (4.46 mmol; 68%) of product as an orange solid. ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.23 (s, 3H), 4.63 (s, 2H), 6.54 (d, J = 10.3 Hz, 1H), 7.32 (m, 5H), 6.78 (s, 1H), 7.98 (d, J = 8.7 Hz, 1H), 8.64 (br, 1H).

***N*¹-Benzyl-5-methylbenzene-1,2-diamine:**



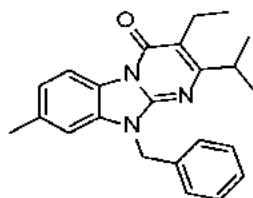
[0174] To a solution of *N*-benzyl-5-methyl-2-nitroaniline (420 mg, 1.71 mmol) in MeOH was added a slurry of Raney nickel (30 mg) and the mixture was stirred vigorously. The flask was flushed with H_2 gas 3 times. The reaction was allowed to stir for 3 hours. The catalyst was removed by filtration through Celite and the reaction was concentrated *in vacuo*, yielding the title compound as a colorless oil (1.68 mmol, 98%). ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.04 (s, 3H), 4.26 (s, 4H), 4.99 (s, 1H), 6.21 (d, J = 7.2 Hz, 2H), 6.45 (d, J = 7.6 Hz, 1H), 7.32 (m, 6H).

1-Benzyl-6-methyl-1*H*-benzo[*d*]imidazol-2-amine:



[0175] The title compound was synthesized from *N*¹-benzyl-5-methylbenzene-1,2-diamine (400 mg, 1.88 mmol) according to the Method D affording 220 mg (0.927 mmol, 49%) of product as a white solid. ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.27 (s, 3H), 5.22 (s, 2H), 6.40 (s, 2H), 6.74 (d, J = 7.9 Hz, 1H), 6.85 (s, 1H), 7.02 (d, J = 8.3 Hz, 1H), 7.26 (m, 5H).

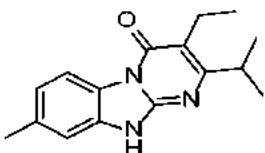
10-Benzyl-3-ethyl-2-isopropyl-8-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



[0176] The title compound was synthesized according to Method F starting from compound 1-benzyl-6-methyl-1*H*-benzo[*d*]imidazol-2-amine (260 mg, 1.10 mmol) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (1.1 mol. equiv). Reaction time: 16 h. After removal of volatiles crude product was purified on silica gel column (heptane:EtOAc – 9:1) affording 183 mg (0.509 mmol; 46%) of product

as an off-white solid. $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ (ppm) 1.19 (t, $J = 7.5$ Hz, 3H), 1.29 (d, $J = 6.6$ Hz, 6H), 2.45 (s, 3H), 2.73 (q, $J = 7.5$ Hz, 2H), 3.25 (sept, $J = 6.6$ Hz, 1H), 5.39 (s, 2H), 7.02–7.44 (m, 7H), 8.49 (d, $J = 8.1$ Hz, 1H).

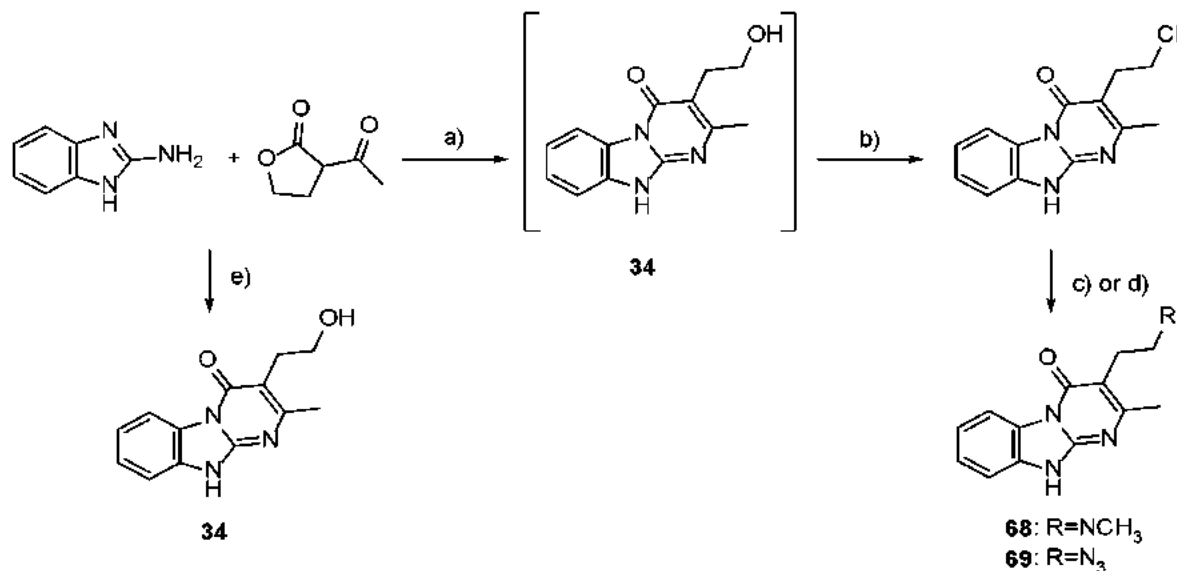
3-Ethyl-2-isopropyl-8-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one



Chemical Formula: $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}$
Exact Mass: 269.15

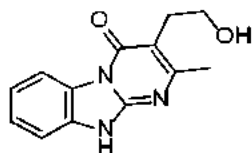
[0177] To a solution of 10-benzyl-3-ethyl-2-isopropyl-7-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one (183 mg, 0.509 mmol) in a mixture of CH_2Cl_2 and MeOH (1:1) was added Pd/C catalyst (10 mol%). The reaction was flushed with H_2 gas and stirred for 5 hours. The mixture was filtered through Celite and the solvent was evaporated. The crude product was purified on silica gel column (heptane: EtOAc – 7:3) yielding 74 mg of the title compound (0.274 mmol, 54%) as a white solid. $^1\text{H NMR}$ (DMSO-d_6 , 300 MHz): δ (ppm) 1.07 (t, $J = 7.0$ Hz, 3H), 1.21 (d, $J = 6.2$ Hz, 6H), 2.44 (s, 3H), 2.57 (q, $J = 6.9$ Hz, 2H), 3.19 (m, 1H), 7.09 (d, $J = 8.2$ Hz, 1H), 7.21 (s, 1H), 8.29 (d, $J = 8.1$ Hz, 1H), 12.53 (br, 1H). $^{13}\text{C NMR}$ (DMSO-d_6 , 75 MHz): δ (ppm) 14.68, 17.87, 21.44, 21.69, 30.70, 111.26, 115.20, 122.31, 135.71, 147.75, 159.61. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}$: 270.1601, found: 270.1592.

Synthesis of 3-substituted 2-methylbenzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-ones:



Scheme: Synthesis of 3-substituted 2-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-ones.
Reagents and conditions: a) *p*-TsOH monohydrate, toluene, reflux; b) POCl₃, reflux; c) 40% aqueous MeNH₂, 90 °C; d) NaN₃, 90 °C; e) DMF, reflux.

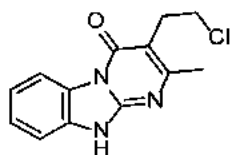
3-(2-Hydroxyethyl)-2-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: C₁₃H₁₃N₃O₂
Exact Mass: 243,10

[0178] The title compound was synthesized according to Method F starting from 2-aminobenzimidazole (100 mg, 0.751 mmol) and 2-acetyl- γ -butyrolactone (1 mol. equiv). Reaction time: overnight. DMF was removed under reduced pressure and crude product was purified using flash column chromatography on silica gel (2–5% of MeOH in CH₂Cl₂) affording 49 mg (0.201 mmol, 27%) of product as brown solid. ¹H NMR (DMSO-*d*₆, 600 MHz): δ (ppm) 2.38 (s, 3H), 2.69 (t, 2H, *J* = 7.2 Hz), 3.51–3.54 (m, 2H), 4.61 (br, 1H), 7.27 (td, 1H, *J* = 8.4 Hz, *J* = 1.2 Hz), 7.41 (td, 1H, *J* = 7.8 Hz, *J* = 1.2 Hz), 7.51 (d, 1H, *J* = 7.8 Hz), 8.39 (d, 1H, *J* = 7.8 Hz), 12.56 (br, 1H). ¹³C NMR (DMSO-*d*₆, 150MHz): δ (ppm) 19.74, 28.85, 59.73, 106.08, 113.90, 115.02, 120.97, 125.41, 126.99, 136.28, 146.75, 154.69, 159.65. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₃H₁₄N₃O₂: 244.1080, found: 244.1081.

3-(2-Chloroethyl)-2-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one (I)

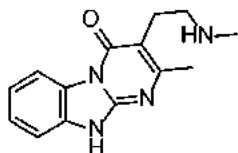


Chemical Formula: C₁₃H₁₂ClN₃O
Exact Mass: 261,07

[0179] Mixture of 2-aminobenzimidazole (666 mg; 5 mmol), 2-acetyl- γ -butyrolactone (539 μ l; 5 mmol) and *p*-toluenesulfonic acid monohydrate (15 mg) in 10 ml of toluene was refluxed for 20 h. Solid was filtered-off and dried *in vacuo* at 80 °C for 15 h. Crude product was refluxed in POCl₃ (15 ml) for 3 h. Excess of POCl₃ was removed *in vacuo*. Ice-cold water was added to the residue, mixture was brought to pH 8–9 using solid Na₂CO₃ and stirred for further 2 h. Solid was filtered-off, washed with water and dried. Crude product was purified on silica gel column (CH₂Cl₂:MeOH – 100:3). Fractions containing product were combined and concentrated. Solid was suspended in MeOH, filtered-off and dried affording 516 mg (1.971 mmol; 39% after 2 steps) of product as a pale yellow solid. ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) 2.40 (s, 3H), 2.99 (t, *J* = 7.5 Hz, 2H), 3.76 (t, *J* = 7.5 Hz, 2H), 7.27–7.32 (m, 1H), 7.44 (td, *J* = 7.5, 1.2 Hz, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 8.39 (d, *J* = 8.1 Hz, 1H), 12.75 (s, 1H). ¹³C

NMR (DMSO- d_6 , 75 MHz): δ (ppm) 20.25, 28.82, 43.09, 105.58, 113.33, 115.12, 121.29, 125.66, 126.60, 134.88, 146.87, 156.75, 159.28. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{13}N_3ClO$: 262.0742, found: 262.0741, 264.0719.

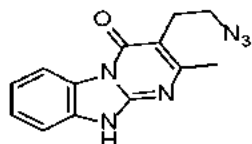
2-Methyl-3-(2-(methylamino)ethyl)benzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{14}H_{16}N_4O$
Exact Mass: 256.13

[0180] Mixture of 3-(2-chloroethyl)-2-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one (50 mg; 0.191 mmol) and 40% aqueous $MeNH_2$ (2 ml) was stirred at 90 °C for 2.5 h. Volatiles were removed under reduced pressure and crude product was purified using column chromatography on silica gel (16–20% MeOH in CH_2Cl_2 with 1.5% (vol.) addition of 7 N ammonia in MeOH) afforded 30 mg (0.117 mmol; 61%) of product as an off-white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.29 (s, 3H), 2.54 (s, 3H), 2.79 (br t, $J = 6.9$ Hz), 2H, 2.93 (br t, $J = 6.9$ Hz, 2H), 6.50 (br, 2H), 7.05 (td, $J = 8.1, 0.9$ Hz, 1H), 7.26 (td, $J = 8.1, 1.2$ Hz, 1H), 7.45 (d, $J = 7.8$ Hz, 1H), 8.35 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 21.47, 23.68, 33.85, 49.25, 101.77, 114.42, 114.77, 118.39, 124.01, 127.68, 140.38, 151.62, 158.30, 160.73. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{14}H_{17}N_4O$: 257.1397, found: 257.1398.

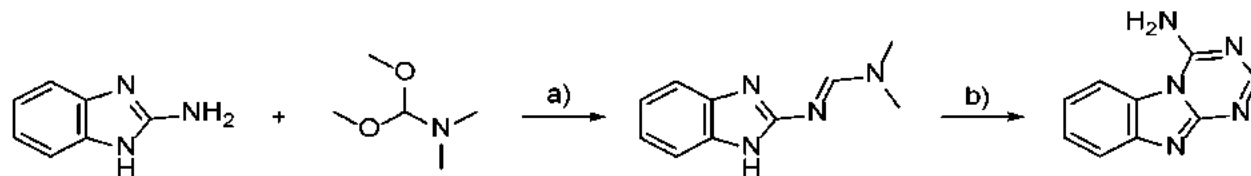
3-(2-Azidoethyl)-2-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one



Chemical Formula: $C_{13}H_{12}N_6O$
Exact Mass: 268.11

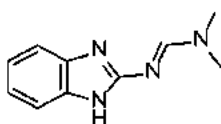
[0181] Mixture of 3-(2-chloroethyl)-2-methylbenzo[4,5]imidazo[1,2-a]pyrimidin-4(10H)-one (50 mg; 0.191 mmol) and NaN_3 (25 mg; 0.382 mol) in DMF (2 ml) was stirred at 90 °C for 18 h (product and starting material has the same R_f value in various solvent system). Volatiles were removed *in vacuo* and crude product was purified on silica gel column (1–2% of MeOH in CH_2Cl_2) afforded 27 mg (0.101 mmol; 53%) of product as an off-white solid. 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 2.38 (s, 3H), 2.79 (t, $J = 7.2$ Hz, 2H), 3.46 (t, $J = 7.2$ Hz, 2H), 7.22 (m, 1H), 7.42 (td, $J = 7.8, 0.9$ Hz, 1H), 7.49 (bd, $J = 7.8$ Hz, 1H), 8.38 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 20.04, 24.98, 49.39, 105.44, 113.36, 115.11, 121.20, 125.60, 126.64, 135.04, 146.89, 156.26, 159.36. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{13}N_6O$: 269.1145, found: 269.1144.

Synthesis of benzo[4,5]imidazo[1,2-a][1,3,5]triazin-4-amine:



Scheme. Synthesis of benzo[4,5]imidazo[1,2-a][1,3,5]triazin-4-amine. Reagents and conditions: a) CHCl_3 , reflux; b) NaOMe, MeOH, H_2NCN , reflux.

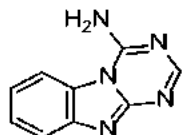
N¹,N¹-Dimethyl-N²-benzimidazolyl-2-formamidine:



Chemical Formula: $\text{C}_{10}\text{H}_{12}\text{N}_4$
Exact Mass: 188,11

[0182] Mixture of 2-aminobenzimidazole (1.5 g, 11.26 mmol) and *N,N*-dimethylformamide dimethyl acetal (1.1 mol. equiv.) in CHCl_3 (10 ml) was heated at reflux for 24 h. Volatiles were removed under reduced pressure and solid residue was chromatographed on silica gel (10% of MeOH in CH_2Cl_2) affording 2.03 g (10.78 mmol, 96%). ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 3.01 (s, 3H), 3.13 (s, 3H), 6.94–6.96 (m, 2H), 7.20–7.24 (m, 2H), 8.65 (s, 1H), 11.53 (br, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 34.24, 40.18, 112.63 (br), 119.79, 157.56, 158.52. ^1H NMR (CDCl_3 , 300 MHz): δ (ppm) 3.09 (s, 3H), 3.12 (s, 3H), 7.07–7.13 (m, 2H), 7.38–7.40 (m, 2H), 8.78 (s, 1H), 11.53 (br, 1H). ^{13}C NMR (CDCl_3 , 75 MHz): δ (ppm) 34.96, 41.10, 112.05 (br), 121.13, 157.94, 158.29. HRMS (ESI): m/z [$\text{M}+\text{H}$] $^+$ calcd for $\text{C}_{10}\text{H}_{13}\text{N}_4$: 189.1135, found: 189.1139.

Benzo[4,5]imidazo[1,2-a][1,3,5]triazin-4-amine

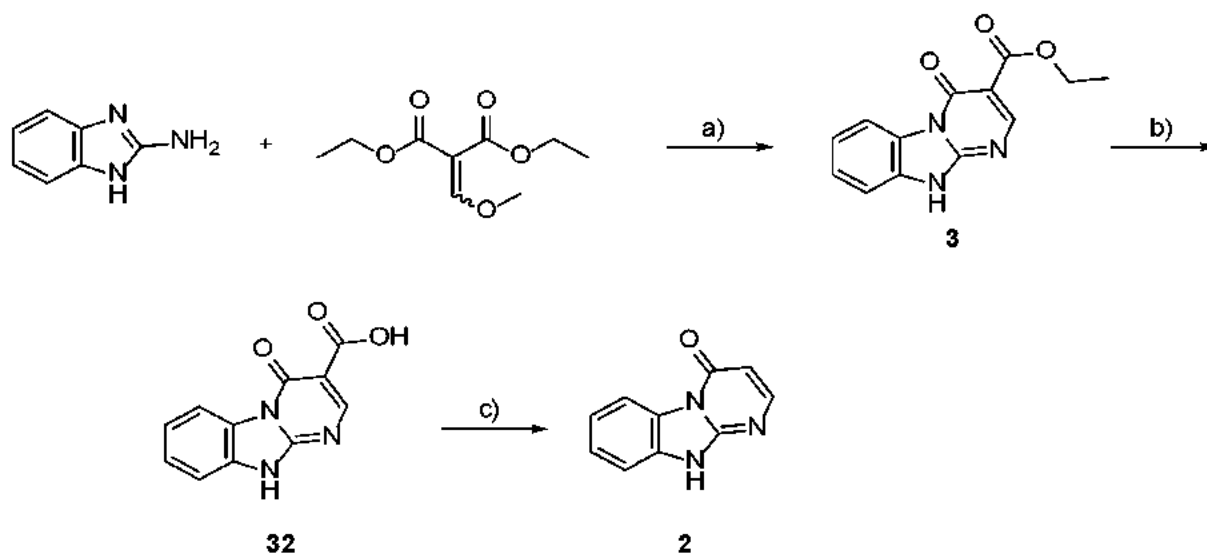


Chemical Formula: $\text{C}_9\text{H}_7\text{N}_5$
Exact Mass: 185,07

[0183] *N¹,N¹-Dimethyl-N²-benzimidazolyl-2-formamidine* (200 mg, 1.062 mmol) and cyanamide (2 mol. equiv.) were added to the solution of metallic sodium (2 mol. equiv.) dissolved in anhydrous MeOH (3.5 ml). Resulting mixture was heated at reflux for 24 h. Then volatiles were removed in vacuo. Solid residue was dissolved in H_2O and acidified (to pH 3) using concentrated HCl. After 30 min of stirring at 0 °C, solid was filtered-off, washed with H_2O and recrystallized from DMF affording 108 mg (0.583 mmol, 55%) of title compound as a white solid. ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 7.37 (t, $J =$

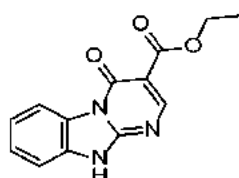
7.5 Hz, 1H), 7.51 (t, $J = 7.5$ Hz, 1H), 7.74 (d, $J = 8.1$ Hz, 1H), 8.26 (s, 1H), 8.41 (d, $J = 7.8$ Hz, 1H), 8.61 (br, 2H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 114.16, 118.45, 121.36, 125.08, 125.85, 143.34, 154.08, 153.49, 159.09. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_9\text{H}_8\text{N}_5$: 186.0774, found: 186.0777.

Synthesis of Examples A-2, A-3 and B-32:



Scheme. The synthesis of 4-oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine. Reagents and conditions: a) MeOH, reflux; b) NaOH, H₂O, 85 °C; c) Cu powder, quinoline, 200 °C.

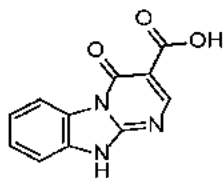
Ethyl 4-oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidine-3-carboxylate



Chemical Formula: $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}_3$
Exact Mass: 257.08

[0184] Mixture of 2-aminobenzimidazole (500 mg, 3.756 mmol) and diethyl ethoxymethylenemalonate (829 mg, 3.831 mmol) in dry MeOH was heated at reflux for 5 h. The mixture was cooled down in an ice-water bath. Precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel (2–20% gradient of MeOH in CH_2Cl_2 with 5% vol. addition of 7 N ammonia in MeOH). ^1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) 1.30 (t, $J = 7.2$ Hz, 3H), 4.25 (q, $J = 7.2$ Hz, 2H), 7.39–7.45 (m, 1H), 7.53 (td, $J = 7.2, 0.9$ Hz, 1H), 7.58 (br d, $J = 8.1$ Hz, 1H), 8.51 (d, $J = 8.1$ Hz, 1H), 8.68 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) 14.32, 59.66, 103.41, 112.49, 116.05, 122.76, 126.34, 126.69, 131.58, 150.61, 156.28, 158.63, 164.27. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{12}\text{N}_3\text{O}_3$: 258.0873, found: 258.0879.

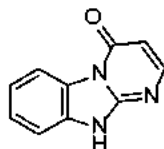
4-Oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carboxylic acid



Chemical Formula: C₁₁H₇N₃O₃
Exact Mass: 229,05

[0185] Mixture of ethyl 4-oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carboxylate (; 99 mg, 0.385 mmol) and 0.2 M aqueous NaOH (5.4 ml) was heated at 85 °C for 2.5 h. Next, the mixture was cooled in an ice-water bath and acidified using concentrated HCl. Precipitate was filtered-off, washed with H₂O and dried affording 54 mg (0.236 mmol, 61%) of title compound as pale yellow solid. ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) 7.48 (br t, *J* = 7.2 Hz, 1H), 7.53 (m, 1H), 7.57–7.66 (m, 2H), 8.48 (d, *J* = 7.8 Hz, 1H), 8.77 (s, 1H), 13.19 (br, 1H). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) 102.48, 112.58, 116.17, 123.26, 126.00, 126.98, 131.14, 150.29, 159.14, 160.35, 165.04. HRMS (ESI): *m/z* [M-H]⁻ calcd for C₁₁H₆N₃O₃: 228.0414, found: 228.0417.

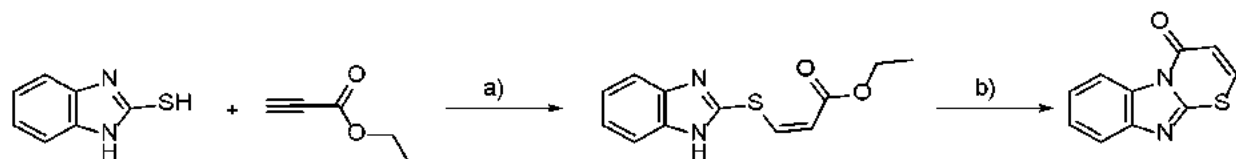
Benzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10H)-one



Chemical Formula: C₁₀H₇N₃O
Exact Mass: 185,06

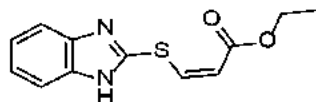
[0186] Mixture of 4-oxo-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carboxylic acid (; 47 mg, 0.205 mmol) and Cu powder (8 mg) in quinoline (0.8 ml) was heated at 200 °C for 1 h. The hot mixture was filtered through paper filter and quinoline was removed by vacuum distillation. The residue was purified on silica gel column (2–4% gradient of MeOH in CH₂Cl₂). Fractions containing product were combined, concentrated and repurified using preparative TLC (3% of MeOH in CH₂Cl₂) affording 6 mg (0.032 mmol, 16%) of product as an off-white solid. ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) 5.96 (d, *J* = 6.9 Hz, 1H), 7.32 (td, *J* = 8.1, 1.2 Hz, 1H), 7.47 (td, *J* = 7.8, 1.2 Hz, 1H), 7.55 (br d, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 6.9 Hz, 1H), 8.43 (d, *J* = 8.1 Hz, 1H), 12.96 (br, 1H). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) 100.07, 113.39, 115.40, 121.49, 125.77, 126.73, 134.67, 148.74, 149.03, 159.46. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₀H₈N₃O: 186.0662, found: 186.0662.

Synthesis of Example B-58:



Scheme. The synthesis of 4H-benzo[4,5]imidazo[2,1-b][1,3]thiazin-4-one. Reagents and conditions: a) EtOH, rt to 60 °C; b) Ph₂O, 220 °C.

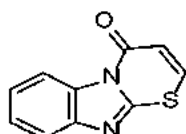
Ethyl (Z)-3-((1H-benzo[d]imidazol-2-yl)thio)acrylate:



Chemical Formula: C₁₂H₁₂N₂O₂S
Exact Mass: 248.06

[0187] Mixture of 2-mercaptobenzimidazole (200 mg, 1.332 mmol) and ethyl propiolate (1.1 mol equiv.) in EtOH (3.3 ml) was stirred overnight at room temperature and next at 60 °C for 5 h. The mixture was concentrated *in vacuo* affording 312 mg (1.256 mmol, 94%) of crude product that was pure enough to be used in the next step without further purification. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 1.26 (t, *J* = 7.2 Hz, 3H), 4.20 (q, *J* = 7.2 Hz, 2H), 6.29 (d, *J* = 9.9 Hz, 1H), 7.16–7.22 (m, 2H), 7.51–7.54 (m, 2H), 8.37 (d, *J* = 9.9 Hz, 1H), 12.96 (br, 1H). ¹³C NMR (DMSO-d₆, 75 MHz): δ (ppm) 14.12, 60.34, 115.18, 122.05, 141.89, 147.73, 165.93. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₂H₁₃N₂O₂S: 249.0692, found: 249.0680.

4H-benzo[4,5]imidazo[2,1-b][1,3]thiazin-4-one

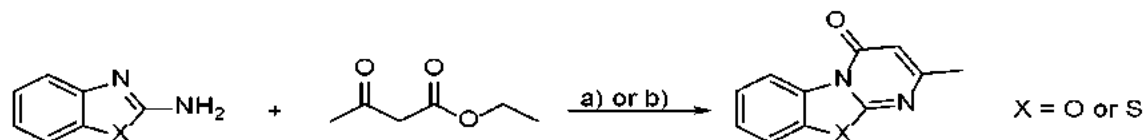


Chemical Formula: C₁₀H₆N₂OS
Exact Mass: 202.02

[0188] Ethyl (Z)-3-((1H-benzo[d]imidazol-2-yl)thio)acrylate (294 mg, 1.184 mmol) was heated in diphenyl ether (5 ml) at 220 °C for 4 h. After cooling to room temperature mixture was diluted with heptane (20 ml). The resulting solid was filtered-off, washed with heptane and dried under vacuum overnight affording crude product. Filtrate, heptane and ethereal washing were combined and kept at 4 °C for 48 h. Resulted precipitate was collected by filtration and dried affording additional amount of crude product. Combined precipitates were purified on silica gel column (0–50% AcOEt in heptane) affording 71 mg (0.351 mmol, 30%) of target compound as an off-white solid. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 6.76 (d, *J* = 10.4 Hz, 1H), 7.45 (td, *J* = 7.7, 1.2 Hz, 1H), 7.51 (td, *J* = 7.2, 1.2 Hz, 1H), 7.68 (d, *J* = 10.4 Hz, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 8.58 (d, *J* = 8.0 Hz, 1H). ¹³C NMR (CDCl₃, 75 MHz):

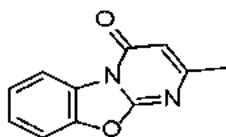
δ (ppm) 116.29, 117.15, 118.62, 124.40, 126.03, 130.98, 134.88, 141.96, 146.21, 159.61. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{10}H_7N_2OS$: 203.0274, found: 203.0280.

Synthesis of Examples B-59-60:



Scheme. Synthesis of 4*H*-benzo[4,5]oxazolo- and 4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidin-4-one. Reagents and conditions: a) Polyphosphoric acid (PPA), 120 °C (for both analogues); b) AcOH, reflux (only for thiazolo analogue).

2-Methyl-4H-benzo[4,5]oxazolo[3,2-a]pyrimidin-4-one



Chemical Formula: $C_{11}H_8N_2O_2$
Exact Mass: 200,06

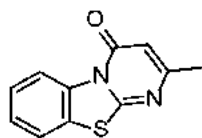
[0189] A mixture of 2-aminobenzoxazole (200 mg, 1.491 mmol) and ethyl acetoacetate (1.1 mol. equiv.) in polyphosphoric acid (1.7 g) was heated at 120 °C overnight. After cooling the mixture in an ice-water bath, ice cold H_2O was added to the flask and mixture was neutralized using 10 M aq. NaOH. Precipitate that formed was filtered-off, washed with H_2O and dried. Crude product was suspended in MeOH, solid was filtered-off and dried affording 41 mg (0.205 mmol, 14%) of pale yellow solid.

Method using AcOH:

[0190] A mixture of 2-aminobenzoxazole (200 mg, 1.491 mmol) and ethyl acetoacetate (1.5 mol. equiv.) in AcOH (1.7 ml) was heated at reflux for 15 h. Volatiles were removed under reduced pressure and the residue was brought pH ca. 8 using saturated aqueous $NaHCO_3$, and extracted with AcOEt. Organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (0–10% gradient of AcOEt in CH_2Cl_2) affording 31 mg (0.155 mmol, 10%) of pale yellow solid.

[0191] 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 2.42 (s, 3H), 6.24 (s, 1H), 7.42–7.55 (m, 3H), 8.38–8.41 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 24.46, 106.44, 111.08, 116.66, 125.44, 126.31, 127.14, 144.71, 155.29, 159.47, 164.94. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{11}H_9N_2O_2$: 201.0658, found: 201.0662.

2-Methyl-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin-4-one



Chemical Formula: C₁₁H₉N₂OS
Exact Mass: 216.04

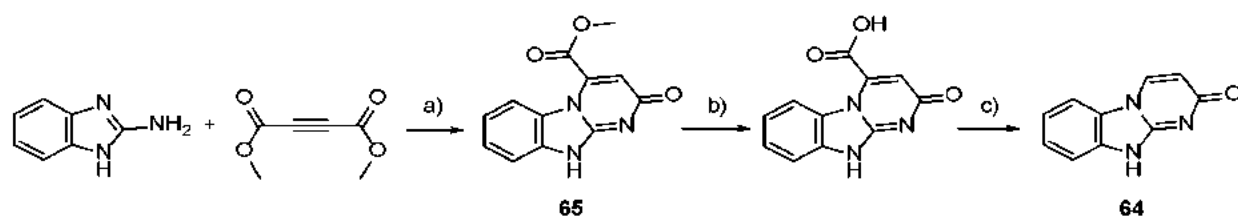
[0192] Mixture of 2-aminobenzothiazole (200 mg, 1.332 mmol) and ethyl acetoacetate (1.1 mol. equiv.) in polyphosphoric acid (PPA; 1.5 g) was heated at 120 °C overnight. After cooling the mixture in an ice-water bath, ice cold H₂O was added to the flask and mixture was neutralized using 10 M aq. NaOH. Precipitate that formed was filtered-off, washed with H₂O and dried. Crude product was suspended in MeOH, solid was filtered-off and dried affording 90 mg (0.416 mmol, 31%) of product as a pale yellow solid.

Method using AcOH:

[0193] To a solution of 2-aminobenzothiazole (200 mg, 1.332 mmol) in AcOH (1.5 ml) was added ethyl acetoacetate (1.5 mol. equiv.) and resulting mixture was heated at reflux for 15 h. Volatiles were removed under reduced pressure and the residue was brought pH ca. 8 using saturated aqueous NaHCO₃, and extracted with AcOEt. Organic layers were combined, washed with brine, dried over MgSO₄, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (0–10% gradient of AcOEt in CH₂Cl₂) affording 112 mg (0.518 mmol, 39%) of product as a pale yellow solid.

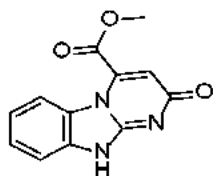
[0194] ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 2.39 (s, 3H), 6.26 (s, 1H), 7.45–7.54 (m, 2H), 7.65–7.68 (m, 1H), 9.05–9.08 (m, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) 23.84, 107.31, 120.19, 121.87, 124.22, 127.05, 127.14, 136.25, 161.30, 161.56, 163.02. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₁H₉N₂OS: 217.0430, found: 217.0436.

Synthesis of Example B-64:



Scheme. Benzo[4,5]imidazo[1,2-*a*]pyrimidin-2(10*H*)-one. Reagents and conditions: a) MeOH, reflux; b) NaOH, H₂O, 85 °C; c) Cu powder, quinoline, 200 °C.

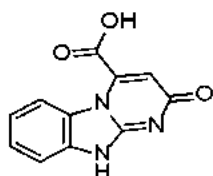
Methyl 2-oxo-2,10-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidine-4-carboxylate



Chemical Formula: C₁₂H₉N₃O₃
Exact Mass: 243,06

[0195] Mixture of 2-aminobenzimidazole (200 mg, 1.502 mmol) and dimethyl acetylenedicarboxylate (271 mg, 1.903 mmol) in 4.5 ml of anhydrous MeOH was heated at reflux overnight. The mixture in an ice-water bath, precipitate was filtered-off and dried affording 245 mg (1.007 mmol, 67%) of the title compound as a yellow solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 4.06 (s, 3H), 6.52 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.53–7.58 (m, 2H). ¹³C NMR (DMSO-d₆, 75 MHz): δ (ppm) 53.94, 109.56, 113.36, 116.53, 121.17, 124.64, 127.47, 137.52, 139.93, 148.02, 161.17, 161.76. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₂H₁₀N₃O₃: 244.0717, found: 244.0714.

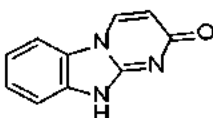
2-Oxo-2,10-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidine-4-carboxylic acid :



Chemical Formula: C₁₁H₇N₃O₃
Exact Mass: 229,05

[0196] Mixture of methyl 2-oxo-2,10-dihydrobenzo[4,5]imidazo[1,2-a]pyrimidine-4-carboxylate (: 206 mg, 0.799 mmol) and 0.2 M aqueous NaOH (3.8 ml) was heated at 85 °C for 2.5 h. Next, the mixture was cooled in an ice-water bath and acidified using concentrated HCl. Precipitate was filtered-off, washed with H₂O and dried affording 153 mg (0.668 mmol, 84%) of an off-white solid. ¹H NMR (DMSO-d₆, 300 MHz): δ (ppm) 6.44 (s, 1H), 7.23 (t, *J* = 7.8 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H). ¹³C NMR (DMSO-d₆, 75 MHz): δ (ppm) 107.58, 113.45, 116.50, 121.24, 124.71, 127.52, 139.84, 147.96, 162.22, 162.26. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₁H₈N₃O₃: 230.0560, found: 230.0552.

Benzo[4,5]imidazo[1,2-a]pyrimidin-2(10H)-one



Chemical Formula: C₁₀H₇N₃O
Exact Mass: 185,06

[0197] A mixture of 2-oxo-2,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-4-carboxylic acid (133 mg, 0.580 mmol) and Cu powder (22 mg) in quinoline (1.1 ml) was heated at 170 °C for 25 min. Then, the hot mixture was filtered through filter paper. After cooling to room temperature, filtrate was diluted with Et₂O. Solid was filtered-off, washed with ether and dried. The crude product was purified on silica gel column (2–5% gradient of MeOH in CH₂Cl₂) affording 38 mg (0.205 mmol, 35%) of title compound as an off-white solid. ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) 6.11 (d, *J* = 7.8 Hz, 1H), 7.22–7.34 (m, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 1H), 8.79 (d, *J* = 7.8 Hz, 1H), 12.59 (br, 1H). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) 105.80, 110.13, 117.01, 120.95, 124.15, 128.46, 134.75, 140.98, 147.28, 162.04. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₀H₈N₃O: 186.0662, found: 186.0664.

[0198] Inhibitory potency data of the compounds according to the invention are compiled in the following table:

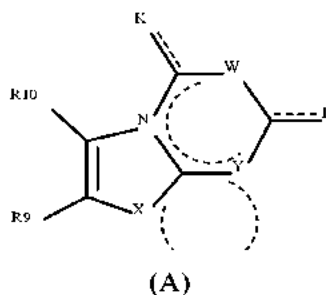
	<i>β</i>-arrestin assay (CHO <i>β</i>-arrestin human MrgX4 L83S)	calcium assay (LN229 cells recombinantly expressing human wildtype MrgX2)
	IC₅₀ ± SEM [μM]^a (% inhibition ± SEM)^c	IC₅₀ ± SEM [μM]^a (% inhibition ± SEM)^d
A-1	> 100 (4%)	
A-2	> 100 (18%)	
A-3	> 100 (25%)	
A-4	> 100 (35%)	
A-5	> 10 (-5%)	
A-6	> 10 (14%)	
A-7	> 100 (43%)	
A-8	11.6 ± 3.7	2.89 ± 0.24
A-9	3.23 ± 0.36	0.662 ± 0.102
A-10	2.42 ± 0.22	0.683 ± 0.174
A-11	> 100 (37%)	
A-12	> 10 (-25%)	
A-13	> 100 (24%)	
A-14	> 10 (19%)	
A-15	1.40 ± 0.88	> 100 (3%)
A-16	> 100 (26%)	
A-17	3.22 ± 0.82	> 100 (70%)
A-18	> 100 (48%)	
A-19	14.7 ± 1.3	> 100 (27%)
A-20	> 100 (-2%)	
A-21	0.583 ± 0.049	0.0121 ± 0.0015
A-22	> 100 (2%)	
A-23	> 10 (4%)	
A-24	> 100 (16%)	> 100 (2%)
A-25	> 100 (22%)	
A-26	> 100 (-13%)	
A-27	> 100 (-18%)	
A-28	> 100 (4%)	

A-29	> 100 (-33%)	
A-30	> 10 (2%)	> 100 (31%)
B-31	> 100 (40%)	
B-32	> 100 (45%)	
B-33	> 100 (24%)	
B-34	> 100 (22%)	> 100 (-2%)
B-35	> 10 (20%)	
B-36	> 100 (48%)	> 100 (10%)
B-37	0.290 ± 0.098	0.00248 ± 0.00075
B-38	> 100 (15%)	0.0540 ± 0.0192
B-39	0.0877 ± 0.0368	0.0184 ± 0.0054
B-40	0.0225 ± 0.0024	0.000420 ± 0.000054
B-41	0.477 ± 0.087	0.00827 ± 0.00082
B-42	0.512 ± 0.107	> 100 (68%)
B-43	0.278 ± 0.042	
B-44	1.40 ± 0.36	0.199 ± 0.025
B-45	0.214 ± 0.032	> 100 (89%)
B-46	0.250 ± 0.061	
B-47	1.05 ± 0.11	
B-48	0.0926 ± 0.0426	
B-49	0.169 ± 0.017	0.0132 ± 0.0074
B-50	0.129 ± 0.007	
B-51	0.213 ± 0.079	0.0132 ± 0.0020
B-52	> 100	
B-53	0.0711 ± 0.0152	
B-54	1.26 ± 0.31	
B-55	5.66 ± 1.15	> 100 (20%)
B-56	0.148 ± 0.042	0.00394 ± 0.00167
B-57	> 100 (-4%)	
B-58	> 100 (-44%)	
B-59	> 100 (13%)	
B-60	> 100 (14%)	
B-61	> 10 (2%)	
B-62	> 100 (18%)	
B-63	> 100 (6%)	
B-64	> 100 (0%)	
B-65	> 100 (48%)	
B-66	> 100 (4%)	
C-67	8.04 ± 1.63	
C-68	> 100 (8%)	
C-69	> 100 (9%)	
C-70	0.147 ± 0.015	0.000352 ± 0.000070
C-71	0.0910 ± 0.0292	0.000446 ± 0.000142
C-72	> 10 (39%)	
C-73	> 10 (40%)	
C-74	0.0180 ± 0.0044	0.00126 ± 0.00029
C-75	0.890 ± 0.184	0.0368 ± 0.0084
C-76	0.349 ± 0.065	
C-77	1.18 ± 0.33	
C-78	0.468 ± 0.052	0.0513 ± 0.0191
C-79	0.540 ± 0.076	> 10 (105%)
C-80	> 10 (34%)	
C-84	0.0582 ± 0.0189	

C-85	0.223 ± 0.019	
C-86	0.130 ± 0.029	
C-87	0.246 ± 0.068	
C-88	5.54 ± 1.74	> 10 (86%)

Patent claims:

- I. A method for preventing or treating a disease or disorder that is associated with the MrgX2 receptor comprising administering to a subject in need thereof a therapeutically effective amount of an MrgX2 antagonist according to general formula (A)



wherein

K means =O or =S or =NH, **W** means -CR1=, and **L** means -R2; or

K means -R2, **W** means -CR1=, and **L** means =O; or

K means -R1, **W** means -N=, and **L** means -R2; or

K means -R1, **W** means -CH=, and **L** means -R2;

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -NR4- and **Y** means -CR0=; or

X means -N= and **Y** means -S-; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=;

R0, **R1**, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -NH₂; -S-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

R9 and **R10** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R9** and **R10** together with the carbon atoms to which they are attached form a phenyl ring optionally substituted with **R5**, **R6**, **R7** and **R8**;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

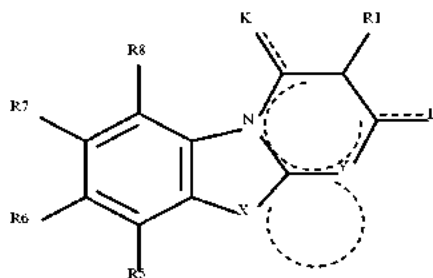
wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

or a physiologically acceptable salt thereof.

2. The method according to claim 1, wherein the disease or disorder that is associated to the MrgX2 receptor is selected from any of the groups consisting of
- pain, especially acute, nociceptive, neuropathic or chronic pain, inflammatory pain or itch; and/or
 - anxiety, stress and stress-associated syndromes, depression, epilepsy, Alzheimer's disease, senile dementia, general cognitive dysfunctions, learning and memory disorders (as a nootropic), withdrawal symptoms, alcohol and/or drug and/or medicament abuse and/or dependency, sexual dysfunctions, cardiovascular diseases, hypotension, hypertension, tinnitus, pruritus, migraine, impaired hearing, deficient intestinal motility, impaired food intake, anorexia, obesity, locomotor disorders, diarrhoea, cachexia, urinary incontinence or as a muscle relaxant, anticonvulsive or anaesthetic or for co-administration in the case of treatment with an opioid analgesic or with an anaesthetic, for diuresis or antinatriuresis, anxiolysis, for modulation of motor activity, for modulation of neurotransmitter secretion and treatment of neurodegenerative diseases associated therewith, for the treatment of withdrawal symptoms and/or for reducing the addictive potential of opioids; and/or
 - asthma, urticaria, skin inflammation, dry skin, atopic eczema, psoriasis, urticaria, scabies, non-allergic hypersensitivity reactions, fibrosis and itch.

3. The method according to claim 1, wherein the MrgX2 antagonist is according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -R2; or

K means -R2 and **L** means =O; and

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -NR4- and **Y** means -CR0=; or

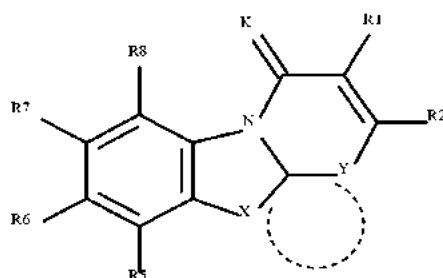
X means -N= and **Y** means S; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

4. The method according to claim 1, wherein the MrgX2 antagonist is according to general formula (C)



(C)

wherein **K** means =O or =S; and

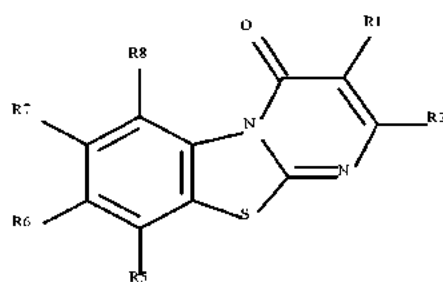
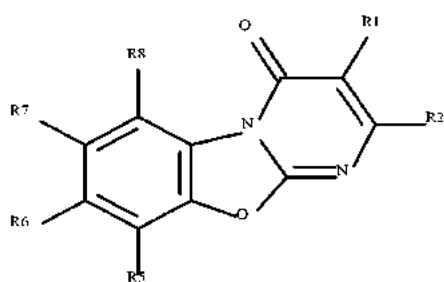
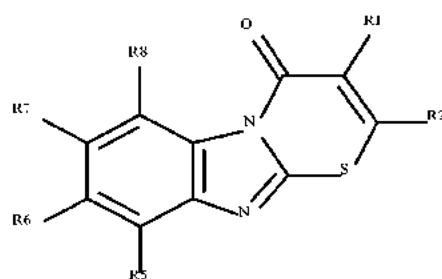
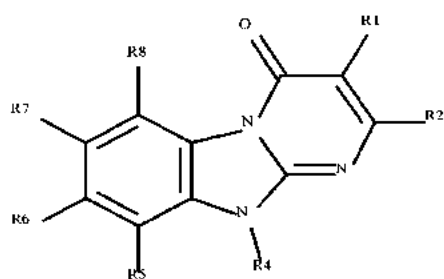
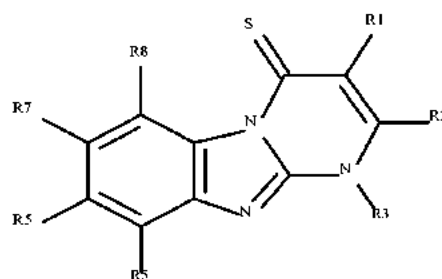
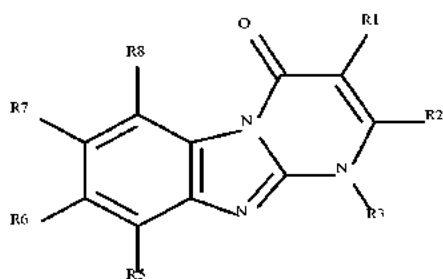
X means -N= and **Y** means -NR3-; or

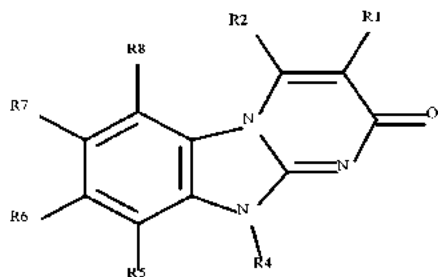
X means -NR4- and **Y** means -N=; or

X means -NR4- and **Y** means -CR0=; or

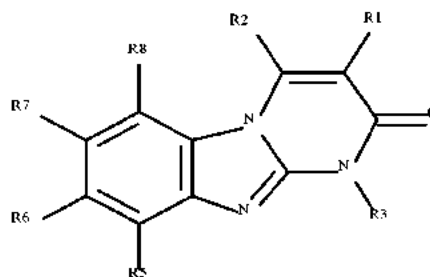
X means -N= and Y means S; or
X means -O- and Y means -N=; or
X means -S- and Y means -N=; or
X means -N= and Y means -N=.

5. The method according to claim 1, wherein the MrgX2 antagonist is selected from the group consisting of compounds according to general formula (I), (II), (III), (IV), (V), (VI), (VII), (VIII), (IX), (X), (XI), and (XII):

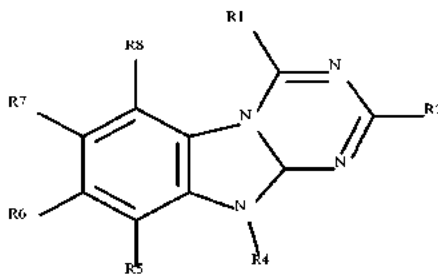




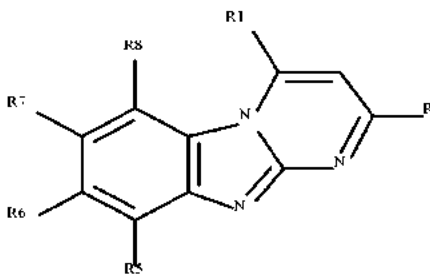
(VII)



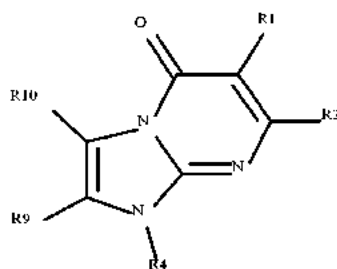
(VIII)



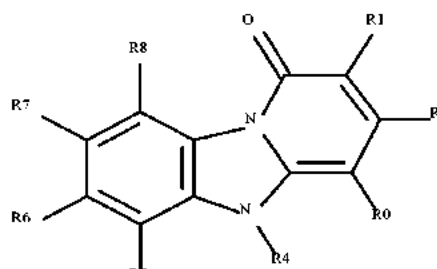
(IX)



(X)



(XI)



(XII)

6. The method according to claim 1, wherein

R0 means -H or -CN;

R1 means -H, -CH₃, -CH₂CH₃, -CH₂CH₂CH₃, -CH₂CH₂CH₂CH₃, -CH₂CH₂CH(CH₃)₂, -CH₂CH₂C(=O)OCH₂CH₃, -CH₂CH₂-CN, -CH₂CH₂-Cl, -CH₂CH₂-OH, -CH₂CH₂-OCH₃, -CH₂CH₂-N₃, -CH₂CH₂-NHCH₃, -C(=O)OH, -C(=O)OCH₃, -C(=O)OCH₂CH₃, -CH₂-phenyl with phenyl being unsubstituted, -CH₂CH₂-phenyl with phenyl being unsubstituted, -S-CH₃ or -NH₂;

and/or

R2 means -H, -CH₃, -CH₂CH₂CH₃, -CH(CH₃)₂, -CH₂CH(CH₃)₂, -C(CH₃)₃, -cyclopropyl, -phenyl with phenyl being unsubstituted, -para-methoxyphenyl, -2-thienyl, -CH₂C(=O)OH, -C(=O)OCH₃, -CH₂C(=O)OCH₃, -CH₂C(=O)OCH₂CH₃, -C(CH₃)₂(OH), -CH₂OCH₃, -SCH₃, -NH₂ or -OH;

or

R1 and **R2** together with the atoms to which they are attached form a cyclohexyl ring and mean $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$;

and/or

R3 means $-\text{H}$, $-\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{CH}_3$, $-\text{CH}_2$ -phenyl, $-\text{CH}_2$ -*p*-chlorophenyl or $-\text{CH}_2$ -CN;

and/or

R4 means $-\text{H}$, $-\text{CH}_3$ or $-\text{CH}_2$ -phenyl with phenyl being unsubstituted;

and/or

R9 means $-\text{H}$; and/or **R10** means $-\text{H}$; or **R9** and **R10** together with the carbon atoms to which they are attached form a phenyl ring optionally substituted with **R5**, **R6**, **R7** and **R8**;

wherein

R5, **R6**, **R7** and **R8** independently of one another mean $-\text{H}$, $-\text{CH}_3$, $-\text{OH}$, $-\text{OCH}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$ or $-\text{CN}$, and preferably

R5 means $-\text{H}$ or $-\text{CH}_3$; and/or

R6 means $-\text{H}$, $-\text{CH}_3$, $-\text{OH}$, $-\text{OCH}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$ or $-\text{CN}$; and/or

R7 means $-\text{H}$, $-\text{CH}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{CN}$, $-\text{OCH}_3$ or $-\text{OH}$; and/or

R8 means $-\text{H}$;

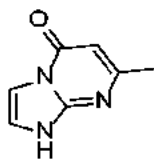
or

R6 and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring.

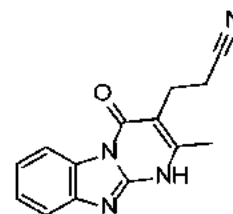
7. The method according to claim 1, wherein the MrgX2 antagonist is selected from compounds

(I) A-1 to A-30 and the physiologically acceptable salts thereof:

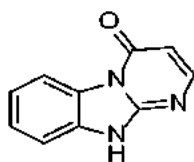
A-1)



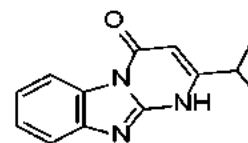
A-16



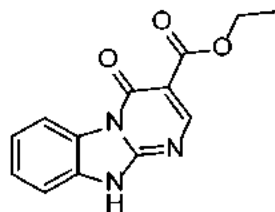
A-2



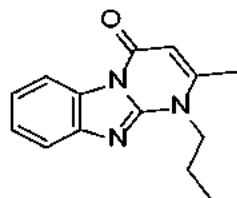
A-17



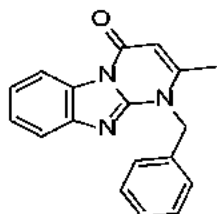
A-3



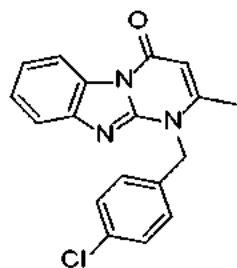
A-4



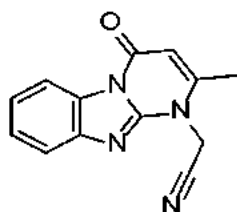
A-5



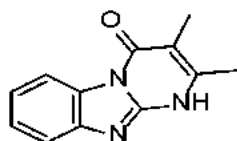
A-6



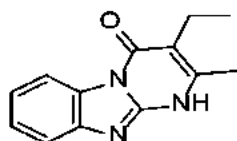
A-7



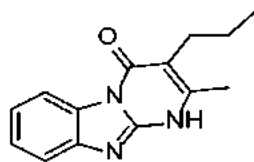
A-8



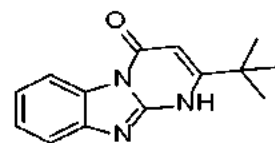
A-9



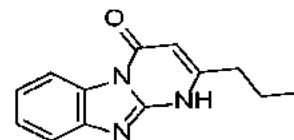
A-10



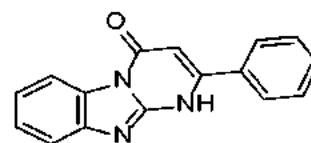
A-18



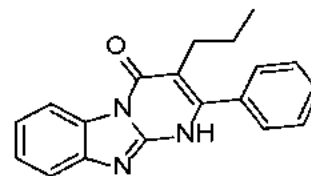
A-19



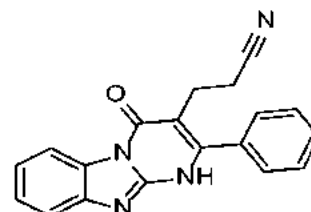
A-20



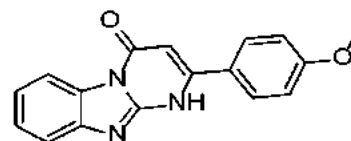
A-21



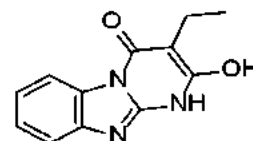
A-22



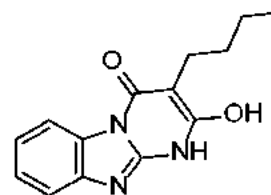
A-23



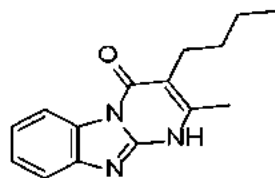
A-24



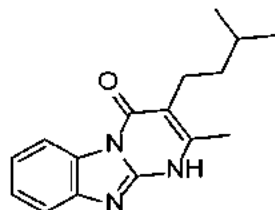
A-25



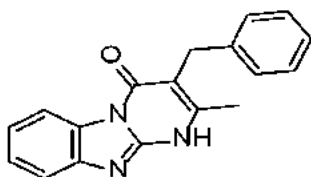
A-11



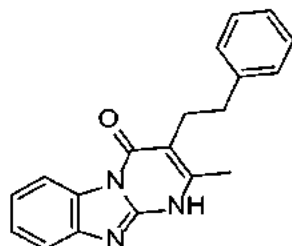
A-12



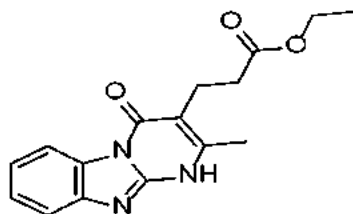
A-13



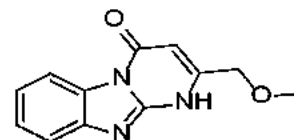
A-14



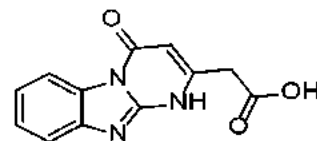
A-15



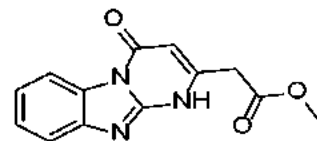
A-26



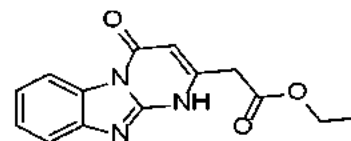
A-27



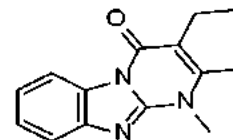
A-28



A-29



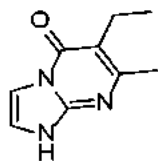
A-30



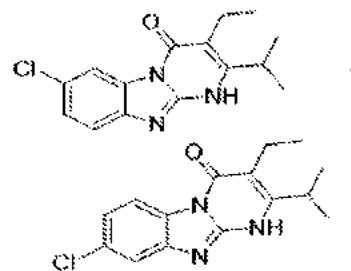
and

(II) B-31 to B-66 and the physiologically acceptable salts thereof:

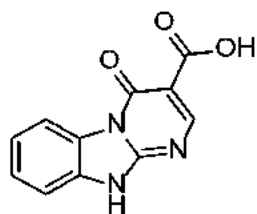
B-31



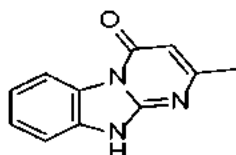
B-49
preferably
ca. 1:1



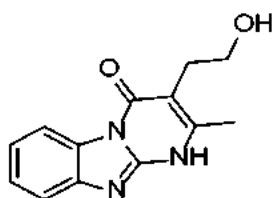
B-32



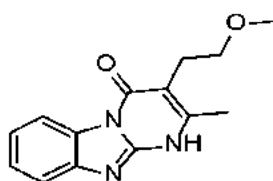
B-33



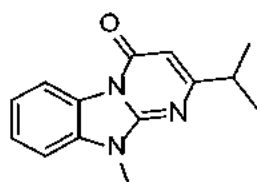
B-34



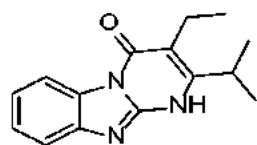
B-35



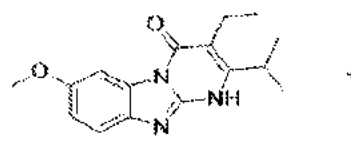
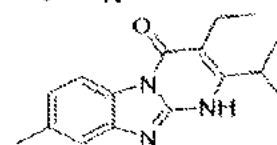
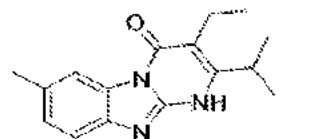
B-36



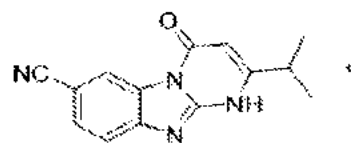
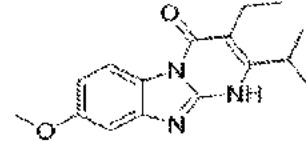
B-37



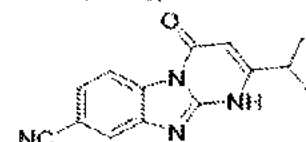
B-50



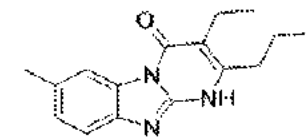
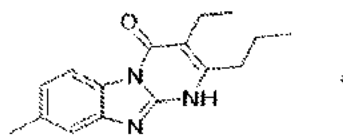
B-51
preferably
ca. 1: 1.5



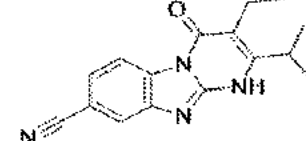
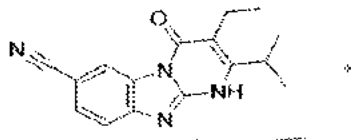
B-52



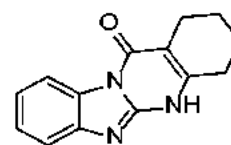
B-53

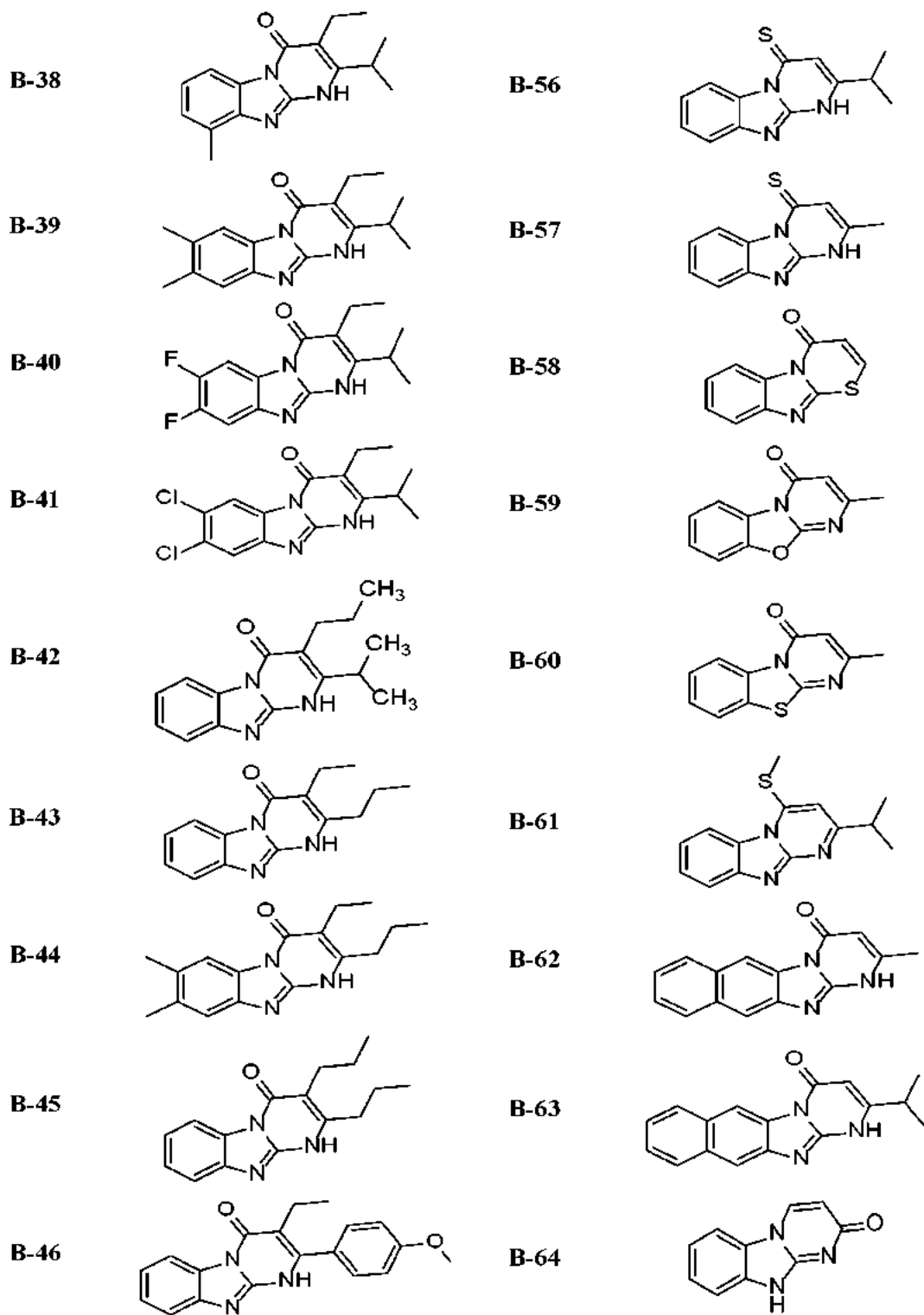


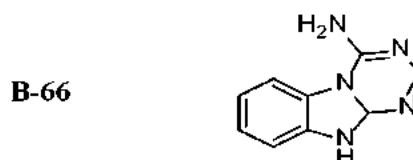
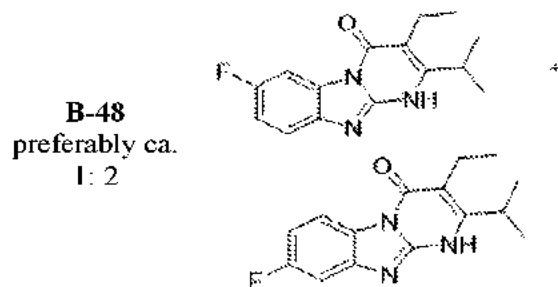
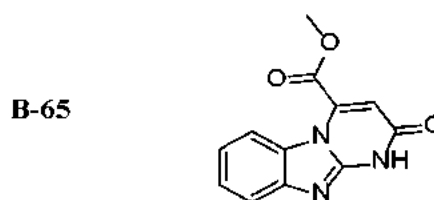
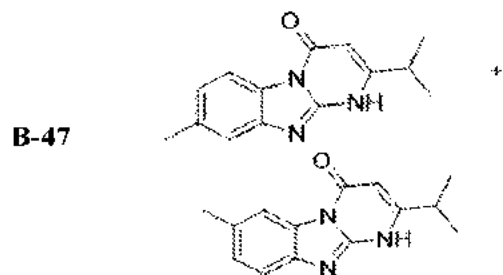
B-54
preferably
ca. 1.5: 1



B-55

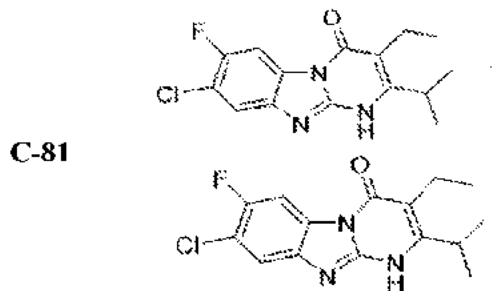
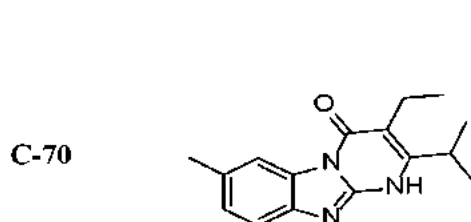
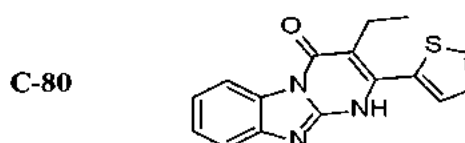
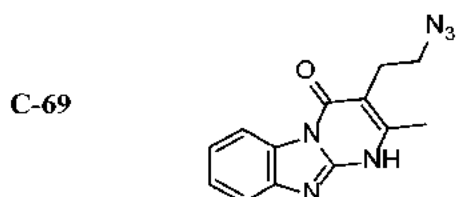
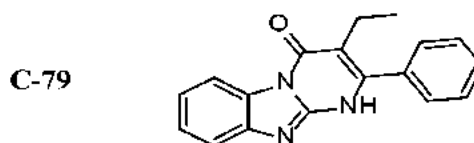
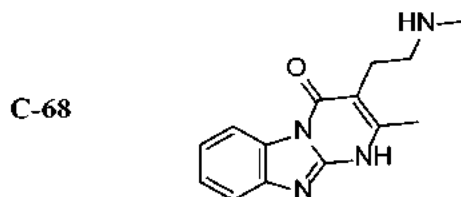
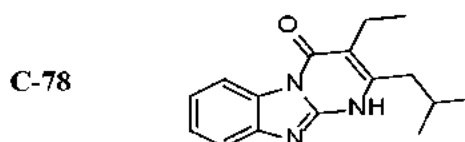
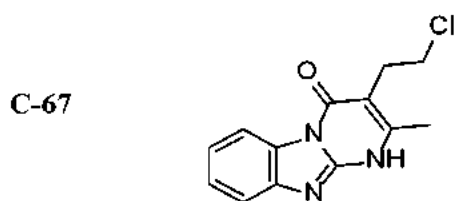




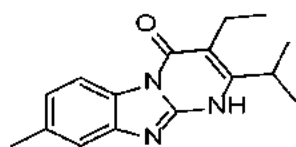


and

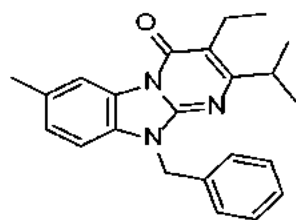
(III) C-67 to C-87 and the physiologically acceptable salts thereof:



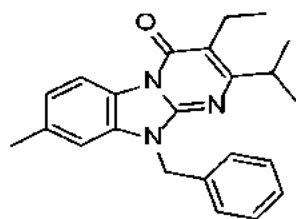
C-71



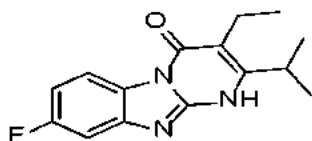
C-72



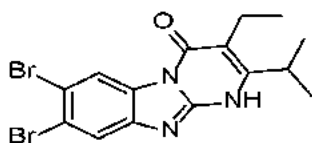
C-73



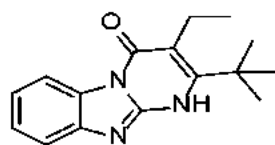
C-74



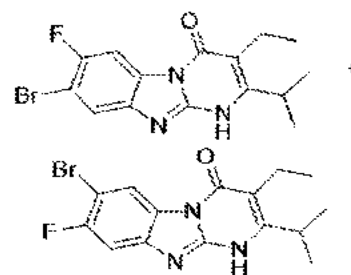
C-75



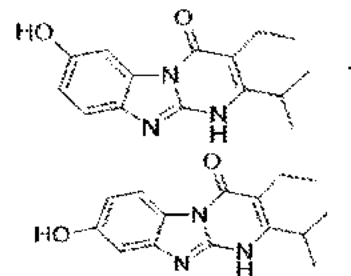
C-76



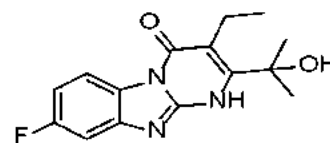
C-82



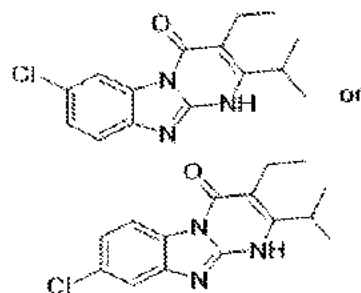
C-83



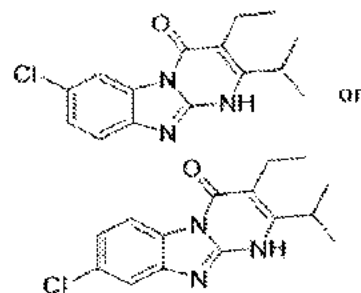
C-84



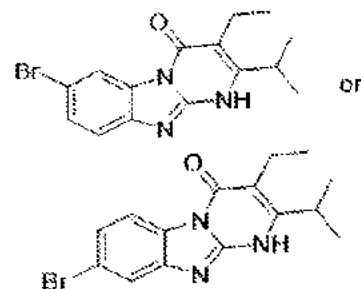
C-84*



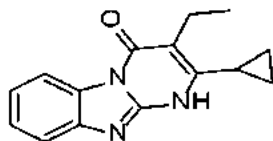
C-85



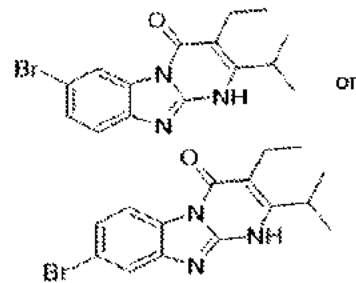
C-86



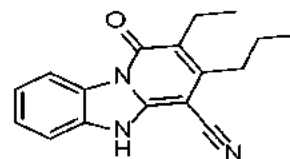
C-77



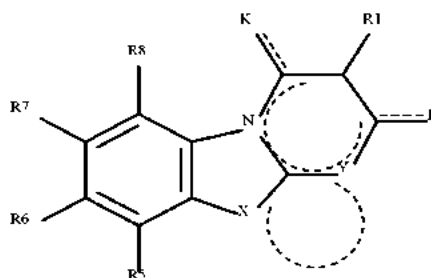
C-87



C-88



8. The method according to claim 1, wherein the MrgX2 antagonist is administered orally.
9. The method according to claim 1, wherein the MrgX2 antagonist is administered once daily, twice daily or thrice daily.
10. An MrgX2 antagonist according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -R2; or

K means -R2 and **L** means =O; and

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -N= and **Y** means S; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

R1, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl;

-C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

with the proviso that

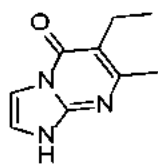
- at least one of **R5**, **R6**, **R7** and **R8** does not mean -H; and/or
- at least three of **R1**, **R2**, **R3**, **R4**, **R5**, **R6**, **R7** and **R8** do not mean -H; and/or
- A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29 and/or A-30 are not included;

or a physiologically acceptable salt thereof.

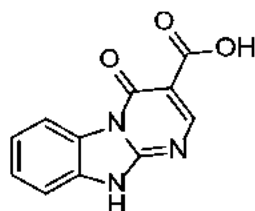
11. An MrgX2 antagonist selected from compounds

(II) B-31 to B-66 and the physiologically acceptable salts thereof:

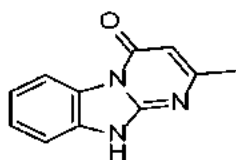
B-31



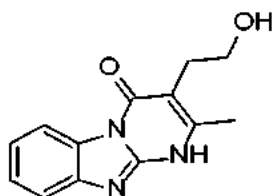
B-32



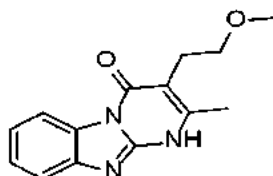
B-33



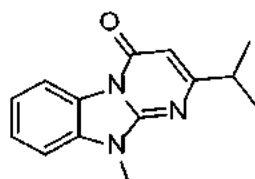
B-34



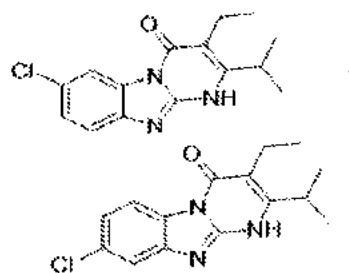
B-35



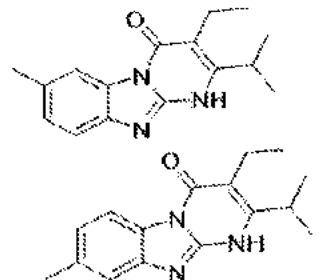
B-36



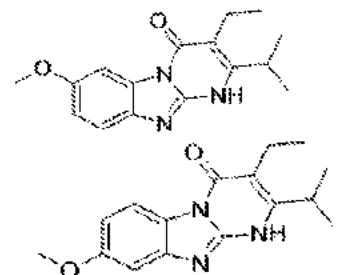
B-49
preferably
ca. 1: 1



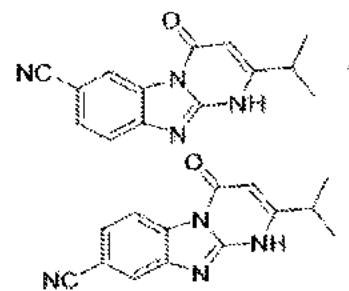
B-50



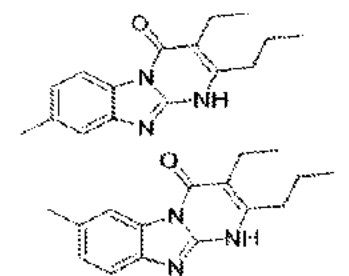
B-51
preferably
ca. 1: 1.5



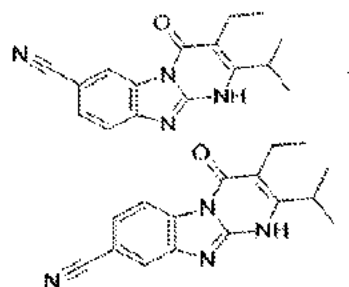
B-52



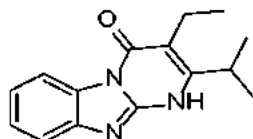
B-53



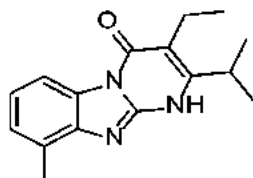
B-54
preferably
ca. 1.5: 1



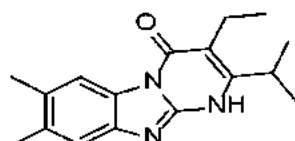
B-37



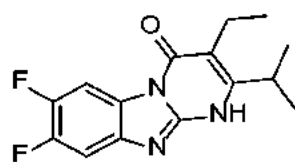
B-38



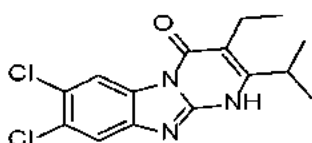
B-39



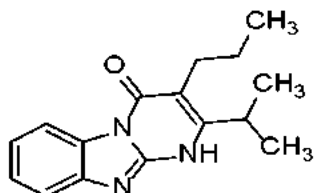
B-40



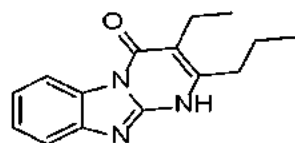
B-41



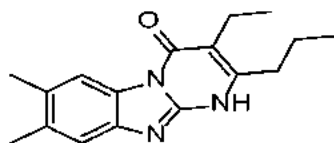
B-42



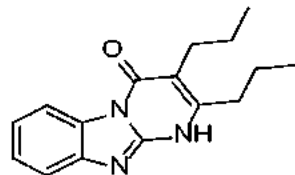
B-43



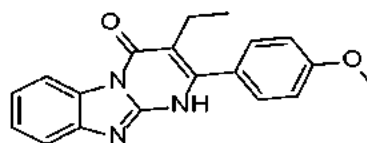
B-44



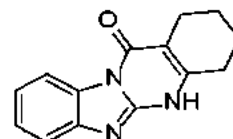
B-45



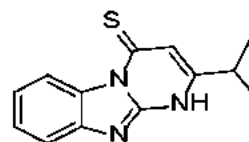
B-46



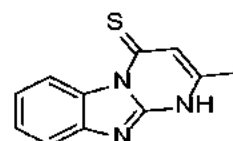
B-55



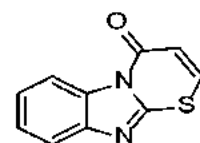
B-56



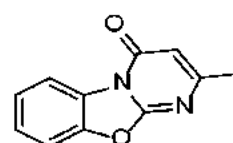
B-57



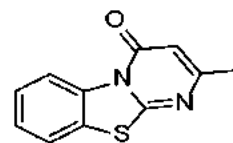
B-58



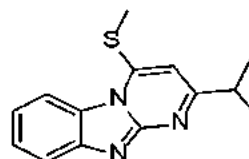
B-59



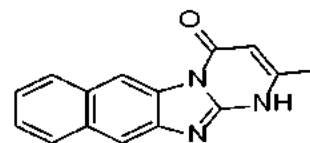
B-60



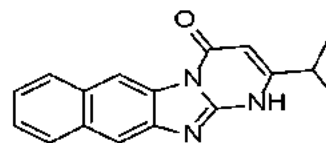
B-61



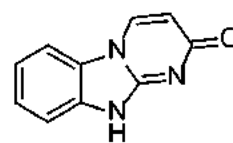
B-62



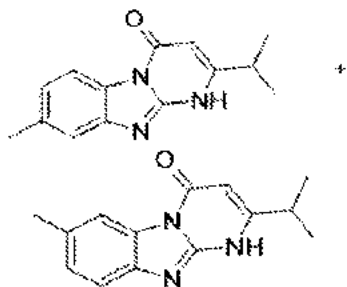
B-63



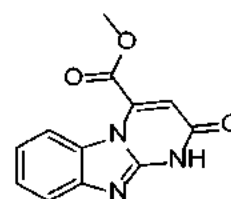
B-64



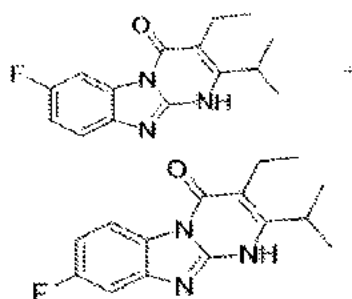
B-47



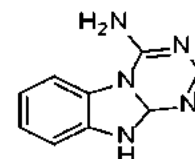
B-65



B-48
preferably ca.
1: 2



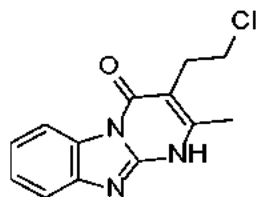
B-66



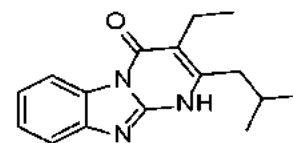
and

(III) C-67 to C-87 and the physiologically acceptable salts thereof.

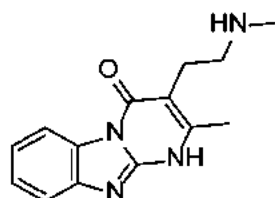
C-67



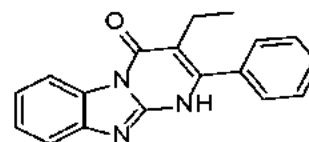
C-78



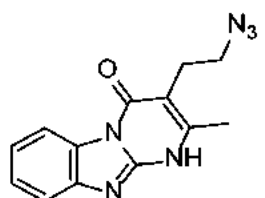
C-68



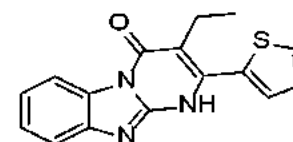
C-79



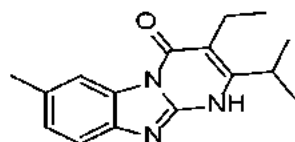
C-69



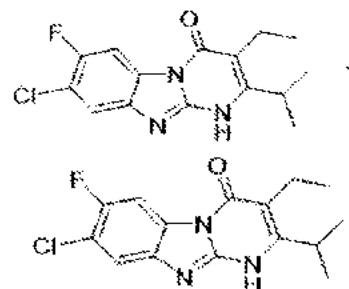
C-80



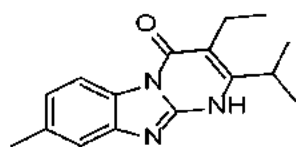
C-70



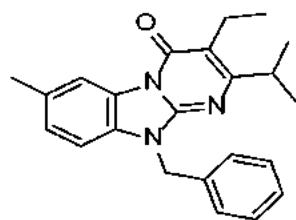
C-81



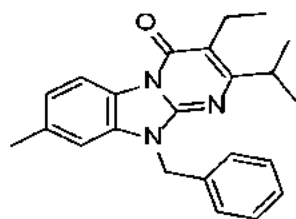
C-71



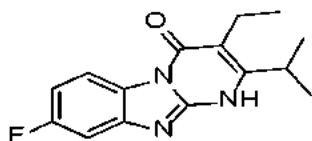
C-72



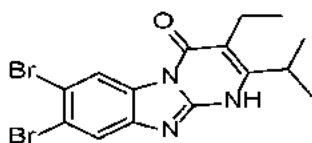
C-73



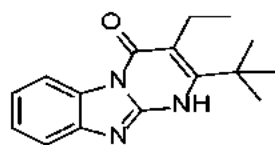
C-74



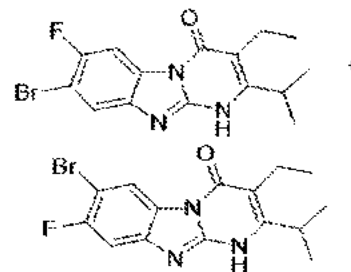
C-75



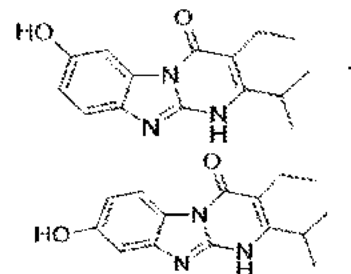
C-76



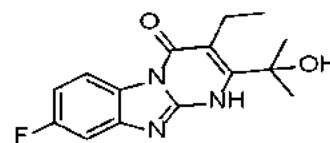
C-82



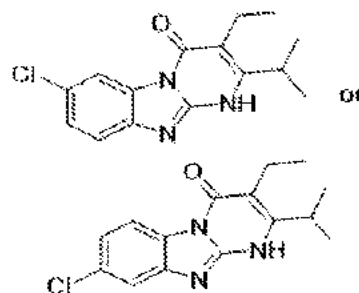
C-83



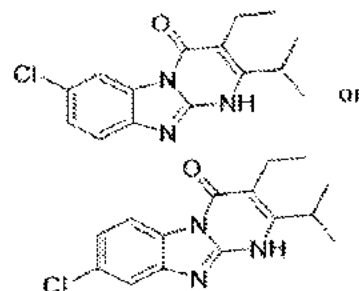
C-84



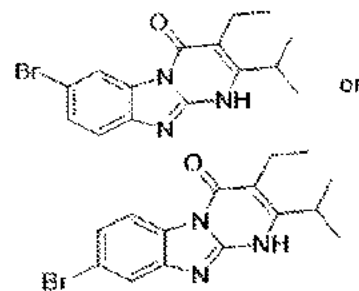
C-84*



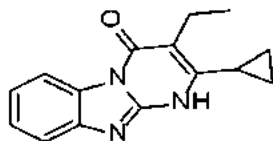
C-85



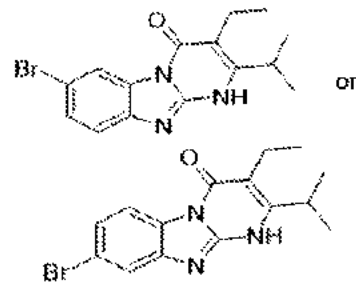
C-86



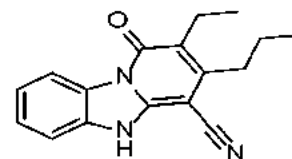
C-77



C-87



C-88



12. A pharmaceutical composition comprising an MrgX2 antagonist according to claim 10 or 11 and a physiologically acceptable excipient.
13. A pharmaceutical dosage form comprising an MrgX2 antagonist according to claim 10 or 11 or a pharmaceutical composition according to claim 12.
14. The pharmaceutical dosage form according to claim 13, which is selected from tablets and capsules.

Abstract:

The invention relates to a method for preventing or treating a disease or disorder that is associated with the MrgX2 receptor. The invention also relates to MrgX2 antagonists and physiologically acceptable salts thereof. The invention also relates to pharmaceutical compositions and dosage forms comprising an MrgX2 antagonist.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US
		Application Number	
Title of Invention	MRGX Receptor Antagonists		
<p>The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.</p>			

Secrecy Order 37 CFR 5.2:

Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor	1				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
Dr. ▾	Christa		Müller	▾	
Residence Information (Select One) US Residency ● Non US Residency Active US Military Service					
City	Bonn		Country of Residence ⁱ	DE	
Mailing Address of Inventor:					
Address 1		Buschstraße 52			
Address 2					
City	Bonn		State/Province		
Postal Code	D-53121	Country ⁱ	DE		
Inventor	2				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
Dr. ▾	Wessam		Alnouri	▾	
Residence Information (Select One) US Residency ● Non US Residency Active US Military Service					
City	Niederau		Country of Residence ⁱ	DE	
Mailing Address of Inventor:					
Address 1		Drosselweg 11			
Address 2					
City	Niederau		State/Province		
Postal Code	61130	Country ⁱ	DE		
Inventor	3				Remove
Legal Name					

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US
		Application Number	
Title of Invention	MRGX Receptor Antagonists		

Prefix	Given Name	Middle Name	Family Name	Suffix
	Yvonne		Riedel	
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Bonn	Country of Residence ⁱ	DE	

Mailing Address of Inventor:				
Address 1	Alte Bonner Str. 39			
Address 2				
City	Bonn	State/Province		
Postal Code	53229	Country ⁱ	DE	
Inventor	4	<input type="button" value="Remove"/>		
Legal Name				

Prefix	Given Name	Middle Name	Family Name	Suffix
Dr.	Dominik		Thimm	
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Bonn	Country of Residence ⁱ	DE	

Mailing Address of Inventor:				
Address 1	Wielstr. 10			
Address 2				
City	Bonn	State/Province		
Postal Code	53115	Country ⁱ	DE	
Inventor	5	<input type="button" value="Remove"/>		
Legal Name				

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniel		Marx	
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Linz am Rhein	Country of Residence ⁱ	DE	

Mailing Address of Inventor:				
Address 1	In der Au 77			
Address 2				
City	Linz am Rhein	State/Province		
Postal Code	53545	Country ⁱ	DE	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US
		Application Number	
Title of Invention	MRGX Receptor Antagonists		

Inventor	6	<input type="button" value="Remove"/>		
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
Dr. ▾	Vigneshwaran		Namasivayam	▾
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Bonn	Country of Residence ⁱ	DE	

Mailing Address of Inventor:

Address 1	Bunispechtweg 22a			
Address 2				
City	Bonn	State/Province		
Postal Code	53123	Country ⁱ	DE	

Inventor	7	<input type="button" value="Remove"/>		
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
▾	Susanne		Gatner	▾
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Bonn	Country of Residence ⁱ	DE	

Mailing Address of Inventor:

Address 1	Oppelner Str. 45			
Address 2				
City	Bonn	State/Province		
Postal Code	53119	Country ⁱ	DE	

Inventor	8	<input type="button" value="Remove"/>		
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
Dr. ▾	Piet		Herdewyn	▾
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Heverlee	Country of Residence ⁱ	BE	

Mailing Address of Inventor:

Address 1	W De Croylaan 35			
Address 2				

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US
		Application Number	
Title of Invention	MRGX Receptor Antagonists		

City	Heverlee		State/Province	
Postal Code	3001	Country ⁱ	BE	
Inventor	9			<input type="button" value="Remove"/>
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
Dr. <input type="checkbox"/>	Steven		De Jonghe	<input type="checkbox"/>
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Tervuren		Country of Residence ⁱ	BE

Mailing Address of Inventor:				
Address 1	Olmenstraat 23			
Address 2				
City	Tervuren		State/Province	
Postal Code	3080	Country ⁱ	BE	
Inventor	10			<input type="button" value="Remove"/>
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
<input type="checkbox"/>	Piotr		Leonczak	<input type="checkbox"/>
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Leuven		Country of Residence ⁱ	BE

Mailing Address of Inventor:				
Address 1	Tiensevest 58/A/303			
Address 2				
City	Leuven		State/Province	
Postal Code	3000	Country ⁱ	BE	
Inventor	11			<input type="button" value="Remove"/>
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
<input type="checkbox"/>	Sven		Verdonck	<input type="checkbox"/>
Residence Information (Select One) US Residency <input checked="" type="radio"/> Non US Residency Active US Military Service				
City	Diest		Country of Residence ⁱ	BE

Mailing Address of Inventor:				
-------------------------------------	--	--	--	--

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US	
		Application Number		
Title of Invention	MRGX Receptor Antagonists			

Address 1	Galgeveldstraat 42			
Address 2				
City	Diest	State/Province		
Postal Code	3293	Country	BE	
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.				<input type="button" value="Add"/>

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).					
<input type="checkbox"/> An Address is being provided for the correspondence information of this application.					
Customer Number	25215				
Email Address	patmail@patentco.com	<input type="button" value="Add Email"/>	<input type="button" value="Remove Email"/>		

Application Information:

Title of the Invention	MRGX Receptor Antagonists			
Attorney Docket Number	2235.001US	Small Entity Status Claimed	<input type="checkbox"/>	
Application Type	Nonprovisional			
Subject Matter	Utility			
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)		

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

<input type="checkbox"/> Request Early Publication (Fee required at time of Request 37 CFR 1.219)
<input type="checkbox"/> Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	2235.001US
	Application Number	
Title of Invention	MRGX Receptor Antagonists	

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	<input checked="" type="radio"/> Customer Number	US Patent Practitioner	<input type="radio"/> Limited Recognition (37 CFR 11.9)
Customer Number	25215		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	<input type="text"/>	<input type="button" value="Remove"/>
Application Number	Continuity Type	Prior Application Number
<input type="text"/>	<input type="text"/>	<input type="text"/>
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.		<input type="button" value="Add"/>

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)	<input type="button" value="Remove"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	
Additional Foreign Priority Data may be generated within this form by selecting the Add button.				<input type="button" value="Add"/>

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	2235.001US
	Application Number	
Title of Invention	MRGX Receptor Antagonists	

<input type="checkbox"/> This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US
		Application Number	
Title of Invention	MRGX Receptor Antagonists		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	2235.001US
	Application Number	
Title of Invention	MRGX Receptor Antagonists	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant	1	<input type="button" value="Remove"/>
<p>If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.</p>		
<input type="button" value="Clear"/>		
Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
<input type="radio"/> Person to whom the inventor is obligated to assign.	<input type="radio"/> Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:		
<div style="border: 1px solid black; height: 20px; width: 100%;"></div>		
Name of the Deceased or Legally Incapacitated Inventor: <input style="width: 90%;" type="text"/>		
If the Applicant is an Organization check here. <input checked="" type="checkbox"/>		
Organization Name	Rheinische-Friedrich-Wilhelms-Universität Bonn	
Mailing Address Information For Applicant:		
Address 1	Regina-Pacis-Weg 3	
Address 2		
City	Bonn	State/Province
Country	DE	Postal Code
Phone Number		Fax Number
Email Address		
Additional Applicant Data may be generated within this form by selecting the Add button. <input type="button" value="Add"/>		

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	2235.001US
	Application Number	
Title of Invention	MRGX Receptor Antagonists	

Applicant	2	<input type="button" value="Remove"/>
<p>If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.</p>		
<input type="button" value="Clear"/>		
Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
<input checked="" type="radio"/> Person to whom the inventor is obligated to assign.		Person who shows sufficient proprietary interest
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:		
▼		
Name of the Deceased or Legally Incapacitated Inventor: <input type="text"/>		
If the Applicant is an Organization check here. <input checked="" type="checkbox"/>		
Organization Name	Katholieke Universiteit Leuven	
Mailing Address Information For Applicant:		
Address 1	KU Leuven Research & Development, Waaistraat 6	
Address 2	Box 5105	
City	Leuven	State/Province
Country	BE	Postal Code
Phone Number		Fax Number
Email Address		
Additional Applicant Data may be generated within this form by selecting the Add button. <input type="button" value="Add"/>		

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee	1	<input type="button" value="Remove"/>
<p>Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.</p>		
348		
If the Assignee or Non-Applicant Assignee is an Organization check here. <input type="checkbox"/>		

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	2235.001US
		Application Number	
Title of Invention	MRGX Receptor Antagonists		

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a juristic entity (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Kristen L. Pursley/		Date (YYYY-MM-DD)	2019-10-31	
First Name	Kristen L.	Last Name	Pursley	Registration Number	61161

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Christa Müller

Group Art Unit:

Serial No.: To be assigned

Examiner:

Filed: Herewith

For: MRGX Receptor Antagonists

Attorney Docket No.: 2235.001US

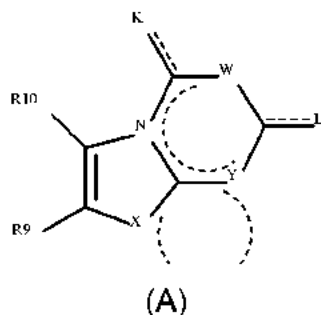
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Prior to the examination of the present application, please amend the application as follows.

AMENDMENTS TO CLAIMS

Claim 1 (Original): A method for preventing or treating a disease or disorder that is associated with the MrgX2 receptor comprising administering to a subject in need thereof a therapeutically effective amount of an MrgX2 antagonist according to general formula (A)



wherein

K means =O or =S or =NH, **W** means -CR1=, and **L** means -R2; or

K means -R2, **W** means -CR1=, and **L** means =O; or

K means -R1, **W** means -N=, and **L** means -R2; or

K means -R1, **W** means -CH=, and **L** means -R2;

X means -N= and **Y** means -NR3-; or

X means -NR4- and **Y** means -N=; or

X means -NR4- and **Y** means -CR0=; or

X means -N= and **Y** means -S-; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=;

R0, **R1**, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl, -NH₂, -S-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

R9 and **R10** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R9** and **R10** together with the carbon atoms to which they are attached form a phenyl ring optionally substituted with **R5**, **R6**, **R7** and **R8**;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

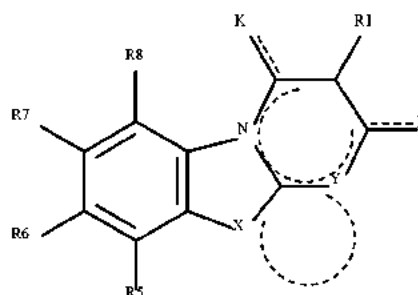
or a physiologically acceptable salt thereof.

Claim 2 (Original): The method according to claim 1, wherein the disease or disorder that is associated to the MrgX2 receptor is selected from any of the groups consisting of

- pain, especially acute, nociceptive, neuropathic or chronic pain, inflammatory pain or itch; and/or

- anxiety, stress and stress-associated syndromes, depression, epilepsy, Alzheimer's disease, senile dementia, general cognitive dysfunctions, learning and memory disorders (as a nootropic), withdrawal symptoms, alcohol and/or drug and/or medicament abuse and/or dependency, sexual dysfunctions, cardiovascular diseases, hypotension, hypertension, tinnitus, pruritus, migraine, impaired hearing, deficient intestinal motility, impaired food intake, anorexia, obesity, locomotor disorders, diarrhoea, cachexia, urinary incontinence or as a muscle relaxant, anticonvulsive or anaesthetic or for co-administration in the case of treatment with an opioid analgesic or with an anaesthetic, for diuresis or antinatriuresis, anxiolysis, for modulation of motor activity, for modulation of neurotransmitter secretion and treatment of neurodegenerative diseases associated therewith, for the treatment of withdrawal symptoms and/or for reducing the addictive potential of opioids; and/or
- asthma, urticaria, skin inflammation, dry skin, atopic eczema, psoriasis, urticaria, scabies, non-allergic hypersensitivity reactions, fibrosis and itch.

Claim 3 (Original): The method according to claim 1, wherein the MrgX2 antagonist is according to general formula (B)



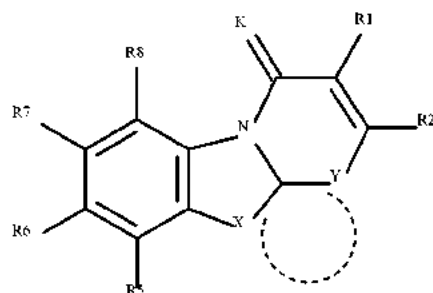
(B)

wherein

K means =O or =S, and **L** means -R2; or

K means **-R2** and **L** means =O; and
X means **-N=** and **Y** means **-NR3-**; or
X means **-NR4-** and **Y** means **-N=**; or
X means **-NR4-** and **Y** means **-CR0=**; or
X means **-N=** and **Y** means **S**; or
X means **-O-** and **Y** means **-N=**; or
X means **-S-** and **Y** means **-N=**; or
X means **-N=** and **Y** means **-N=**.

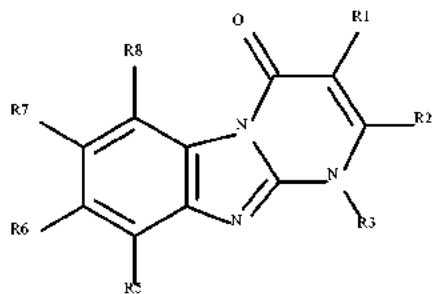
Claim 4 (Original): The method according to claim 1, wherein the MrgX2 antagonist is according to general formula (C)



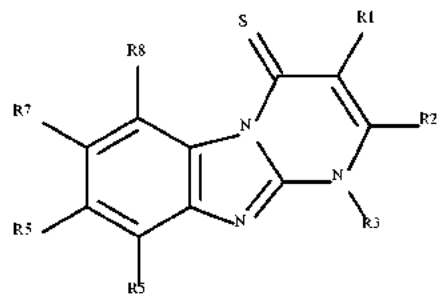
(C)

wherein **K** means =O or =S; and
X means **-N=** and **Y** means **-NR3-**; or
X means **-NR4-** and **Y** means **-N=**; or
X means **-NR4-** and **Y** means **-CR0=**; or
X means **-N=** and **Y** means **S**; or
X means **-O-** and **Y** means **-N=**; or
X means **-S-** and **Y** means **-N=**; or
X means **-N=** and **Y** means **-N=**.

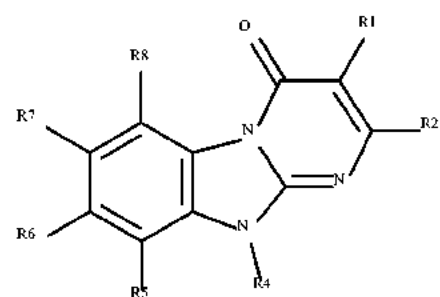
Claim 5 (Original): The method according to claim 1, wherein the MrgX2 antagonist is selected from the group consisting of compounds according to general formula (I), (II), (III), (IV), (V), (VI), (VII), (VIII), (IX), (X), (XI), and (XII):



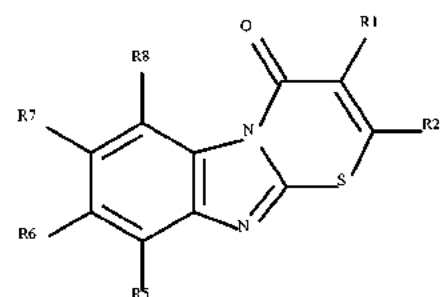
(I)



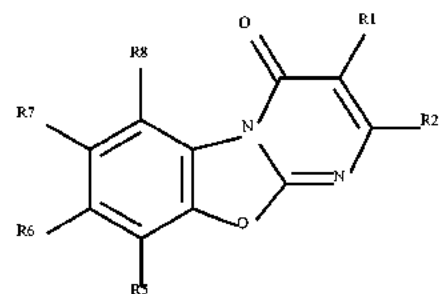
(II)



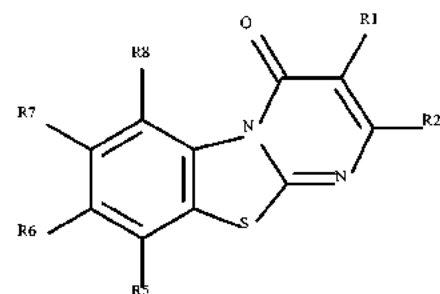
(III)



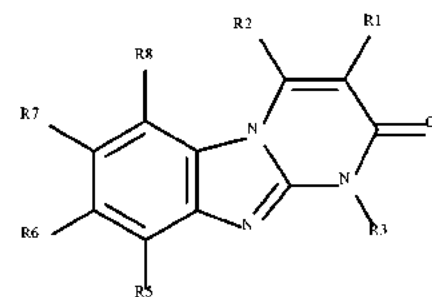
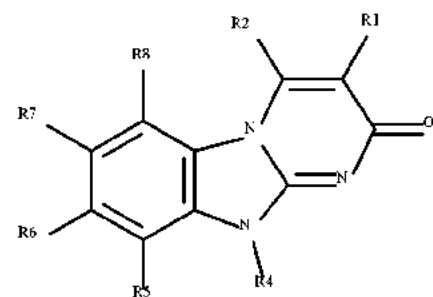
(IV)



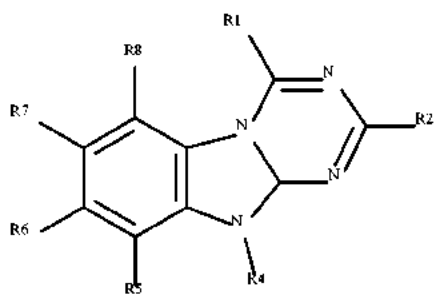
(V)



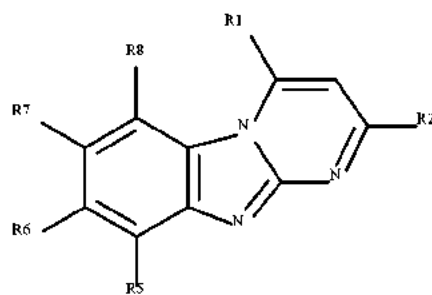
(VI)



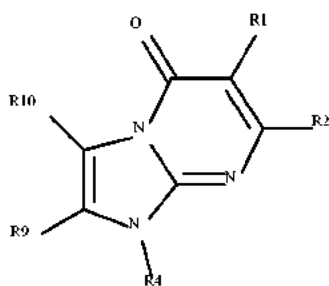
(VII)



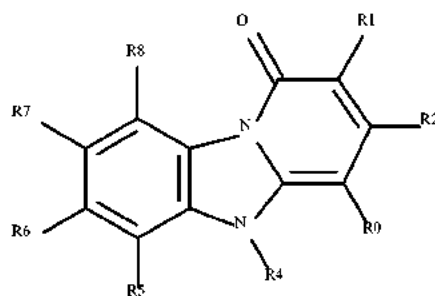
(VIII)



(IX)



(X)



(XI)

(XII)

Claim 6 (Original): The method according to claim 1, wherein

R0 means -H or -CN;

R1 means -H, -CH₃, -CH₂CH₃, -CH₂CH₂CH₃, -CH₂CH₂CH₂CH₃, -CH₂CH₂CH(CH₃)₂, -CH₂CH₂C(=O)OCH₂CH₃, -CH₂CH₂-CN, -CH₂CH₂-Cl, -CH₂CH₂-OH, -CH₂CH₂-OCH₃, -CH₂CH₂-N₃, -CH₂CH₂-NHCH₃, -C(=O)OH, -C(=O)OCH₃, -C(=O)OCH₂CH₃, -CH₂-phenyl with phenyl being unsubstituted, -CH₂CH₂-phenyl with phenyl being unsubstituted, -S-CH₃ or -NH₂;

and/or

R2 means -H, -CH₃, -CH₂CH₂CH₃, -CH(CH₃)₂, -CH₂CH(CH₃)₂, -C(CH₃)₃, -cyclopropyl, -phenyl with phenyl being unsubstituted, -para-methoxyphenyl, -2-thienyl, -CH₂C(=O)OH, -C(=O)OCH₃, -CH₂C(=O)OCH₃, -CH₂C(=O)OCH₂CH₃, -C(CH₃)₂(OH), -CH₂OCH₃, -SCH₃, -NH₂ or -OH;

or

R1 and **R2** together with the atoms to which they are attached form a cyclohexyl ring and mean -CH₂CH₂CH₂CH₂-;

and/or

R3 means -H, -CH₃, -CH₂CH₂CH₃, -CH₂-phenyl, -CH₂-*p*-chlorophenyl or -CH₂-CN;

and/or

R4 means -H, -CH₃ or -CH₂-phenyl with phenyl being unsubstituted;

and/or

R9 means -H; and/or **R10** means -H; or **R9** and **R10** together with the carbon atoms to which they are attached form a phenyl ring optionally substituted with **R5**, **R6**, **R7** and **R8**;

wherein

R5, **R6**, **R7** and **R8** independently of one another mean -H, -CH₃, -OH, -OCH₃, -F, -Cl, -Br or -CN, and preferably

R5 means -H or -CH₃; and/or

R6 means -H, -CH₃, -OH, -OCH₃, -F, -Cl, -Br or -CN; and/or

R7 means -H, -CH₃, -F, -Cl, -Br, -CN, -OCH₃ or -OH; and/or

R8 means -H;

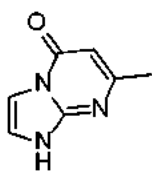
or

R6 and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring.

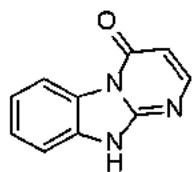
Claim 7 (Original): The method according to claim 1, wherein the MrgX2 antagonist is selected from compounds

(I) A-1 to A-30 and the physiologically acceptable salts thereof:

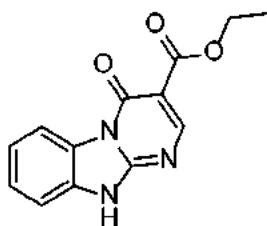
A-1)



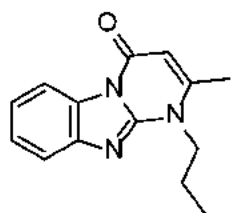
A-2



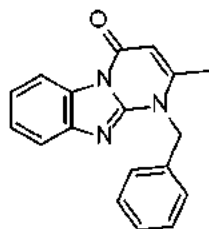
A-3



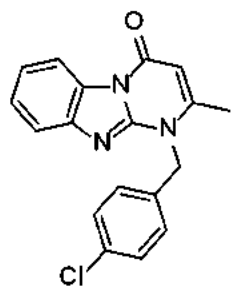
A-4



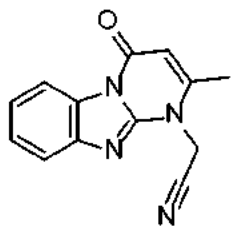
A-5



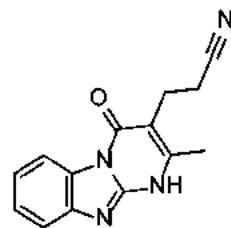
A-6



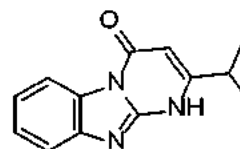
A-7



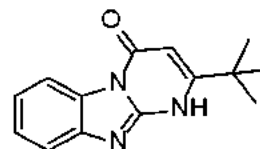
A-16



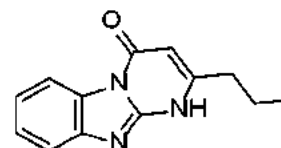
A-17



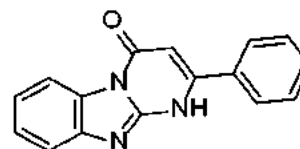
A-18



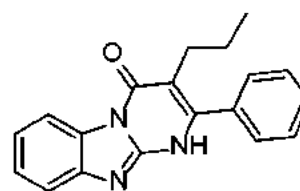
A-19



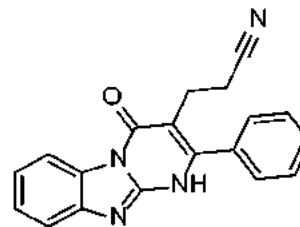
A-20

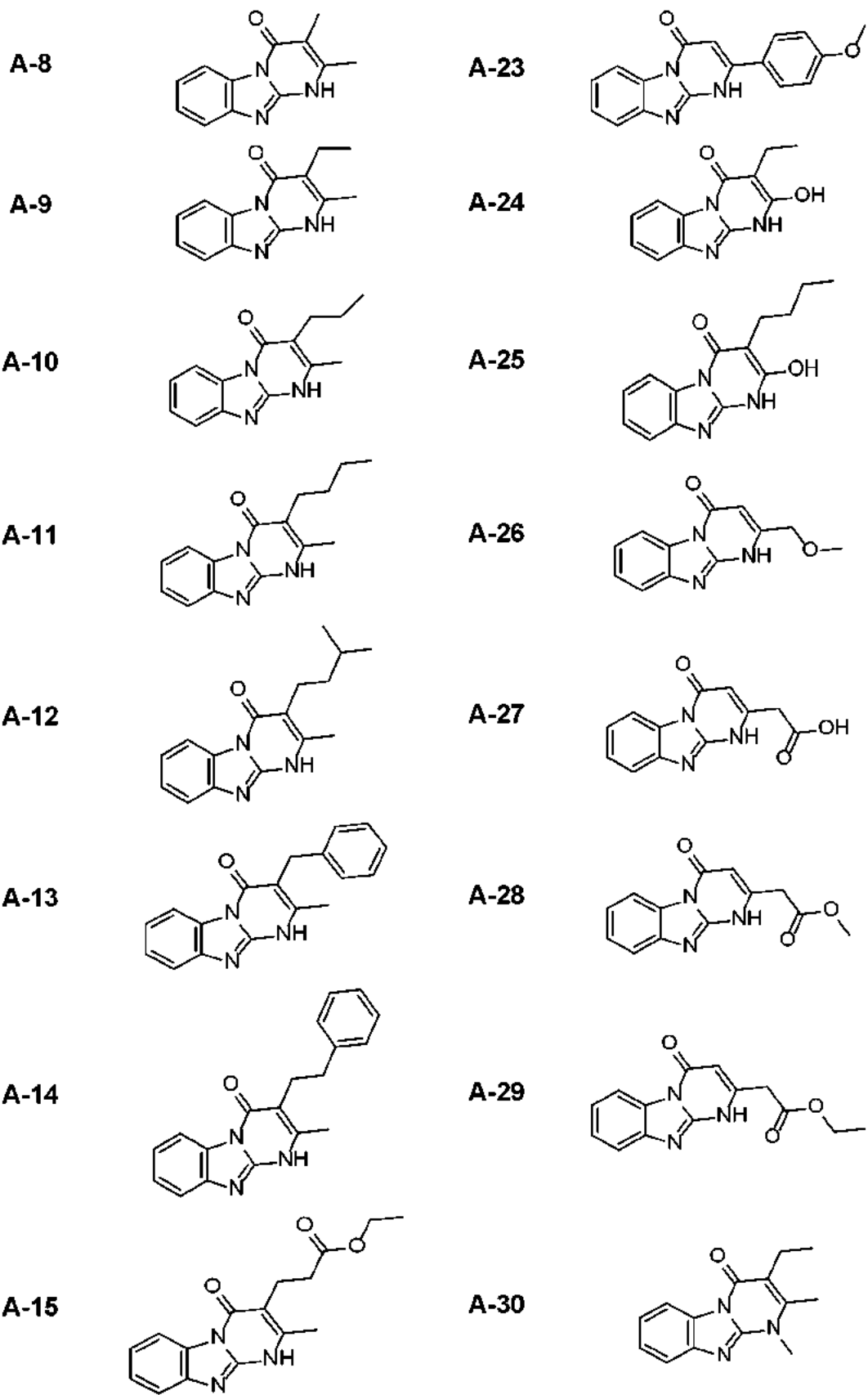


A-21



A-22

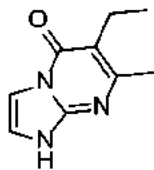




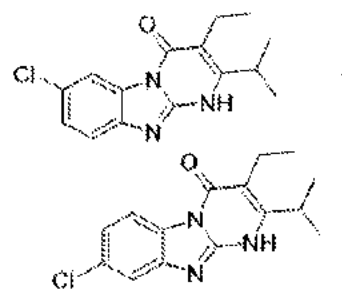
and

(II) B-31 to B-66 and the physiologically acceptable salts thereof:

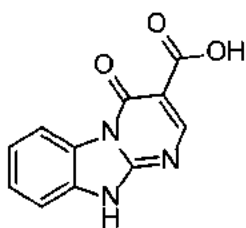
B-31



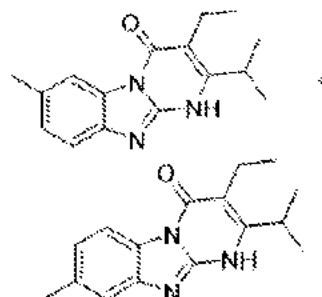
B-49
preferably
ca. 1: 1



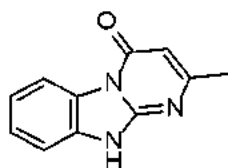
B-32



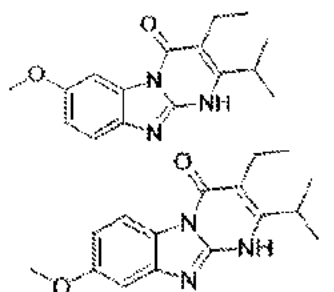
B-50



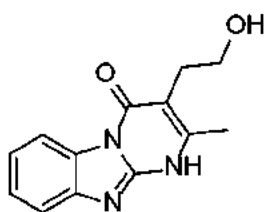
B-33



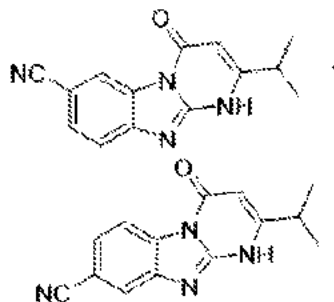
B-51
preferably
ca. 1: 1.5



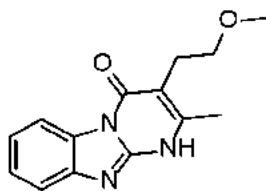
B-34



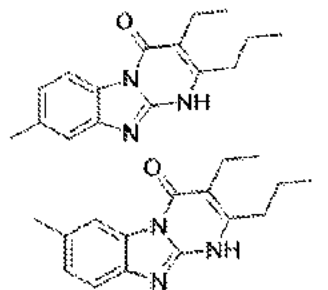
B-52



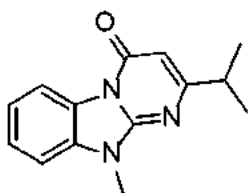
B-35



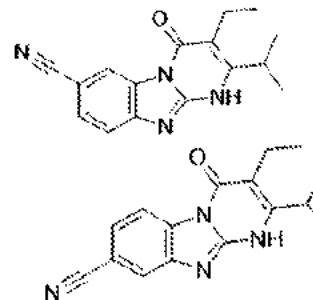
B-53



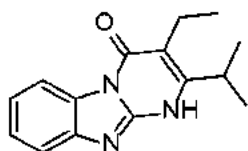
B-36



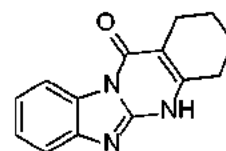
B-54
preferably
ca. 1.5: 1



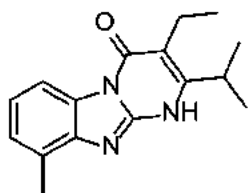
B-37



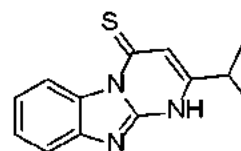
B-55



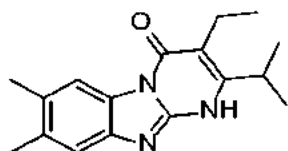
B-38



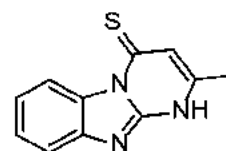
B-56



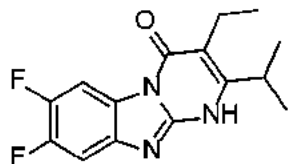
B-39



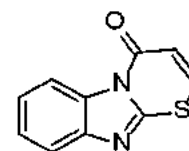
B-57



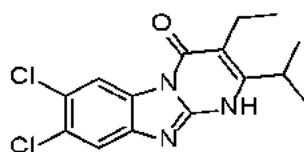
B-40



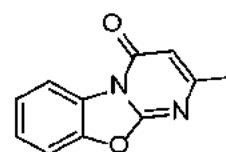
B-58



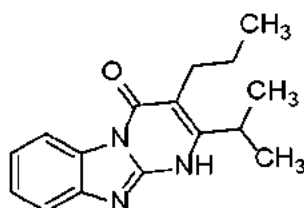
B-41



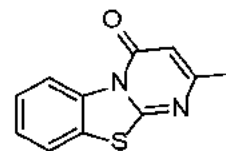
B-59



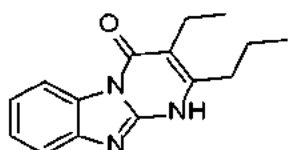
B-42



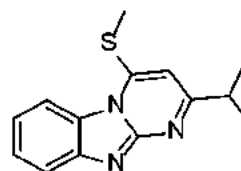
B-60

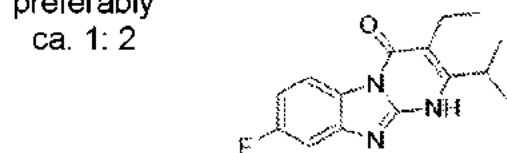
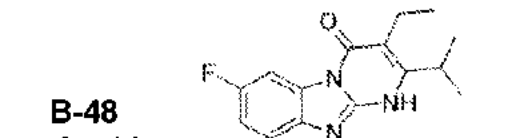
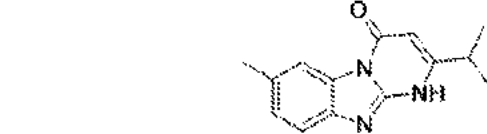
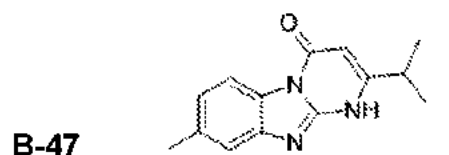
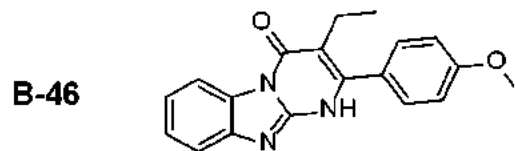
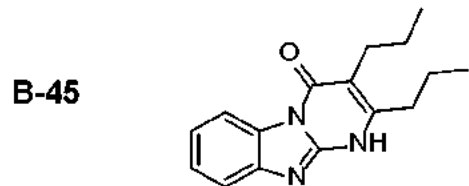
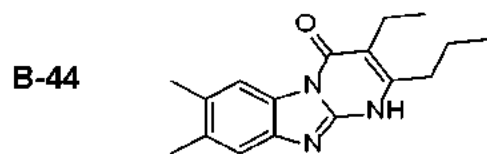


B-43



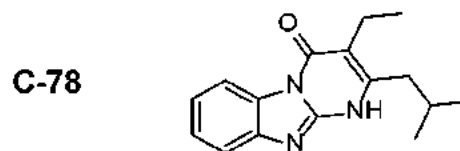
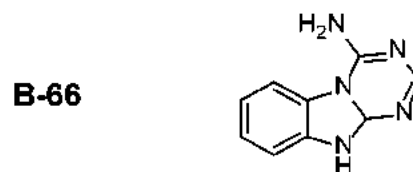
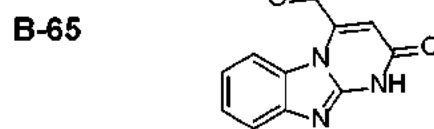
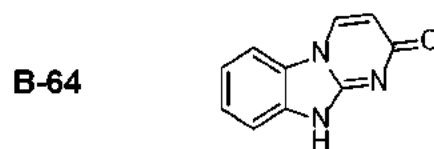
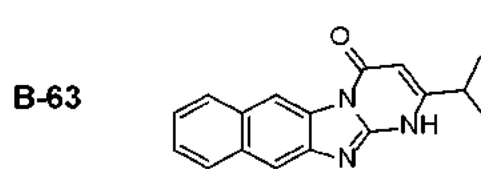
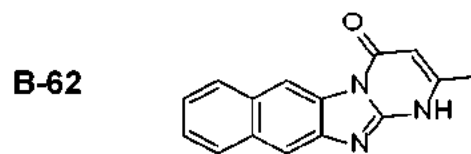
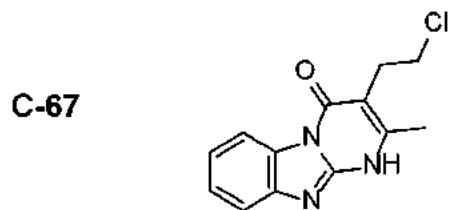
B-61

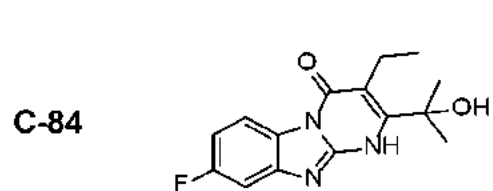
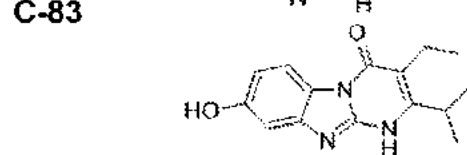
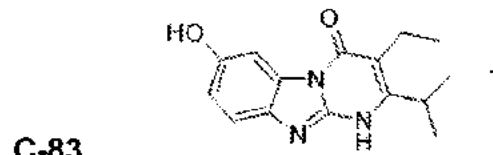
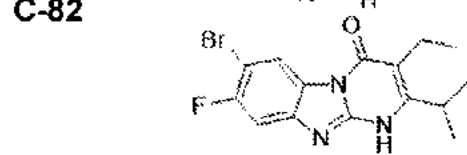
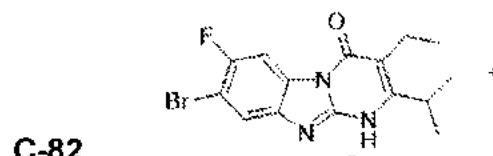
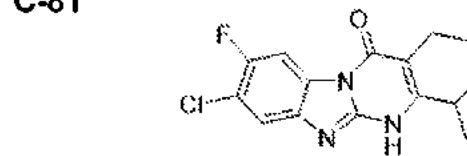
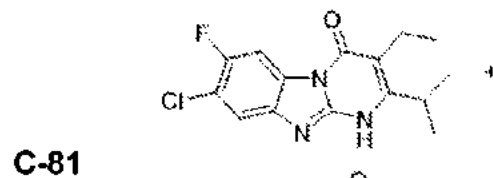
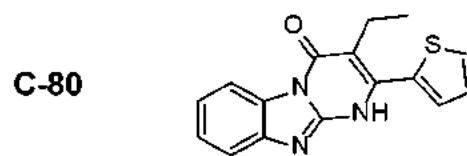
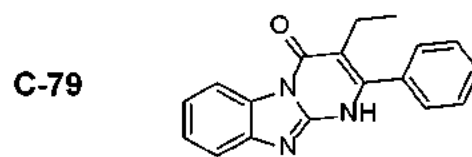
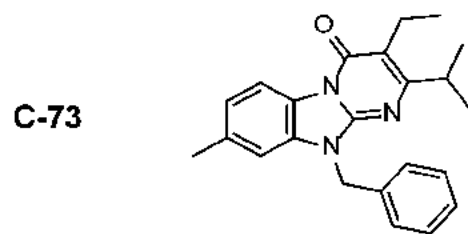
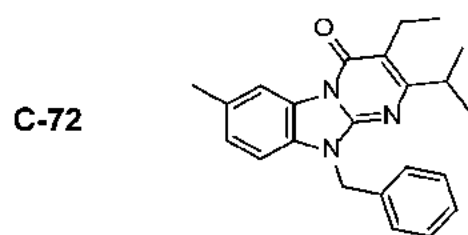
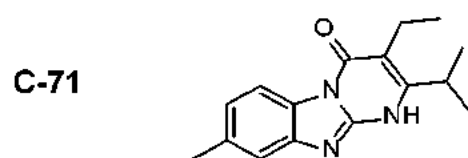
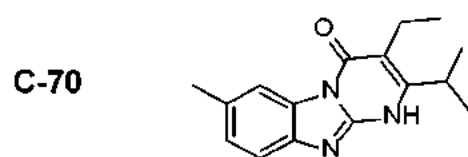
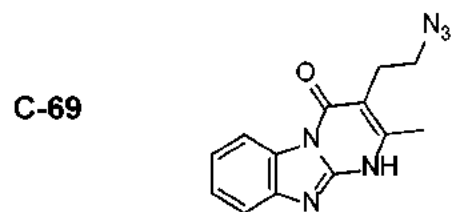
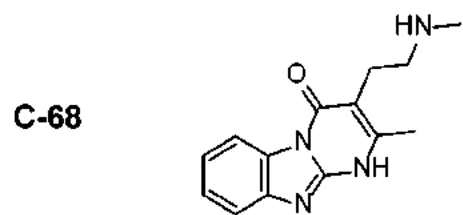




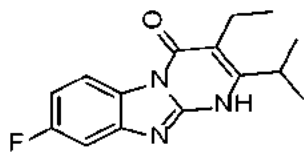
and

(III) C-67 to C-87 and the physiologically acceptable salts thereof:

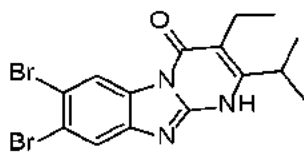




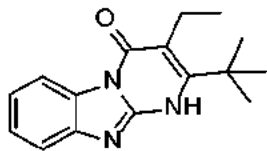
C-74



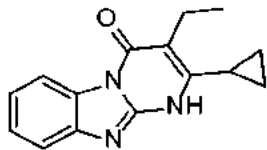
C-75



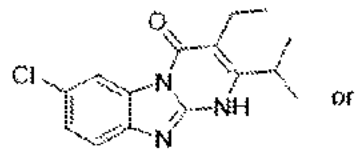
C-76



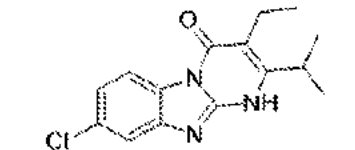
C-77



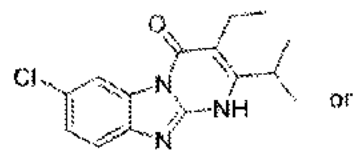
C-84*



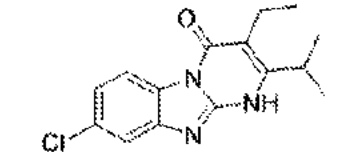
C-85



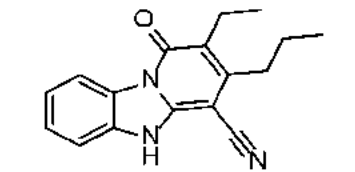
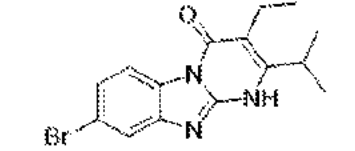
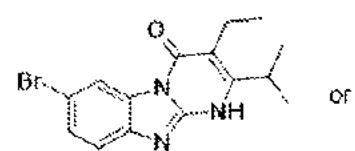
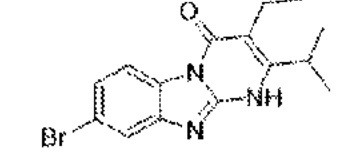
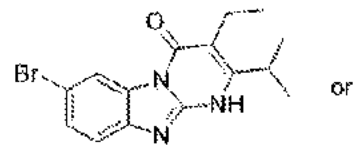
C-86



C-87



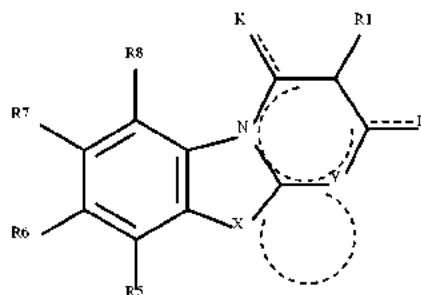
C-88



Claim 8 (Original): The method according to claim 1, wherein the MrgX2 antagonist is administered orally.

Claim 9 (Original): The method according to claim 1, wherein the MrgX2 antagonist is administered once daily, twice daily or thrice daily.

Claim 10 (Original): An MrgX2 antagonist according to general formula (B)



(B)

wherein

K means =O or =S, and **L** means -**R2**; or

K means -**R2** and **L** means =O; and

X means -N= and **Y** means -**NR3**-; or

X means -**NR4**- and **Y** means -N=; or

X means -N= and **Y** means S; or

X means -O- and **Y** means -N=; or

X means -S- and **Y** means -N=; or

X means -N= and **Y** means -N=.

R1, **R2**, **R3**, **R4** independently of one another mean -H; -C₁₋₆-alkyl; -C₁₋₆-cycloalkyl; -phenyl; -C₁₋₆-alkyl-phenyl; -heteroaryl selected from the group consisting of thienyl, furanyl and pyrrolyl; -C(=O)OH; -C(=O)O-C₁₋₆-alkyl; -CN; -OH; -O-C₁₋₆-alkyl; -F, -Cl, -Br, or -I; or **R1** and **R2** together with the atoms to which they are attached form a six membered saturated unsubstituted alicyclic ring;

wherein **R5**, **R6**, **R7** and **R8** independently of one another mean -H, -C₁₋₆-alkyl, -CN, -OH, -O-C₁₋₆-alkyl, -F, -Cl, -Br, -I; or **R6** and **R7** together with the carbon atoms to which they are attached form an unsubstituted phenyl ring;

wherein in each case the "C₁₋₆-alkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "C₁₋₆-cycloalkyl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "phenyl" may be unsubstituted, mono- or disubstituted with a substituent independently selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

wherein in each case the "heteroaryl" may be linear or branched, saturated or unsaturated, unsubstituted or monosubstituted with a substituent selected from -CN, -C(=O)OH, -C(=O)O-C₁₋₆-alkyl, -OH, -O-C₁₋₆-alkyl, -NH₂, -NH-C₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -N₃, -F, -Cl, -Br, -I;

with the proviso that

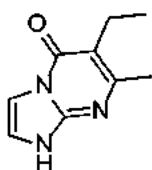
- at least one of **R5**, **R6**, **R7** and **R8** does not mean -H; and/or
- at least three of **R1**, **R2**, **R3**, **R4**, **R5**, **R6**, **R7** and **R8** do not mean -H; and/or
- A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29 and/or A-30 are not included;

or a physiologically acceptable salt thereof.

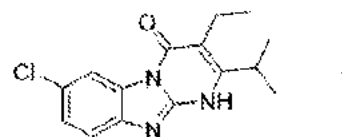
Claim 11 (Original): An MrgX2 antagonist selected from compounds

(II) B-31 to B-66 and the physiologically acceptable salts thereof:

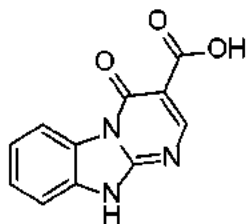
B-31



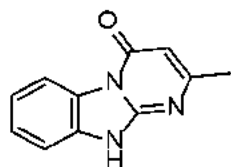
B-49
preferably
ca. 1: 1



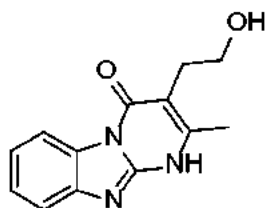
B-32



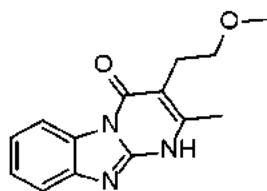
B-33



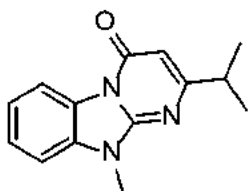
B-34



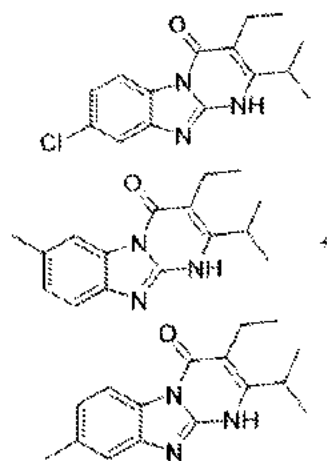
B-35



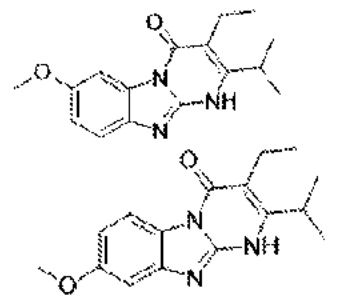
B-36



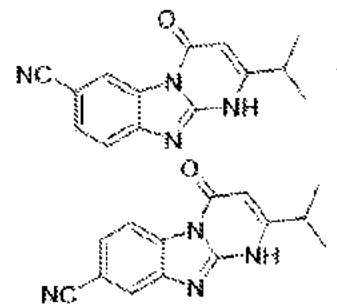
B-50



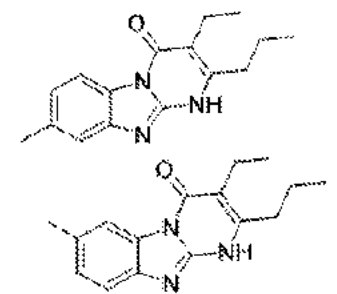
B-51
preferably
ca. 1: 1.5



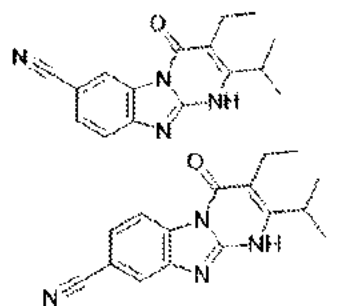
B-52

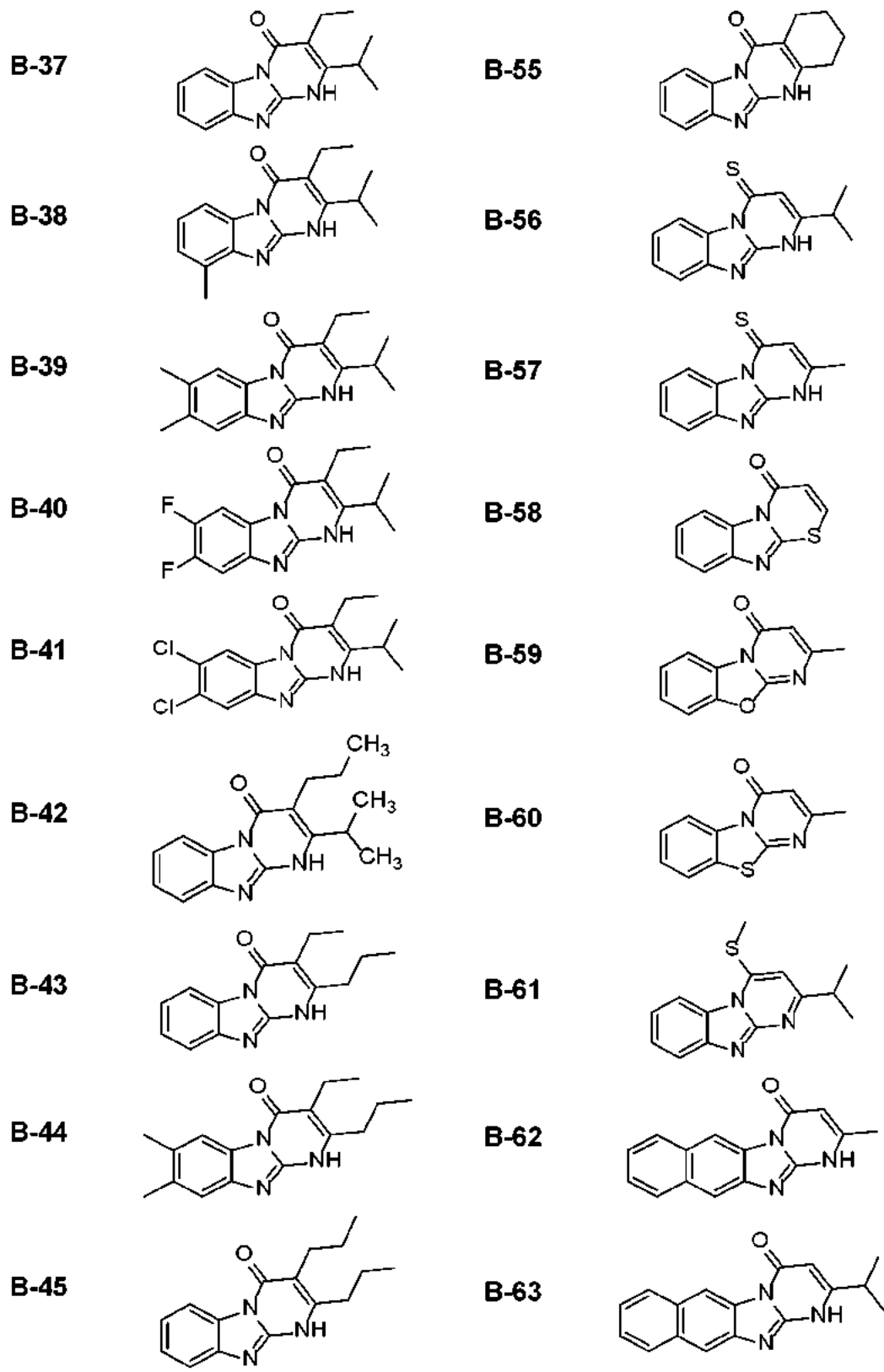


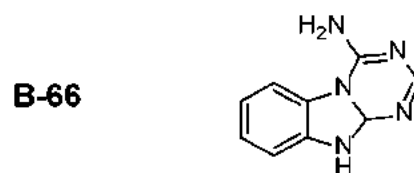
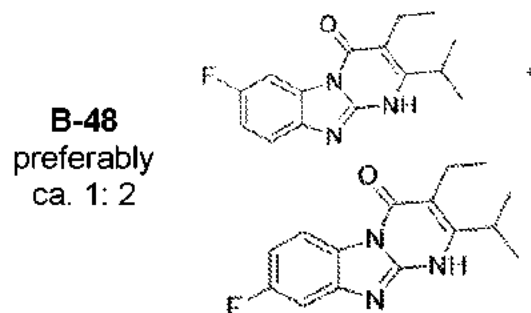
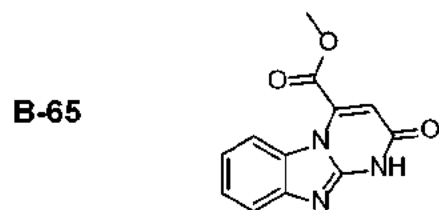
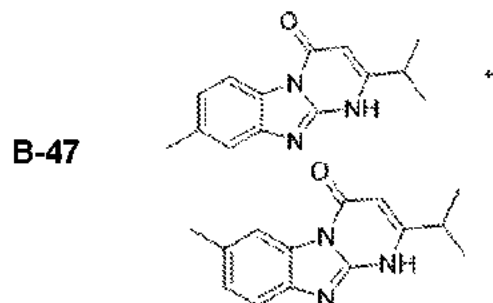
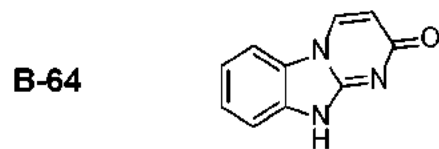
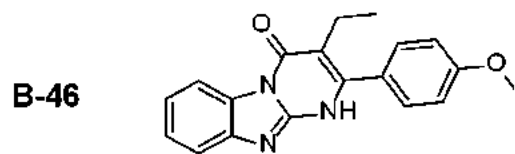
B-53



B-54
preferably
ca. 1.5: 1

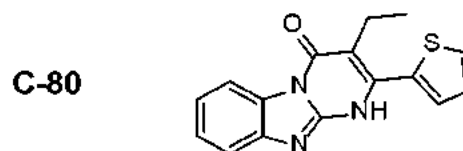
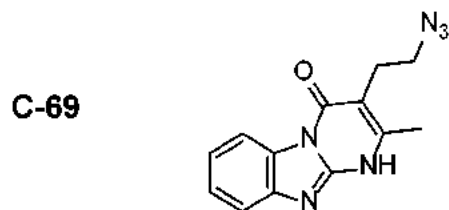
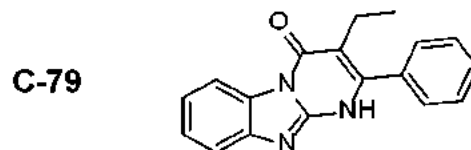
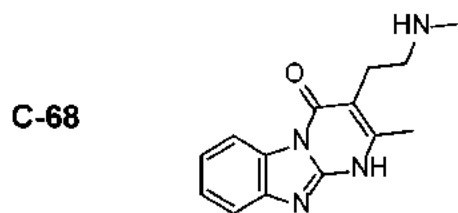
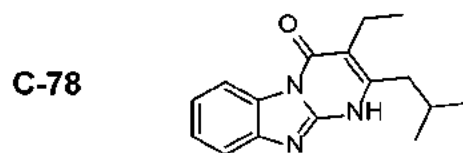
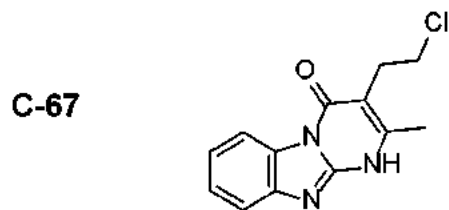




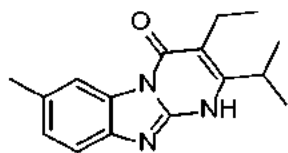


and

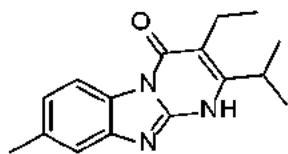
(III) C-67 to C-87 and the physiologically acceptable salts thereof:



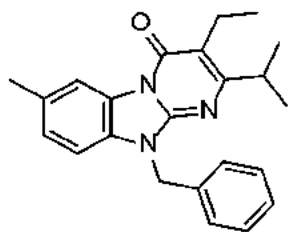
C-70



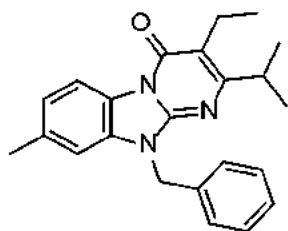
C-71



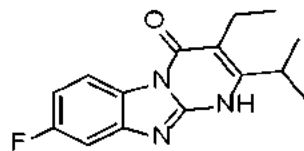
C-72



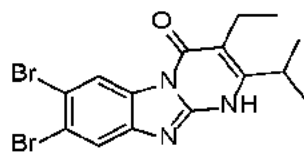
C-73



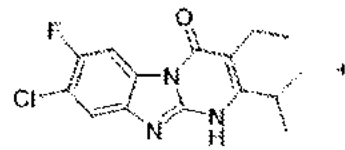
C-74



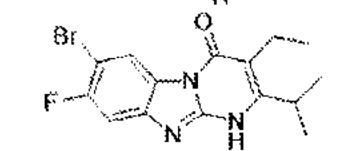
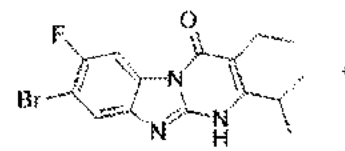
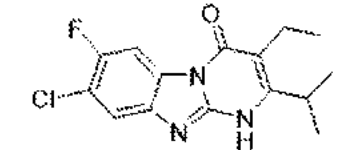
C-75



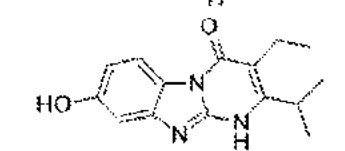
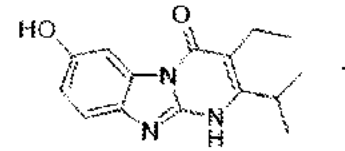
C-81



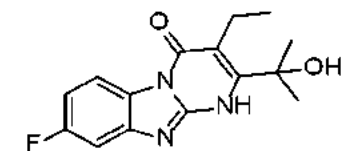
C-82



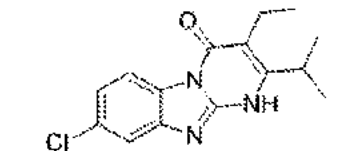
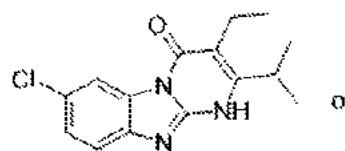
C-83



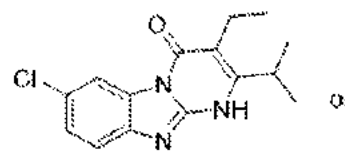
C-84



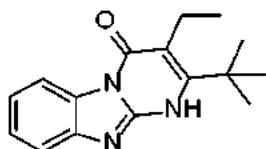
C-84*



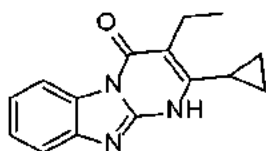
C-85



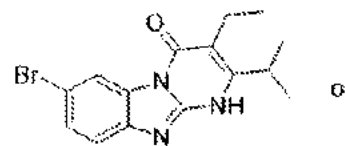
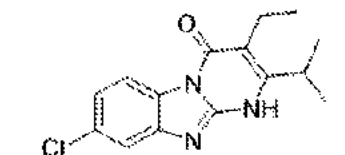
C-76



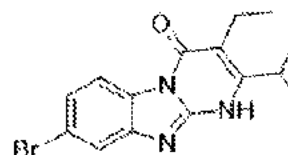
C-77



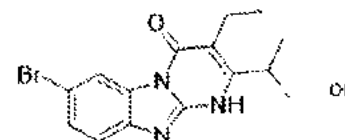
C-86



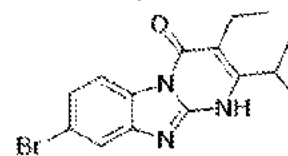
or



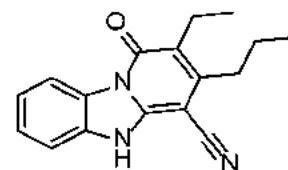
C-87



or



C-88



Claim 12 (Currently Amended): A pharmaceutical composition comprising an MrgX2 antagonist according to claim 10 [[or 11]] and a physiologically acceptable excipient.

Claim 13 (Currently Amended): A pharmaceutical dosage form comprising an MrgX2 antagonist according to claim 10 [[or 11]] ~~or a pharmaceutical composition according to claim 12.~~

Claim 14 (New): The pharmaceutical dosage form according to claim 13, which is selected from tablets and capsules.

Claim 15 (New): A pharmaceutical composition comprising an MrgX2 antagonist according to claim 11 and a physiologically acceptable excipient.

Claim 16 (New): A pharmaceutical dosage form comprising an MrgX2 antagonist according to claim 11.

Claim 17 (New): A pharmaceutical dosage form comprising a pharmaceutical composition according to claim 12.

REMARKS

After entry of this preliminary amendment, claims 1-17 will be pending. Applicants believe that the new set of claims is patentable. Accordingly, Applicants submit that the present application is in condition for allowance and request that the Examiner pass the case to issue at the earliest convenience. Should the Examiner have any question or wish to further discuss this application, Applicants request that the Examiner contact the undersigned at (248) 292-2920.

If for some reason Applicants have not requested a sufficient extension and/or have not paid a sufficient fee for this response and/or for the extension necessary to prevent the abandonment of this application, please consider this as a request for an extension for the required time period and/or authorization to charge our Deposit Account No. 50-1097 for any fee which may be due.

Respectfully submitted,

Date: October 31, 2019

/Kristen L. Pursley/

Kristen L. Pursley (Reg. No. 61,161)
The Dobrusin Law Firm, PC
29 W. Lawrence St., Suite 210
Pontiac, Michigan 48342
(248) 292-2920
kpursley@patentco.com
Customer No. 25215

8. Enhancement of the water solubility of MRGPRX2 antagonists

This chapter describes the optimization of the patented MRGPRX2 antagonists (chapter 7). A major drawback of the previously described compounds (Patent II) is their low water solubility, which complicates their further development into an orally bioavailable drug. This is an important factor, since up to 85% of the most top-selling drugs in the USA and Europe are administered orally. If a compound is poorly water-soluble, the applied dose must be increased, and large variabilities in intestinal absorption and thus in bioavailability are typically observed. Water solubility is also important for other routes of administration, e.g. intravenous or bronchial application. Water solubility is therefore an important parameter in the development of new drugs, which poses severe problems for pharmaceutical companies.¹

8.1. Synthesis

To improve the water solubility of MRGPRX2 antagonists, the benzene ring in the benzo[4,5]imidazo[1,2-*a*]pyrimidin-4(10*H*)-one derivatives (chapter 7) were to be substituted by a pyridine moiety. Different positions for the introduction of the nitrogen atom can be considered, leading on the one hand to pyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one derivatives, and on the other hand to pyrido[3',4':4,5]imidazo[1,2-*a*]pyrimidin-4(1*H*)-one derivatives (Figure 1).

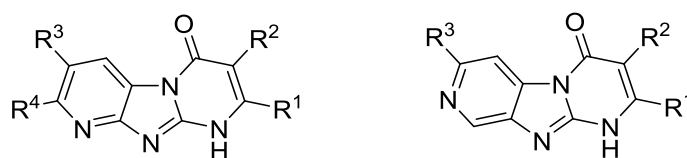
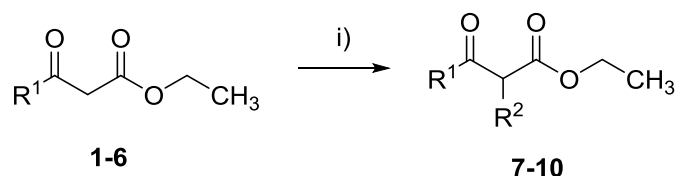


Figure 1: Chemical structures of new proposed MRGPRX2 antagonists

The synthesis of the proposed compounds is similar to the synthetic route already presented in chapter 7. Starting material can be differently substituted diaminopyridine derivatives with adjacent amino groups. This is followed by two successive ring-closure reactions, first with cyanogen bromide to form 1*H*-imidazo[4,5-*b*]pyridin-2-amine or 1*H*-imidazo[4,5-*c*]pyridin-2-amine and then the final cyclization reaction using an appropriate 3-oxocarboxylic ester to produce the final products shown in Figure 1. The non-commercially available 3-oxocarboxylic esters **7–10** were synthesized by an α -alkylation of different β -ketoesters (**1–6**, Scheme 1).

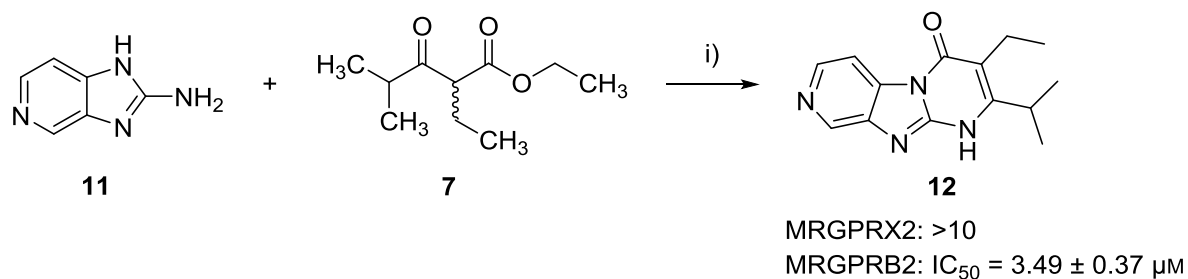


- 7: R¹ = isopropyl; R² = ethyl
 8: R¹ = *n*-butyl; R² = ethyl
 9: R¹ = *n*-propyl; R² = ethyl
 10: R¹ = cyclobutyl; R² = ethyl

^aReagents and conditions: (i) alkyl iodide, K₂CO₃, DMF, 60 °C, 12 h, 45–69%

Scheme 1: Synthesis of different 2-substituted 3-oxoesters **7–10**.

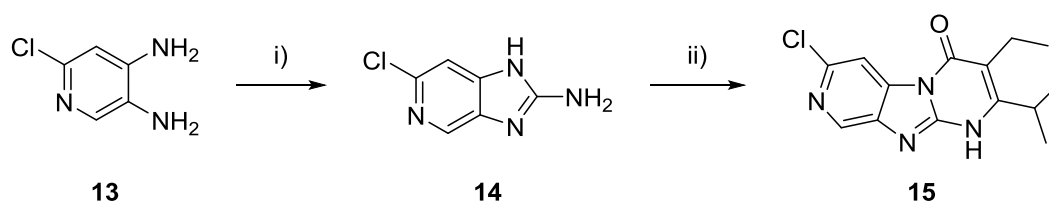
The first reaction was tested with the commercially available 1*H*-imidazo[4,5-*c*]pyridin-2-amine (**11**) and ethyl 2-ethyl-4-methyl-3-oxopentanoate (**7**) to yield compound **12** as the first pyrido-annelated MRGPRX2 antagonist. The final compounds were tested on both human MRPGRX2 and mouse MRGPRB2. Compound **12** was inactive in the β-arrestin assay up to 10 μM at the human MRGPRX2 but showed a weak antagonistic potency at the murine MRGPRB2 with an IC₅₀ value of 3.49 ± 0.37 μM determined in a calcium mobilization assay in recombinant 1321N1 astrocytoma cells.



^aReagents and conditions: (i) DMF, DIPEA, 160 °C, 18 h, 9%

Scheme 2: Synthesis of 3-ethyl-2-isopropylpyrido[3',4':4,5]imidazo[1,2-*a*]pyrimidin-4(1*H*)-one (**12**)

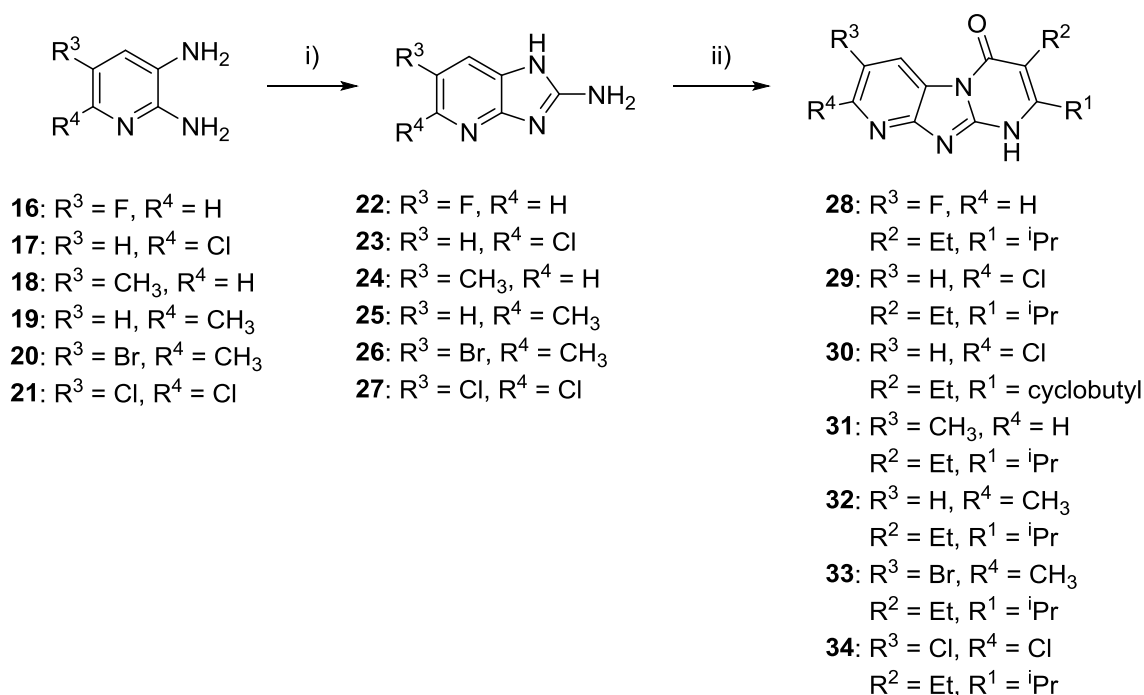
Since 6-chloropyridine-3,4-diamine (**13**) was commercially available, this compound was used as starting material in order to explore the role of a chloro substitution in position 7 of the corresponding MRGPRX2 antagonist **15**.



^aReagents and conditions: (i) BrCN, MeOH/H₂O, 90 °C, 1 h, 80%; ii) 7, DIPEA, DMF, 160 °C, 7%

Scheme 3: Synthesis of 7-chloro-3-ethyl-2-isopropylpyrido[3',4':4,5]imidazo[1,2-*a*]pyrimidin-4(1*H*)-one (**15**).

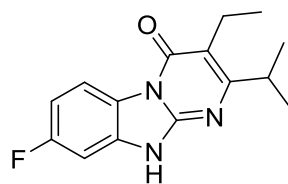
In order to increase the potency at MRGPRX2, it was considered useful to design the target compounds and their substitution pattern based on the structure-activity relationships observed for the analogous compounds described in Chapter 7 lacking the additional nitrogen atom. For this purpose, the nitrogen atom had to be shifted by one position to obtain the appropriate substitution profile. Different commercially available 5- and 6-substituted 2,3-diaminopyridine derivatives (**16–21**) were used as starting material for the synthesis of the potential MRGPRX2 antagonists **28–34**.



^aReagents and conditions: (i) BrCN, MeOH/H₂O, 90 °C, 1 h; ii) 7 or 10, DIPEA, DMF, 160 °C, 3–34% over two steps.

Scheme 3: Synthesis of pyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin 6(9*H*)-one derivatives (**28–34**).

However, the substitution pattern of the most potent MRGPRX2 antagonist obtained to date (**RCM40**) required a fluorine atom for R4.



RCM40

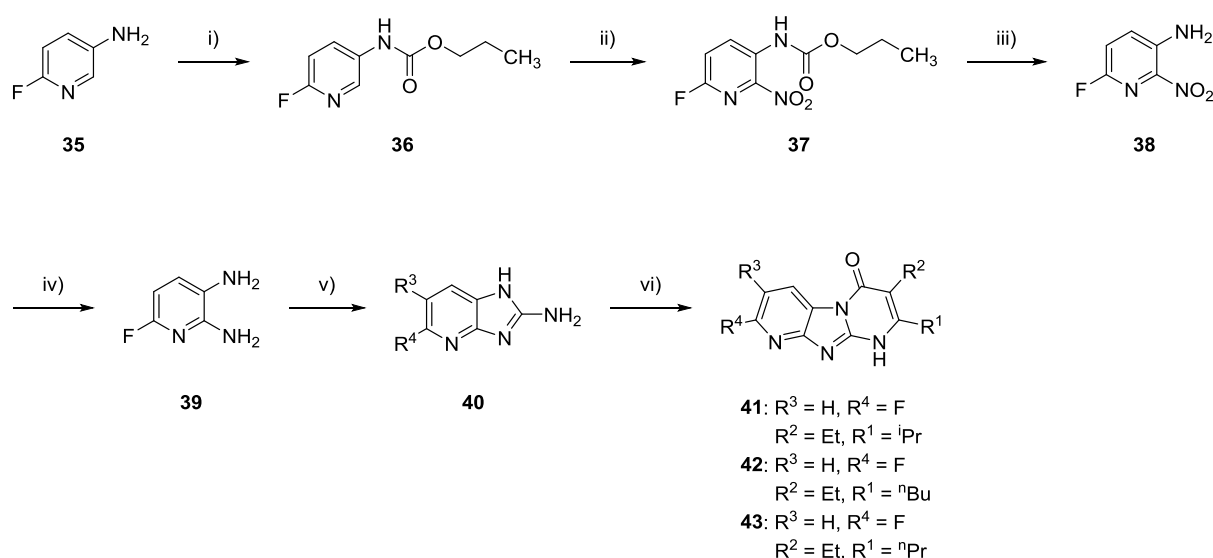
$IC_{50} = 0.0181 \pm 0.0044 \mu\text{M}$ (β -arrestin assay MRGPRX2)

$IC_{50} = 0.0013 \pm 0.0003 \mu\text{M}$ (Ca^{2+} -assay MRGPRX2)

$IC_{50} = 0.0155 \pm 0.0024 \mu\text{M}$ (Ca^{2+} -assay MRGPRB2)

Figure 2: Structure and potency of **RCM40**, the most potent MRGPRX2 antagonist to date.

The required 6-fluoro-2,3-diaminopyridine (**39**) was not commercially available and needed to be synthesized from 6-fluoropyridin-3-amine (**35**). In the next steps the second amino group was introduced by a selective aromatic nitration in the second position after protection of the 3-amino moiety as a carbamate group (**36**, **37**). The deprotection of the amino group was carried out under basic conditions, and the reduction of the nitro group using hydrogen and palladium on charcoal as a catalyst yielded the diaminopyridine derivative **39** as an important building block for the synthesis of pyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one derivatives **41**–**43** (Scheme 5).

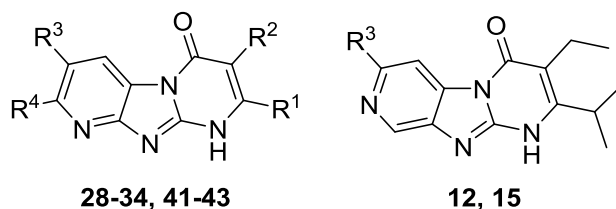


^aReagents and conditions: (i) Propyl chloroformate, NaHCO_3 , THF, 0°C , 30 min, 92%; (ii) H_2SO_4 , HNO_3 , 0°C to rt, 43%; (iii) $\text{Ba}(\text{OH})_2$, 2N NaOH , acetonitrile, 90°C , overnight, 48%; (iv) H_2 , 10% Pd/C, MeOH, rt, 95% (v) BrCN , MeOH/ H_2O , 90°C , 1 h; (vi) **7–9**, DIPEA, DMF, 160°C , 3–34% over two steps.

Scheme 5: Synthesis of F-substituted (R⁴) potential MRGPRX2 antagonists **41–43**.

All synthesized compounds were tested in β -arrestin assays at MRGPRX2, and most of the finale compounds were additionally tested in calcium assays at MRGPRB2 (Table 1). Screening of the compounds in calcium assays at MRGPRX2 is currently in progress. The following results SARs were observed: The first synthesized compound, **12**, showed no potency at MRGPRX2 but moderate antagonistic activity at MRGPRB2 with an IC₅₀ value of 3.49 ± 0.37 μ M. Substitution of the hydrogen atom in position R³ by a chlorine atom (**15**) increased potency at both MRGPRX2 and MRGPRB2 the compound showing IC₅₀ values of 2.27 ± 0.39 μ M and 0.222 ± 0.084 μ M, respectively. Shifting of the nitrogen atom by one position and inserting of a fluorine substituent in position R³ (**28**) resulted in an MRGPRX2 antagonist with an IC₅₀ value of 2.53 ± 0.85 μ M. Substitution of the fluorine atom by a methyl group (**31**) yielded an MRGPRX2 antagonist with similar potency displaying an IC₅₀ value of 2.25 ± 0.42 μ M at MRGPRX2, and 0.585 ± 0.215 μ M at MRGPRB2. A disubstituted compound with two chlorine atoms in *ortho*- and *meta*-position with respect to the nitrogen atom as in compound **34** was similarly active as **28** (IC₅₀ = 2.38 ± 0.67 μ M for the MRGPRX2 and IC₅₀ = 0.370 ± 0.073 μ M for the MRGPRB2). Shifting the fluorine substituent into the *ortho*-position with respect to the nitrogen atom as in compound **41** decreased the potency at MRGPRX2 (IC₅₀ = 4.94 ± 1.36 μ M) but resulted in good inhibitory activity at the mouse MRGPRB2 with an IC₅₀ value of 0.276 ± 0.169 μ M (n = 2). Based on the results of the previously described compounds described in Patent II, a substitution of the ethyl moiety did not seem to be useful. A substitution of the isopropyl group also promised only little improvement but was nevertheless tested for the substitution pattern of **41**. Substitution of the isopropyl moiety by ⁿBu and ⁿPr group resulted in two inactive compounds, **42** and **43**. Substitution of the fluorine atom in compound **41** by chlorine (compound **29**) could not significantly improve the IC₅₀ value at MRGPRX2 (4.66 ± 0.28 μ M). A substitution of the isopropyl linker by cyclobutyl also resulted in an inactive derivative, compound **30**. Compound **40** with R³ = Br and R⁴ = methyl, decreased the IC₅₀ value in both MRGPRX2 (51% inhibition at 10 μ M) and MRGPRB2 (0.837 ± 0.162 μ M). Until now, the inhibitors did not yet show nanomolar potencies, but the water solubility of the compounds has been significantly improved. The water solubility of the compounds presented in Patent II were <5 μ M for all tested compounds. In contrast, the new nitrogen-containing compound **28** showed an aqueous solubility of 47 ± 3 μ M. Thus, the new aza-analogs represent a promising class of compounds for the development of new MRGPRX2 antagonists with improved solubility.

Table 1: Potency of antagonists **12**, **15**, **28–34** and **41–43** at the human MRGPRX2 and murine MRGPRB2.



Cmpd	R ¹	R ²	R ³	R ⁴	MRGPRX2		MRGPRB2
					β-arrestin assay (CHO β-arrestin cells recombinantly expressing MRGPRX2)		calcium assay (1321N1 astrocytoma cells recombinantly expressing MRGPRB2)
					% effect ± SEM	IC ₅₀ ± SEM [μM] ^a (% inhibition ^b ± SEM)	IC ₅₀ ± SEM [μM] ^a (% inhibition ^d ± SEM)
12	-	-	H	-	> 10 (0%)	> 10 (23%)	3.49 ± 0.37
15	-	-	Cl	-	n.d.	2.27 ± 0.39	0.222 ± 0.084
28	ⁱ Pr	Et	F	H	> 10 (-3%)	2.53 ± 0.85	n.d.
29	ⁱ Pr	Et	H	Cl	n.d.	4.66 ± 0.28	n.d.
30	Cyclobutyle	Et	H	Cl	> 10 (1%)	> 10 (33%)	n.d.
31	ⁱ Pr	Et	CH ₃	H	n.d.	2.25 ± 0.42	0.585 ± 0.215
32	ⁱ Pr	Et	H	CH ₃	n.d.	9.68 ± 1.04	1.54 ± 0.50 (n = 2)
33	ⁱ Pr	Et	Br	CH ₃	n.d.	(51 ± 3) (10 μM)	0.837 ± 0.162
34	ⁱ Pr	Et	Cl	Cl	n.d.	2.38 ± 0.67	0.370 ± 0.073
41	ⁱ Pr	Et	H	F	n.d.	4.94 ± 1.36	0.276 ± 0.169 (n = 2)
42	ⁿ Bu	Et	H	F	> 10 (1%)	> 10 (5%)	n.d.
43	ⁿ Pr	Et	H	F	> 10 (0%)	> 10 (34%)	0.179 ± 0.048

^aThe results are the means of three to four independent effects. ^bInhibition of the effect of 1 μM CST-14 (corresponding to its EC₈₀ value). ^cInhibition of the effect of 0.7 μM CST-14 (corresponding to its EC₈₀ value). ^dInhibition of the effect of 2 μM CST-14 (corresponding to its EC₈₀ value). Screenings were performed at a concentration of 10 μM. The results were obtained from, Yvonne Riedel^Y and Susanne Gattner^S.

8.2. Experimental

8.2.1. Crystal structure of **41**

Compound **41** crystallized from THF solution at room temperature in clear colorless plates of the size $0.18 \times 0.1 \times 0.05$ mm. Measurement and analysis of the resulting crystal structure using a Bruker X8-KappaApexII instrument showed a triclinic crystal system within the space group P-1. The crystal is mainly formed by intermolecular hydrogen bonds.

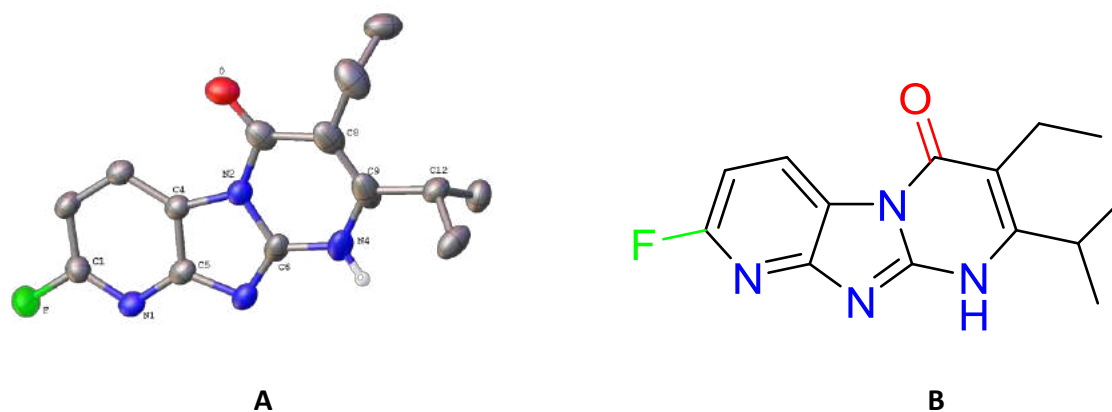


Figure 3: A: Crystal structure of **41**. B: Molecular structure of **41**

8.2.2. Materials and Methods

Melting points were determined with a Büchi-545 melting point apparatus. Chemicals were purchased from Sigma Aldrich, ABCR, Alfa Aesar or TCI. Analytical thin layer chromatography (TLC) was performed on TLC plates F₂₅₄ (Merck) and analyzed using UV light. High resolution mass spectra (HR-MS) were recorded on a micrOTOF-Q mass spectrometer (Bruker), low resolution mass spectra (LR-MS) on an API 2000 (Applied Biosystems) mass spectrometer. ¹H-NMR, ¹³C-NMR and ³¹P NMR spectra were recorded in (CD₃)₂SO on a Bruker Ascend 600 MHz NMR-spectrometer operating at 600.18 MHz (¹H), and 150.93 MHz (¹³C). Chemical shifts (δ) are reported in ppm and are referenced to the chemical shift of the residual solvent proton(s) present in dimethylsulfoxide δ ((CH₃)₂SO) = 2.50 ppm for the ¹H-NMR spectra and δ ((CD₃)₂SO) = 39.52 ppm for the ¹³C-NMR spectra. Multiplicity: s, singlet; d, doublet; q, quartet; m, multiplet. Coupling constants (*J*) are shown in hertz (Hz). The infrared spectra were recorded as solid samples on an ALPHA-T (Bruker) with a Platinum ATR Module using Opus software. Column chromatography was performed on silica gel 60 (35–70 μ m). Thin layer chromatography was carried out using precoated silica gel

F-254 plates (thickness 0.25 mm). All final compounds reported were analyzed using one of these analytical methods and were at least 95% pure. Final compounds were purified using reversed phase HPLC (C18, ϕ 250 x 20 mm, Eurospher 100-10, 60% - 100% aqueous MeOH).

General procedure A: Preparation of 2-substituted 3-oxoesters (**7-10**).

The β -keto ester (1.0 equiv), K_2CO_3 (0.75 equiv) and ethyl iodide (1.1 equiv) were dissolved in a minimum amount of DMF (1-2 ml) and stirred at 60 °C overnight. After complete conversion of the starting material (TLC: petroleum ether/ethyl acetate, 9:1) water (5–10 ml) was added and extracted three times with ethyl acetate (5 ml). The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by silica gel flash chromatography (eluent: 7% ethyl acetate in petroleum ether) yielding the final 2-substituted 3-oxoester derivatives as colorless oils.

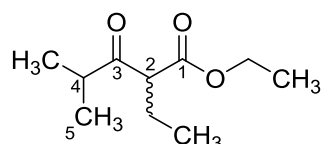
General procedure B: Preparation of 1*H*-imidazo[4,5-*b*]pyridin-2-amine (**14 22–27**).

The diamino substituted pyridine derivatives and BrCN (3.0 equiv) were dissolved in a mixture of MeOH/Water (1:1) and heated to 90 °C for 4–6 h. After complete conversion of the starting material (TLC: 20% MeOH in dichloromethane) the solvent was removed *in vacuo* and the residue purified by automated flash chromatography (gradient, 0-20% MeOH in CH_2Cl_2) to yield the 1*H*-imidazo[4,5-*b*]pyridin-2-amine derivatives as brown solids.

General procedure C: Preparation of pyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (**12, 32–41, 44**).

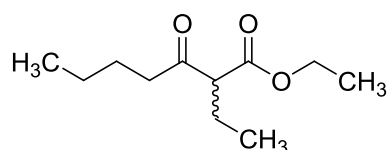
The 1*H*-imidazo[4,5-*b*]pyridin-2-amine derivatives (**12, 15, 28–34** and **41–43**) and DIPEA (2.0 equiv) were dissolved in a minimum amount of DMF (2–3 ml) and the 2-substituted 3-oxoester derivative (**7–10**, 2.0 equiv) was added in portions. The resulting mixture was heated to 165 °C for 18 h until complete conversion of the starting material (TLC: 5% MeOH in CH_2Cl_2). The solvent was removed, and the residue purified by automated flash chromatography (gradient, 0-10% MeOH/ CH_2Cl_2 over 20 min). An additional reversed phase HPLC separation (60-100% MeOH/ H_2O , 20 min) was required to yield the final products as white solids with at least 95% purity.

Synthesis of ethyl 2-ethyl-4-methyl-3-oxopentanoate (**7**)



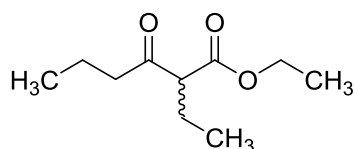
Yield: 45% (colorless oil); $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 4.09 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 3.75 (t, $J = 7.2$ Hz, 1H, H-2), 2.78 (h, $J = 6.8$ Hz, 1H, H-4), 1.70 (tdd, $J = 13.7, 7.2, 5.6$ Hz, 2H, CHCH_2), 1.16 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.01 (dd, $J = 6.9, 5.9$ Hz, 6H, $(\text{CH}_3)_2$ -4), 0.83 (t, $J = 7.4$ Hz, 3H, OCH_2CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 209.1 (C3), 169.4 (C1), 60.6 (OCH_2), 57.3 (C2), 40.0 (C4), 21.2 (CHCH_2), 17.9 (C5), 17.9 (C5), 13.9 (OCH_2CH_3), 11.6 (CHCH_2CH_3). LC-MS (m/z): 186.8 $[\text{M} + \text{H}]^+$.

Synthesis of ethyl ethyl 2-ethyl-3-oxoheptanoate (**8**)



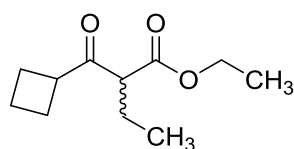
Yield: 65% (colorless oil); $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 4.10 (qd, $J = 7.1, 1.7$ Hz, 1H, OCH_2CH_3), 3.53 (dd, $J = 7.8, 6.6$ Hz, 1H, H-2), 2.54 – 2.50 (m, 2H, CH_2), 1.78 – 1.65 (m, 2H, CH_2), 1.48 – 1.40 (m, 2H, CH_2), 1.23 (dq, $J = 9.2, 7.5, 6.2$ Hz, 2H, CH_2), 1.17 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 0.83 (dt, $J = 9.2, 7.4$ Hz, 6H, 2 x CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 205.4 (C3), 169.4 (C1), 60.6 (OCH_2), 59.4 (C2), 41.0 (CH_2), 25.0 (CH_2), 21.5 (CH_2), 21.0 (CH_2), 14.0 (CH_3), 13.7 (CH_3), 11.6 (CH_3). LC-MS (m/z): 200.7 $[\text{M} + \text{H}]^+$.

Synthesis of ethyl 2-ethyl-3-oxohexanoate (**9**)



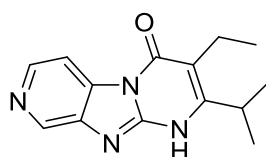
Yield: 61% (colorless oil); $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 4.10 (qd, $J = 7.1, 1.5$ Hz, 2H, OCH_2CH_3), 3.53 (dd, $J = 7.9, 6.6$ Hz, 1H, H-2), 2.52 – 2.50 (m, 2H, CH_2), 1.81 – 1.65 (m, 2H, CH_2), 1.48 (h, $J = 7.3$ Hz, 2H, CH_2), 1.17 (t, $J = 7.1$ Hz, 3H, CH_3), 0.83 (td, $J = 7.4, 1.1$ Hz, 6H, 2 x CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 205.4 (C3), 169.4 (C1), 60.6 (OCH_2), 59.4 (C2), 43.2 (CH_2), 20.9 (CH_2), 16.4 (CH_2), 14.0 (CH_3), 13.3 (CH_3), 11.6 (CH_3). LC-MS (m/z): 186.8 $[\text{M} + \text{H}]^+$.

Synthesis of ethyl 2-(cyclobutanecarbonyl)butanoate (**10**)



Yield: 69% (colorless oil); $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 4.09 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 3.50 (dd, $J = 7.9, 6.6$ Hz, 1H, CH), 3.45 (pd, $J = 8.6, 1.2$ Hz, 1H, CH), 2.16 – 2.02 (m, 4H), 1.91 (dp, $J = 11.0, 8.8$ Hz, 1H), 1.77 – 1.65 (m, 3H), 1.17 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 0.82 (t, $J = 7.4$ Hz, 3H, CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 205.9 (C3), 169.4 (C1), 60.6 (OCH_2), 57.5 (C2), 44.3 (CH), 24.0 (CH_2), 21.0 (CH_2), 17.1 (CH_2), 14.0 (CH_3), 11.6 (CH_3). LC-MS (m/z): 198.8 [$\text{M} + \text{H}$] $^+$.

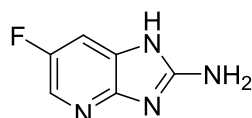
Synthesis of 3-ethyl-2-isopropylpyrido[3',4':4,5]imidazo[1,2-*a*]pyrimidin-4(1*H*)-one (**12**)



Compound **12** was synthesized as described in the general procedure C using commercially available 1*H*-imidazo[4,5-*c*]pyridin-2-amine (**11**, 70 mg, 0.522 mmol) as starting material.

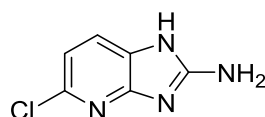
Yield: **12** (12 mg, 9%), white solid. $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ [ppm] = 12.83 (s, 1H, NH,), 9.40 (s, 1H, H_{arom}), 8.45 (d, $J = 5.7$ Hz, 1H, H_{arom}), 7.49 (d, $J = 5.6$ Hz, 1H, H_{arom}), 3.23 – 3.21 (m, 1H, CH), 2.62 (q, $J = 7.4$ Hz, 2H, CH_2), 1.25 (d, $J = 6.8$ Hz, 6H, 2 x CH_3), 1.08 (t, $J = 7.4$ Hz, 3H, CH_2CH_3).

Synthesis of 6-fluoro-1*H*-imidazo[4,5-*b*]pyridin-2-amine (**22**)



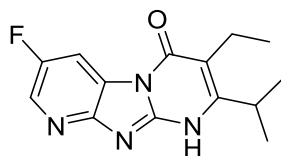
Compound **22** was synthesized as described in the general procedure B. Yield: 63%, brown solid. $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ [ppm] = 8.01 (m, 1H, H_{arom}), 7.78 (s, 2H, NH_2), 7.52 (dd, $J = 9.0, 2.6$ Hz, 1H, H_{arom}). $^{13}\text{C-NMR}$ (126 MHz, $\text{DMSO-}d_6$) δ [ppm] = 156.1 (d, $^1J_{\text{C,F}} = 240.7$ Hz, $\text{C}_{\text{arom-F}}$), 155.2 (C- NH_2), 145.1 (C_{arom}), 128.2 (d, $^3J_{\text{C,F}} = 9.7$ Hz, C_{arom}), 127.6 (d, $^2J_{\text{C,F}} = 27.9$ Hz, C_{arom}), 106.79 (d, $^2J_{\text{C,F}} = 25.0$ Hz, C_{arom}). LC-MS (m/z): 152.8 [$\text{M} + \text{H}$] $^+$. ESI-MS purity: 97.3%.

Synthesis of 5-chloro-1*H*-imidazo[4,5-*b*]pyridin-2-amine (**23**)



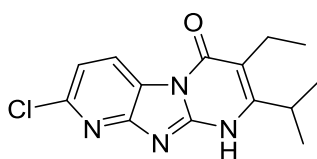
Compound **23** was synthesized as described in the general procedure B. Yield: 99%, brown solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 8.29 (s, 2H, NH_2), 7.69 – 7.62 (m, 1H, H_{arom}), 7.23 – 7.16 (m, 1H, H_{arom}). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 153.2 (C_q), 147.1 (C_q), 142.0 (C_q), 124.1 (C_q), 120.8 (C-H_{arom}), 117.0 (C-H_{arom}). LC-MS (m/z): 169.0 [$\text{M} + \text{H}$] $^+$. ESI-MS purity: 99.2%.

Synthesis of 3-ethyl-7-fluoro-2-isopropylpyrido[3',4':4,5]imidazo[1,2-*a*]pyrimidin-4(1*H*)-one (**28**)



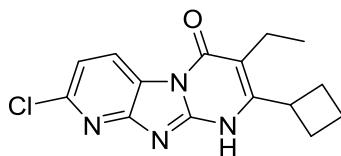
Compound **28** was synthesized as described in the general procedure C. Yield: 10%, white solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 12.91 (br s, 1H, NH), 8.43 (t, $J = 2.4$ Hz, 1H, H_{arom}), 8.41 (dd, $J = 8.3, 2.9$ Hz, 1H, H_{arom}), 3.27 – 3.20 (m, 1H, $\text{CH-(CH}_3)_2$), 2.59 (q, $J = 7.5$ Hz, 2H, CH_2), 1.26 (d, $J = 6.7$ Hz, 6H, 2 x CH_3), 1.07 (t, $J = 7.4$ Hz, 3H, CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 159.53, 154.63, 149.15, 149.11, 133.41, 120.01, 119.95, 110.31, 110.19, 30.18, 21.08, 17.79, 14.63. HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{15}\text{FN}_4\text{O}$ [$\text{M} + \text{H}$] $^+$: 275.1303; found: 275.1320. m.p. 212 – 215 $^\circ\text{C}$.

Synthesis of 2-chloro-7-ethyl-8-isopropylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (**29**)



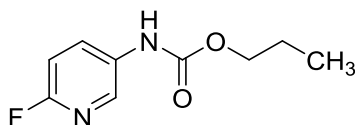
Compound **29** was synthesized as described in the general procedure C. Yield: 12% over 2 steps, white solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 12.81 (s, 1H, NH), 8.54 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.32 (d, $J = 8.2$ Hz, 1H, H_{arom}), 3.29 – 3.24 (m, 1H, $-\text{CH}(\text{CH}_3)_2$), 2.59 (q, $J = 7.4$ Hz, 2H, $-\text{CH}_2$), 1.28 (d, $J = 6.8$ Hz, 6H, $-\text{CH}(\text{CH}_3)_2$), 1.06 (t, $J = 7.4$ Hz, 3H, $-\text{CH}_2\text{CH}_3$). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 159.2, 148.7, 145.7, 124.6 (H_{arom}), 119.5, 116.0 (H_{arom}), 29.6, 20.6, 17.6 (CH_2), 14.4 (2 x CH_3). HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{15}\text{ClN}_4\text{O}$ [$\text{M} + \text{H}$] $^+$: 291.1002; found: 291.1003. m.p. > 300 $^\circ\text{C}$.

Synthesis of 2-chloro-8-cyclobutyl-7-ethylpyrido[2,3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (**30**)



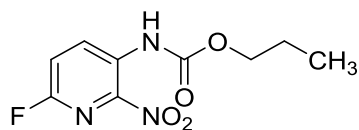
Compound **30** was synthesized as described in the general procedure C. Yield: 13% over 2 steps, yellowish solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 8.54 (d, $J = 8.2$ Hz, 1H), 7.31 (d, $J = 8.3$ Hz, 1H), 6.51 (s, 2H), 3.84 (p, $J = 8.8$ Hz, 1H), 2.53 (q, $J = 7.5$ Hz, 2H), 2.46 (qd, $J = 9.6, 2.8$ Hz, 2H), 2.20 (dtd, $J = 11.3, 8.5, 2.7$ Hz, 2H), 2.02 (h, $J = 9.6$ Hz, 1H), 1.84 (q, $J = 9.7$ Hz, 1H), 1.03 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 159.1, 148.7, 145.6, 124.7, 119.4, 115.9, 111.3, 36.7, 27.1, 17.7, 17.7, 14.4. HRMS (ESI-QTOF) calculated for $\text{C}_{15}\text{H}_{15}\text{ClN}_4\text{O}$ $[\text{M}+\text{H}]^+$: 303.1007; found: 303.1021. m.p. > 300 °C.

Synthesis of propyl (6-fluoropyridin-3-yl)carbamate (**36**)



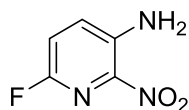
6-Fluoropyridine-3-amine (**35**, 5 g, 44.60 mmol, 1.0 equiv) and NaHCO_3 (11.24 g, 133.80 mmol, 3.0 equiv) was dissolved in dry THF (25 ml) and propyl chloroformate (16.40 g, 133.80 mmol, 3.0 equiv) was added slowly. Reaction was completed after 30 min (TLC: petroleum ether/ethyl acetate = 8:2) and the solvent evaporated *in vacuo*. Then water was added and extracted with ethyl acetate (3 x 10 ml) and the combined organic phases were washed with brine, dried over MgSO_4 , filtered and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (10% ethyl acetate in petroleum ether) to yield compound **36** (7.15 g, 81%) as yellowish solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 9.87 (s, 1H, NH), 8.31 – 8.20 (m, 1H, H_{arom}), 8.07 – 7.95 (m, 1H, H_{arom}), 7.12 (dd, $J = 8.9, 3.2$ Hz, 1H, H_{arom}), 4.05 (t, $J = 6.7$ Hz, 2H, OCH_2), 1.64 (sext, $J = 7.2$ Hz, 2H, CH_2), 0.92 (t, $J = 7.4$ Hz, 3H, CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 158.2 (1C, $^1J_{\text{C,F}} = 231.4$ Hz, $\text{C}_{\text{arom-F}}$), 153.8 (CO), 136.6 (1C, $^3J_{\text{C,F}} = 15.2$ Hz, C_{arom}), 134.3 (1C, $^4J_{\text{C,F}} = 4.4$ Hz, C_q), 131.6 (C_{arom}), 109.3 (1C, $^2J_{\text{C,F}} = 39.5$ Hz, C_{arom}), 66.1 (OCH_2), 21.8 (CH_2), 10.2 (CH_3). LC-MS (m/z): 198.7 $[\text{M} + \text{H}]^+$. ESI-MS purity: 97.1%.

Synthesis of propyl (6-fluoro-2-nitropyridin-3-yl)carbamate (**37**)



Compound **36** (2.0 g, 10.09 mmol, 1.0 equiv) was dissolved in conc. HNO₃ (2 ml) and cooled to 0 °C. Than conc. H₂SO₄ (2 ml) was added dropwise over 30 min and stirred for further 5 h at rt. After complete conversion (TLC: ethyl acetate/ petroleum ether, 1:5) the mixture was basified by the addition of NaOH, extracted with ethyl acetate and the combined organic phases dried over MgSO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (50% ethyl acetate in petroleum ether) to yield compound **37** (1.996 g, 81%) as yellowish solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 10.02 (s, 1H, NH), 8.35 (dd, *J* = 8.7, 6.5 Hz, 1H, H_{arom}), 7.69 (dd, *J* = 8.7, 3.3 Hz, 1H, H_{arom}), 4.06 (t, *J* = 6.7 Hz, 2H, OCH₂), 1.63 (sext, *J* = 7.4, 2H, CH₂), 0.92 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 155.5 (1C, ¹*J*_{C,F} = 231.4 Hz, C_{arom}-F), 153.5 (CO), 144.8 (1C, ³*J*_{C,F} = 12.7 Hz, C_q-NO₂), 141.0 (1C, ³*J*_{C,F} = 7.6 Hz, C_{arom}), 126.3 (1C, ⁴*J*_{C,F} = 4.0 Hz, C_q), 116.4 (1C, ²*J*_{C,F} = 37.8 Hz, C_{arom}), 67.0 (OCH₂), 21.7 (CH₂), 10.1 (CH₃). LC-MS (m/z): 243.9 [M + H]⁺. ESI-MS purity: 73.3%.

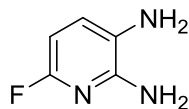
Synthesis of 6-fluoro-2-nitropyridin-3-amine (**38**)



Compound **37** (1.4394 g, 5.919 mmol, 1.0 equiv) and Ba(OH)₂ (1.8 g, 10.654 mmol, 1.8 equiv) were suspended in acetonitrile (10 ml). 2N NaOH was added in portions over 8 h until complete conversion of the starting material (TLC, CH₂Cl₂). The acetonitrile was removed *in vacuo* and the residue neutralized with saturated aq. NH₄Cl (10 ml), extracted with ethyl acetate (3 x 5 ml) and the combined organic phases were washed with brine, dried over MgSO₄ and the solvent removed under reduced pressure. The crude product was purified by automated flash column chromatography (0-60% ethyl acetate in petroleum ether) to yield **38** (445 mg, 48%) as yellowish solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.73 (dd, *J* = 8.9, 6.8 Hz, 1H, H_{arom}), 7.49 (s, 2H, NH₂), 7.41 (dd, *J* = 8.9, 3.7 Hz, 1H, H_{arom}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 149.6 (1C, ¹*J*_{C,F} = 234.0 Hz, C_q-F), 140.4 (1C, ⁴*J*_{C,F} = 1.5 Hz, C_q-NH₂), 135.9 (1C, ³*J*_{C,F}

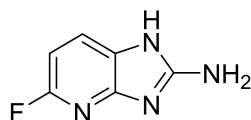
= 6.6 Hz, C_{arom}), 132.8 (1C, ³J_{C,F} = 13.0 Hz, C_q-NO₂), 119.1 (1C, ²J_{C,F} = 42.1 Hz, C_{arom}). LC-MS (m/z): 157.8 [M + H]⁺. ESI-MS purity: 97.5%.

Synthesis of 6-fluoropyridine-2,3-diamine (**39**)



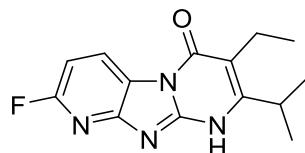
To a solution of **38** (352.0 mg, 2.241 mmol, 1.0 equiv) in MeOH (5 ml), were added 10% palladium of charcoal (238.4 mg, 1.0 equiv). The reaction mixture was stirred for 1 h under hydrogen atmosphere (1 atm) at rt. After complete conversion of the starting material monitored by TLC (10% MeOH in CH₂Cl₂) the catalyst was filtered over celite and washed with MeOH. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography (10% MeOH in CH₂Cl₂) to yield the diamino derivative **39** (270 mg, 95%) as yellowish solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 6.86 (t, *J* = 7.7 Hz, 1H, H_{arom}), 5.98 (dd, *J* = 7.9, 2.9 Hz, 1H, H_{arom}), 5.73 (br s, 2H, NH₂), 5.13 (br s, 2H, NH₂). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 155.1 (1C, ¹J_{C,F} = 222.7 Hz, C_q-F), 147.1 (1C, ³J_{C,F} = 17.9 Hz, C_{arom}), 125.2 (1C, ⁴J_{C,F} = 3.1 Hz, C_q-NH₂), 124.0 (1C, ³J_{C,F} = 6.9 Hz, C_q-NH₂), 93.7 (1C, ²J_{C,F} = 38.6 Hz, C_{arom}). LC-MS (m/z): 127.8 [M + H]⁺. ESI-MS purity: 97.6%.

Synthesis of 5-fluoro-1H-imidazo[4,5-*b*]pyridin-2-amine (**40**)



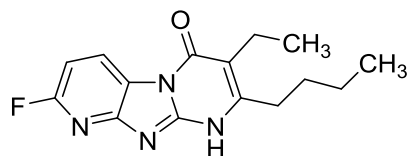
Compound **39** (265 mg, 2.085 mmol, 1.0 equiv) was dissolved in a mixture of MeOH (2 ml) and H₂O (2 ml) and cyanogen bromide (662.3 mg, 6.254 mmol, 3.0 equiv) added in portions. The reaction mixture was heated at reflux for 3 h until complete conversion of the starting material (TLC, 15% MeOH in CH₂Cl₂). The solvent was evaporated under reduced pressure and the residue purified by flash column chromatography (20% EtOH in CH₂Cl₂) to yield compound **40** as yellowish solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.62 (dd, *J* = 8.2, 7.2 Hz, 1H, H_{arom}), 7.49 (s, 2H, NH₂), 6.68 (d, *J* = 8.2 Hz, 1H, H_{arom}). ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 158.7 (1C, ¹J_{C,F} = 228.9 Hz, C_q-F), 155.4 (C_q-NH₂), 148.9 (1C, ³J_{C,F} = 20.2 Hz, C_{arom}), 123.9 (1C, ⁴J_{C,F} = 2.6 Hz, C_{arom}), 121.1 (1C, ³J_{C,F} = 9.6 Hz, C_{arom}), 99.3 (1C, ²J_{C,F} = 39.8 Hz, C_{arom}). LC-MS (m/z): 152.9 [M + H]⁺. ESI-MS purity: 97.5%.

Synthesis of 7-ethyl-2-fluoro-8-isopropylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (41)



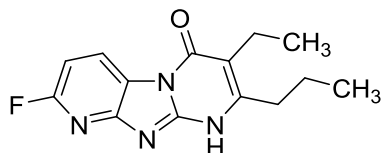
Compound **41** was synthesized as described in the general procedure C. Yield: 14% over 2 steps, yellowish solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 12.73 (br s, 1H), 8.65 (t, J = 7.9 Hz, 1H, H_{arom}), 6.97 (d, J = 8.4 Hz, 1H, H_{arom}), 3.30 – 3.25 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.59 (q, J = 7.4 Hz, 2H, CH_2CH_3), 1.28 (d, J = 6.8 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.07 (t, J = 7.4 Hz, 3H, CH_2CH_3). $^{13}\text{C-NMR}$ (126 MHz, DMSO) δ [ppm] = 160.6 (1C, $^1J_{\text{C,F}}$ = 233.2 Hz, $\text{C}_q\text{-F}$), 159.0, 126.7, 100.8 (1C, $^2J_{\text{C,F}}$ = 40.7 Hz, C_{arom}), 29.5, 20.5, 17.6 (CH_2), 14.4 (2 x CH_3). HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{15}\text{FN}_4\text{O}$ [$\text{M}+\text{H}$] $^+$: 275.1303; found: 275.1320. m.p. > 300 °C.

Synthesis of 8-butyl-7-ethyl-2-fluoropyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (42)



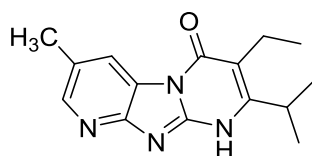
Compound **42** was synthesized as described in the general procedure C. Yield: 30% over 2 steps, off-white solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 13.00 (br s, 1H, NH), 8.64 (t, J = 8.0 Hz, 1H, H_{arom}), 6.95 (d, J = 8.4 Hz, 1H, H_{arom}), 2.66 (dd, J = 9.2, 6.7 Hz, 2H, CH_2), 2.54 (q, 2H, CH_2), 1.63 (tt, J = 8.0, 6.4 Hz, 2H, CH_2), 1.40 (h, J = 7.4 Hz, 2H, CH_2), 1.09 (t, J = 7.4 Hz, 3H, CH_3), 0.93 (t, J = 7.4 Hz, 3H, CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 160.8, 159.2, 126.6, 119.1, 100.7, 100.4, 31.0, 22.1, 18.1, 14.2, 13.9. HRMS (ESI-QTOF) calculated for $\text{C}_{15}\text{H}_{17}\text{FN}_4\text{O}$ [$\text{M}+\text{H}$] $^+$: 289.1459; found: 289.1479. m.p. > 300 °C.

Synthesis of 7-ethyl-2-fluoro-8-propylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one
(**43**)



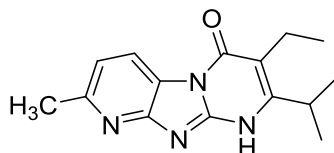
Compound **43** was synthesized as described in the general procedure C. Yield: 34% over 2 steps, off-white solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 12.95 (br s, 1H, NH), 8.64 (t, $J = 7.9$ Hz, 1H, H_{arom}), 6.96 (d, $J = 8.4$ Hz, 1H, H_{arom}), 2.67 – 2.63 (m, 2H, CH_2), 2.53 (q, $J = 8.2, 7.8$ Hz, 2H, CH_2), 1.73 – 1.64 (m, 2H, CH_2), 1.09 (t, $J = 7.3$ Hz, 3H, CH_3), 0.98 (t, $J = 7.3$ Hz, 3H, CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 160.6, 159.0, 148.8, 126.5, 118.9, 110.3, 100.7, 32.4, 22.0, 17.9, 14.0, 13.6. HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{15}\text{FN}_4\text{O}$ $[\text{M}+\text{H}]^+$: 275.1303; found: 275.1327. m.p. > 300 °C.

Synthesis of 7-ethyl-8-isopropyl-3-methylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one
(**31**)



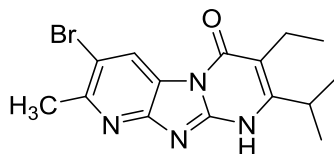
Compound **31** was synthesized as described in the general procedure C. Yield: 3% over two steps (off-white solid). $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 13.00 (s, 1H, NH), 8.44 (d, $J = 2.7$ Hz, 1H, H_{arom}), 8.24 (d, $J = 2.4$ Hz, 1H, H_{arom}), 3.22 (m, $J = 6.9$ Hz, 1H, $\text{CH}(\text{CH}_3)_2$), 2.59 (q, $J = 7.4$ Hz, 2H, CH_2CH_3), 2.43 (s, 3H, Ar- CH_3), 1.23 (d, $J = 6.7$ Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.07 (t, $J = 7.4$ Hz, 3H, CH_2CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ [ppm] = 159.5, 147.6, 145.8, 126.1, 122.7, 119.5, 30.4, 21.2, 18.0, 17.7, 14.5. HRMS (ESI-QTOF) calculated for $\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$: 271.1553; found: 271.1575. m.p. 224 – 227 °C.

Synthesis of 7-ethyl-8-isopropyl-2-methylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (32)



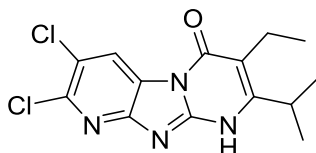
Compound **32** was synthesized as described in the general procedure C. Yield: 8% over two steps, off-white solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.05 (br s, 1H, NH), 8.46 (d, *J* = 8.0 Hz, 1H, H_{arom}), 7.15 (d, *J* = 8.0 Hz, 1H, H_{arom}), 3.26 – 3.18 (m, 1H, CH(CH₃)₂), 2.59 (q, *J* = 7.5 Hz, 2H, CH₂), 2.55 (s, 3H, Ar-CH₃), 1.23 (d, *J* = 6.7 Hz, 6H, CH(CH₃)₂), 1.07 (t, *J* = 7.4 Hz, 3H, CH₂CH₃). ¹³C-NMR (151 MHz, DMSO) δ [ppm] = 159.3, 154.7, 147.4, 122.6, 117.6, 116.2, 30.3, 24.0, 21.3, 17.7, 14.5. HRMS (ESI-QTOF) calculated for C₁₅H₁₈N₄O [M+H]⁺: 271.1553; found: 271.1571. m.p. 263 – 266 °C.

Synthesis of 3-bromo-7-ethyl-8-isopropyl-2-methylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (33)



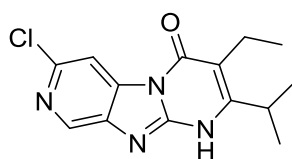
Compound **33** was synthesized as described in the general procedure C. Yield: 9%, off-white solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 13.26 (s, 1H, NH), 8.61 (s, 1H, H_{arom}), 3.24 (m, *J* = 8.8 Hz, 1H, CH(CH₃)₂), 2.65 (s, 3H, Ar-CH₃), 2.58 (q, *J* = 7.4 Hz, 2H, CH₂CH₃), 1.25 (d, *J* = 7.2 Hz, 6H, CH(CH₃)₂), 1.07 (t, *J* = 7.4 Hz, 3H, CH₂CH₃). ¹³C-NMR (151 MHz, DMSO) δ [ppm] = 159.1, 152.4, 125.0, 111.2, 30.2, 24.9, 21.0, 17.6, 14.4. HRMS (ESI-QTOF) calculated for C₁₅H₁₇BrN₄O [M+H]⁺: 349.0658; found: 349.0685. m.p. 271 – 274 °C.

Synthesis of 2,3-dichloro-7-ethyl-8-isopropylpyrido[2',3':4,5]imidazo[1,2-*a*]pyrimidin-6(9*H*)-one (**34**)



Compound **34** was synthesized as described in the general procedure C. Yield: 3% over two steps, off-white solid. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ [ppm] = 12.87 (s, 1H, NH), 8.64 (s, 1H, H_{arom}), 2.59 (q, $J = 7.5$ Hz, 3H, CH_2CH_3), 1.33 – 1.26 (m, 6H, $\text{CH}(\text{CH}_3)_2$), 1.07 (t, $J = 7.4$ Hz, 3H, CH_2CH_3). CH-signal under water peak. $^{13}\text{C-NMR}$ (151 MHz, DMSO) δ [ppm] = 159.1, 142.8, 123.8, 120.6, 29.5, 20.4, 17.6, 14.4. HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{14}\text{Cl}_2\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$: 325.0617; found: 325.0637. m.p. 254 – 257 °C.

Synthesis of 7-chloro-3-ethyl-2-isopropylpyrido[3',4':4,5]imidazo[1,2-*a*]pyrimidin-4(1*H*)-one (**15**)



Compound **15** was synthesized as described in the general procedure C. Yield: 7%, off-white solid. $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ [ppm] = 9.17 (s, 1H), 7.57 (s, 1H), 2.61 (q, $J = 7.5$ Hz, 2H), 1.28 (d, $J = 6.7$ Hz, 6H), 1.08 (t, $J = 7.4$ Hz, 3H). CH-signal under water peak. $^{13}\text{C-NMR}$ (126 MHz, DMSO) δ [ppm] = 159.0, 145.1, 134.6, 29.6, 20.6, 17.6, 14.3, 11.7. HRMS (ESI-QTOF) calculated for $\text{C}_{14}\text{H}_{15}\text{ClN}_4\text{O}$ $[\text{M}+\text{H}]^+$: 291.1007; found: 291.1006. m.p. > 300 °C.

8.3. References

- (1) Nishikawa, T.; Nakagami, H.; Maeda, A.; Morishita, R.; Miyazaki, N.; Ogawa, T.; Tabata, Y.; Kikuchi, Y.; Hayashi, H.; Tatsu, Y. Development of a novel antimicrobial peptide, AG-30, with angiogenic properties. *J. Cell. Mol. Med.* **2009**, *13* (3), 535–546.
- (2) Šali, A.; Blundell, T. L. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.* **1993**, *234* (3), 779–815.
- (3) Webb, B.; Sali, A. Comparative protein structure modeling using MODELLER. *Curr. Protoc. Bioinformatics* **2014**, *47* (1), 5.6. 1–5.6. 32.
- (4) Zhang, D.; Gao, Z.-G.; Zhang, K.; Kiselev, E.; Crane, S.; Wang, J.; Paoletta, S.; Yi, C.; Ma, L.; Zhang, W. Two disparate ligand-binding sites in the human P2Y 1 receptor. *Nature* **2015**, *520* (7547), 317.
- (5) Chojnacki, S.; Cowley, A.; Lee, J.; Foix, A.; Lopez, R. Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Res.* **2017**, *45* (W1), W550–W553.
- (6) Costanzi, S., Homology modeling of class a G protein-coupled receptors. In *Methods. Mol. Biol.*, Springer: 2011; 259–279.
- (7) Lovell, S. C.; Davis, I. W.; Arendall, W. B.; de Bakker, P. I.; Word, J. M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C. Structure validation by C α geometry: phi,psi and C β deviation. *Proteins* **2002**, *50*, 437–450.
- (8) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30* (16), 2785–2791.
- (9) *Molecular Operating Environment (MOE), 2013.08*, Chemical Computing Group ULC: 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7,, 2019.
- (10) Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T. J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Söding, J. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **2011**, *7* (1), 539.

9. Summary and Outlook

The G protein-coupled receptor (GPCR) superfamily with more than 800 members is one of the most important target classes for drug development. After an agonist binds to the receptor, it induces a conformational change leading to the activation of a heterotrimeric guanine nucleotide-binding protein (G protein). G proteins play a key role in signal transduction from GPCRs activated by extracellular signals to intracellular second messenger systems. GPCRs are involved in almost all physiological and pathophysiological processes. About 100 of the more than 800 human GPCRs are so-called orphan receptors, whose endogenous ligands remain to be identified or confirmed.

In 2001, Dong *et al.* discovered a subfamily of orphan GPCRs comprising over 50 rodent and human receptors, which were termed Mas-related gene receptors (MRGs). Later, in 2002, Lembo *et al.* discovered the same GPCR family to be expressed in rat primary cultures of dorsal root ganglia (DRG) and designated them sensory neuron-specific receptors (SNSRs). The current nomenclature declared by the International Union of Pharmacology (IUPHAR) of these orphan receptors is Mas-related G protein-coupled receptors (MRGPR).

This receptor family can be divided into nine distinct subfamilies (MRGPRA-H and -X). They belong to the δ -branch of class A, rhodopsin-like GPCR subgroup. Among the nine subfamilies of MRGPRs, four members (MRGPRX1-4) are primate-specific. Due to their expression profile, they are supposed to be involved, e.g., in pain transmission, immune responses, itching and wound healing.

A proprietary compound library was used to identify a potential lead compound that activates MRGPRX4. Both the MRGPRX4 wild type (WT) receptor as well as the rare natural variant L83S were employed for compound screening. The xanthine derivative MSX-3 ((*E*)-phosphoric acid mono-[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester disodium salt), a prodrug of the A_{2A} antagonist MSX-2 ((*E*)-3-(3-hydroxypropyl)-8-[2-(3-methoxyphenyl)vinyl]-7-methyl-1-prop-2-ynyl-3,7-di-hydropurine-2,6-dione), was discovered to activate as the rare natural variant L83S of MRGPRX4. In order to increase the agonist's potency on the rare natural variant L83S, and to identify an agonist for the MRGPRX4 wild type receptor, various side group modifications were performed. Since the phosphate group of MSX-3 is metabolically unstable due to hydrolysis by phosphatases, the phosphate group was to be replaced by a phosphonate or other bioisosteric functional groups. To further increase the stability of MSX-3 derivatives, the photosensitive double bond linker

between C8 and the aromatic moiety should be replaced by a single bond linker. Since there was no synthesis known in the literature for this kind of compounds, various chemical procedures were investigated to obtain the desired target compounds by a 6- to 9-step synthetic procedure.

The synthesis of the xanthine derivative is complex and involves several synthetic steps. The synthesis usually starts with 6-aminouracil, which can be selectively substituted at the 3-position by a previously developed silylation-alkylation method. Nitrosylation at the 5-position and subsequent reduction enables the synthesis of the corresponding 3-substituted 5,6-diaminouracil derivatives, which can subsequently be subjected to a selective amide coupling reaction in the 5-position (see figure 1).

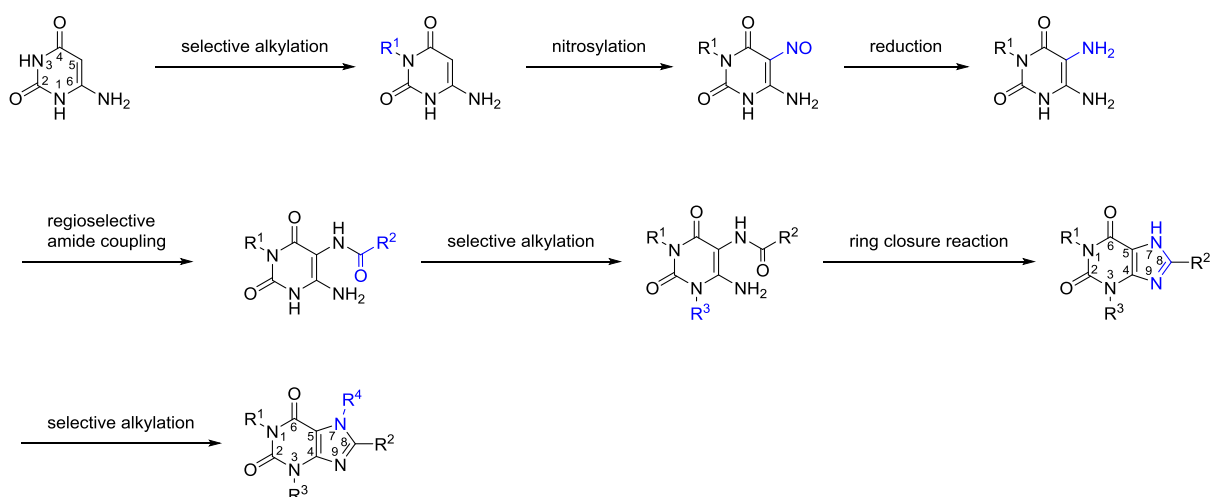


Figure 1: General synthetic procedure for xanthine derivatives

The resulting 6-amino-5-carboxamidouracil derivatives are key intermediates in the synthesis of the targeted xanthine derivatives. They show high thermodynamic stability, and can thus be stored for long time at room temperature. Different substitutions in the C8-position of the xanthine core were explored. For amide formation, hazardous coupling reagents are typically used, which often result in only moderate yields requiring long reaction times. With COMU as a novel coupling reagent for this reaction, the reaction time could be reduced to less than 10 min, and quantitative yields could be achieved. No complex column chromatographic purification was necessary to obtain the products in high purity of over 95%, simply by precipitation with water. This optimization of the synthesis was published in the journal *Frontiers in Chemistry* (Marx, D., Wingen, L.M., Schnakenburg, G., Müller, C.E., Scholz, M.S.

Synthesis of 6-amino-5-carboxamidouracils as precursors for 8-substituted xanthines. *Front. Chem.*, **2019**, *7*, 56, 1–15).

NMR analyses of some of these intermediate compounds showed an unexpected duplication of peaks, which was dependent on the substitution pattern of the carboxylic acids used for the coupling reaction. This phenomenon was investigated by dynamic NMR experiments, 2D NMR spectroscopy and density-function theory (DFT), and the results were published in the journal *Molecules* (Marx, D., Schnakenburg, G., Grimme, S., Müller, C.E. Structural and conformational studies on carboxamides of 5,6-diaminouracils–precursors of biologically active xanthine derivatives. *Molecules* **2019**, *24*, 2168).

This optimization of the xanthine synthesis sequence enabled the preparation of many different xanthine derivatives which were evaluated for their agonistic activity at the orphan receptor MRGPRX4.

Using the 3-substituted 6-amino-5-carboxamidouracil derivatives described above as starting points, a polar phosphonate residue, or bioisosteric analogs, respectively, can be introduced in the 1-position (which later becomes the 3-position in the final xanthine) by an alkylation reaction introducing the desired linker length. The phosphonate group was found to be the best moiety resulting in MRGPRX4 agonists with nanomolar potencies. The diethyl-(4-iodobutyl)phosphonate required for the alkylation could be obtained by an Arbuzov reaction of 1,4-dibromobutane and subsequent Finkelstein reaction with NaI. Alkylation with 1,4-dibromobutane and subsequent Arbuzov reaction was not possible because an intramolecular ring-closure reaction with the 6-amino group takes place instead. The subsequent ring-closure reaction with hexamethyldisilazane (HMDS) to obtain the corresponding xanthine derivative leads to a mixture of the desired double-deprotected phosphonic acid derivative, the single-protected phosphonate ester and the double-protected phosphonate ester, which was difficult to separate by HPLC. Instead of HMDS, NaOH was therefore used as a reagent for the ring-closure reaction leading to the double-protected xanthine derivatives in good yields. Optimized reaction conditions were 80 °C for 30 min resulting in the best yields. These compounds have the decisive advantage that the *N7*-position can subsequently be alkylated using various alkylating reagents. Different *N7*-substitutions showed a dramatic improvement in the agonistic activity. If the reaction time of 30 min is exceeded, and the temperature is increased, a cleavage of the side group at the *N3*-position can be observed. After alkylation of the *N7*-position, which in many cases showed quantitative yields, the phosphonic acid ester could be deprotected using

trimethylsilyl bromide yielding the desired xanthine derivative bearing a phosphonic acid moiety attached via an alkyl (optimally a butyl) linker to the 3-position of the xanthine scaffold (see figure 2).

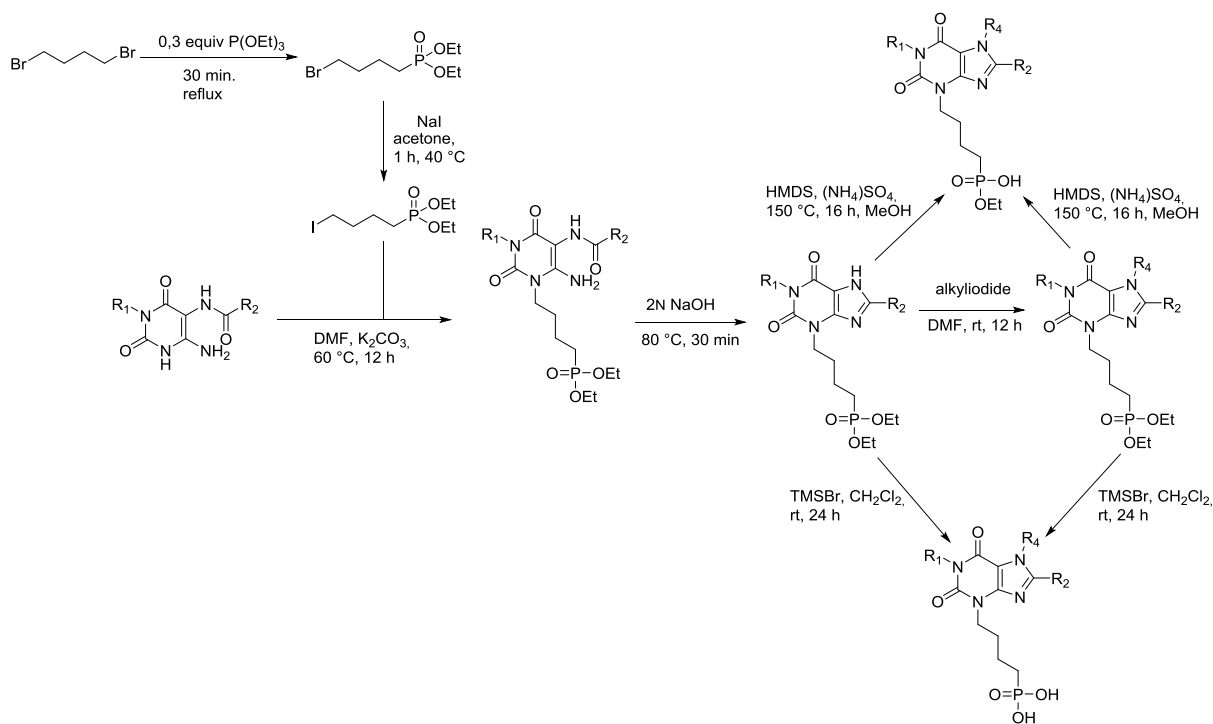


Figure 2: Synthetic route for the newly developed highly potent and selective MRGPRX4 agonists.

The design and synthesis of 56 different xanthine derivatives and the analysis of their structure-activity relationships employing computer-based docking studies (see figure 3) enabled the preparation of the most potent MRGPRX4 agonist currently available with an EC_{50} value of 25.9 nM in β -arrestin assays and 4.26 nM in Ca^{2+} -assay. The new compounds have been protected in a patent application (Marx, D.; Müller, C.; Alnouri, W.; Riedel, Y.; Namasivayam, V.; Pillaiyar, T.; Thimm, D.; Hockemeyer, J. MRGPRX4 agonists and antagonists. *UBN0005(EP)* 2020). The newly synthesized highly potent and selective MRGPRX4 agonists will be useful as tool compounds to study the (patho)physiological roles of the so far sparsely investigated MRGPRX4.

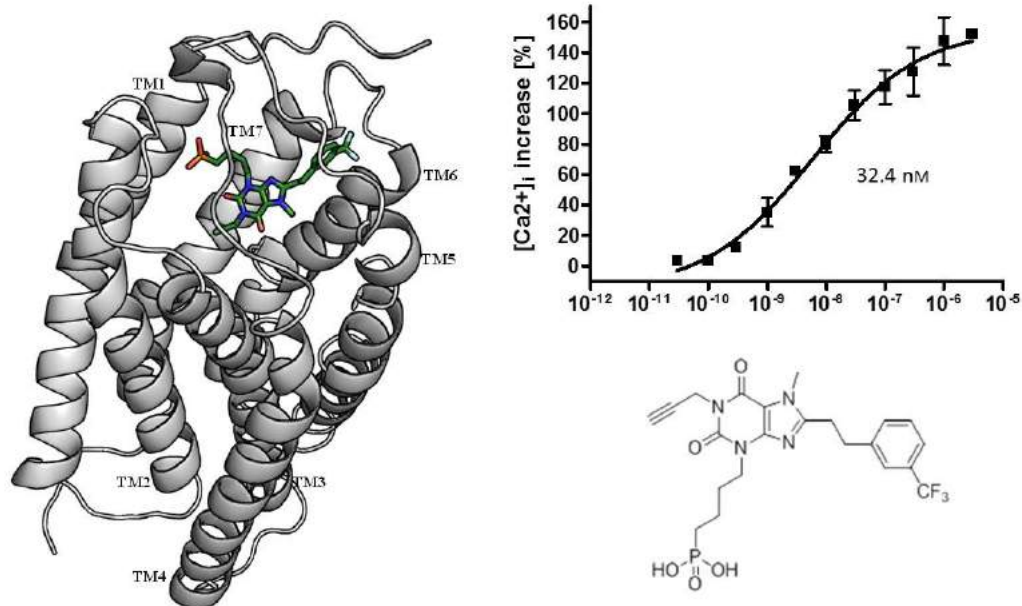


Figure 3: Docking pose and concentration-response curve of one of the most active and selective MRGPRX4 agonist

Moreover, a further orphan receptor from the MRGPRX family was investigated. MRGPRX2, like the other MRGPRX subtypes, is expressed in the dorsal root ganglia of the spinal cord, and also in mast cells. Antagonists of this subclass of receptors are therefore promising potential therapeutics for the treatment of pseudoallergic reactions and itching, as well as chronic pain. In fact, they might be used to replace opioids which have led to the opioid crisis in the USA. The new compounds were synthesized in collaboration with the research group of Prof. Herdewijn, and a modification to increase their water solubility, is described in the last chapter of this thesis.

The synthesis of the tricyclic benzimidazole derivatives, disclosed in the second patent application, usually starts with the corresponding phenylenediamine derivatives. However, the corresponding pyridine derivatives are commercially available only to a very limited extent. In many cases, they had to be synthesized from the corresponding aminopyridines. The amino function was initially protected as a carbamate function. Subsequently, nitration can be carried out via an electrophilic aromatic substitution in the *ortho*-position to the carbamate function. The protective group can then be removed using Ba(OH)₂ under basic conditions, and after reduction over palladium, the corresponding *o,o*-diamines can be obtained. The subsequent ring-closure reaction was performed using an excess of BrCN. A further ring-closure reaction with a β -keto ester allows the synthesis of the final target compounds. By introducing the

nitrogen atom, the water solubility of the subsequent compound was increased from 5 μM to 59 μM (12-fold).

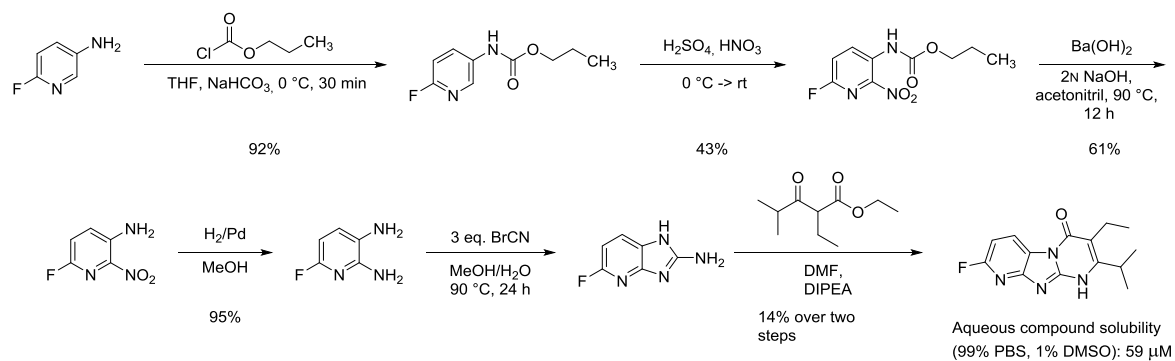


Figure 4: Synthetic procedure of new MRGPRX2 antagonists with improved physicochemical properties.

In summary, the optimization of the synthesis of xanthine derivatives with substituents in various positions, including polar phosphonate groups, was achieved, which enabled the preparation of a large number of MRGPRX4 agonists. The most potent MRGPRX4 agonist described to date with low nanomolar potency could be synthesized, which is currently under investigation for its pharmacological and therapeutic potential. Furthermore, I not only was a leading author on the patent application of the first highly potent and selective MRGPRX2 antagonists, but, in addition, I was also able to further improve the water solubility of the patented compounds, which constitutes a significant improvement of the physicochemical properties of these MRGPRX2 antagonists.

The synthesis and structure-activity relationship of the most active and selective MRGPRX4 agonists and MRGPRX2 antagonists so far has been described. These data represent an important basis for further research to better understand the sparsely known pharmacological properties of these receptors.

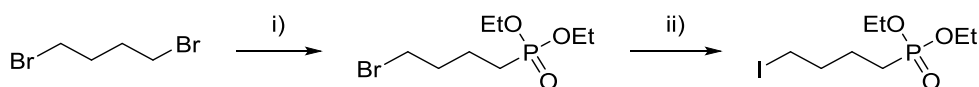
Since clinical tool compounds must not only be potent, but also have other important physicochemical properties the moderate water solubility of the disclosed MRGPRX2 antagonists was further addressed. By the synthesis of a completely new compound class, the water solubility and other physicochemical properties could be considerably improved. The compounds display low micromolar potencies at present and need to be further optimized to

gain nanomolar IC₅₀ values. However, with the optimization of their water solubility, a crucial step has already been taken to develop a potential clinical candidate for stopping mast cell degranulation and thus, the possibility of treating itching and allergic reactions via this receptor.

10. Supporting Information to MRGPRX4 Agonists

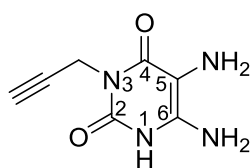
1. Synthesis of intermediates

Scheme S1. Synthesis of 4-iodobutylphosphonic acid diethyl ester.



^aReagents and conditions: (i) 0.25 eq. P(OEt)₃, reflux, 30 min (ii) NaI, acetone, 70 °C, 1 h, 21% over two steps.¹

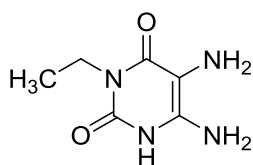
5,6-diamino-3-(prop-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (DM027)



Synthesis of compound **DM027** was done as described in the literature.²

Yield: 86%, yellowish solid (Lit.: 67%²). ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.69 (s, 2H, NH₂), 4.43 (d, *J* = 2.5 Hz, 2H, N3-CH₂), 2.97 (t, *J* = 2.4 Hz, 1H, H_{propargyl}). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 159.4 (CO), 148.7 (CO), 143.3 (C5), 95.3 (C6), 80.3 (C_{propargyl}), 72.3 (C_{propargyl}), 29.0 (N3-CH₂). LC-MS (m/z): 181.0³⁺. Purity (LC-MS): 99.3%.

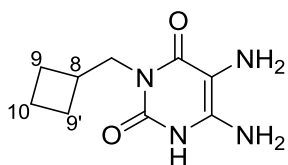
5,6-diamino-3-ethylpyrimidine-2,4(1H,3H)-dione (DM065)



Synthesis of compound **DM065** was done as described in the literature.

Yield: 58%, brown solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.54 (s, 2H, NH₂), 3.73 (q, *J* = 7.0 Hz, 2H, N3-CH₂), 1.03 (t, *J* = 7.0 Hz, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 160.2 (CO), 149.0 (CO), 142.5 (C5), 95.7 (C6), 34.5 (N3-CH₂), 13.4 (CH₃). LC-MS (m/z): 171.0 [M + H]⁺. Purity (LC-MS): 94.5%.

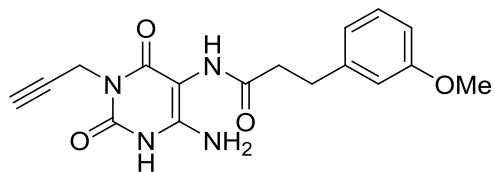
5,6-diamino-3-(cyclobutylmethyl)pyrimidine-2,4(1H,3H)-dione (DM063)



Synthesis was done as described in the literature. Yield: 95%,

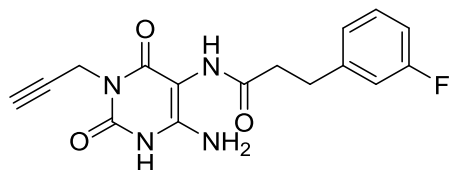
yellowish solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.54 (s, 2H, NH₂), 3.75 (d, *J* = 7.2 Hz, 2H, N3-CH₂), 2.58 (dt, *J* = 15.5, 7.7 Hz, 1H, 8-CH), 1.89 – 1.83 (m, 2H, 10-CH₂), 1.77 – 1.69 (m, 4H, 9-CH₂, 9'-CH₂). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 160.6 (C4), 149.5 (C2), 142.4 (C5), 95.5 (C6), 44.2 (C7), 34.1 (C8), 25.6 (C9, C9'), 17.8 (C10).

***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-methoxyphenyl)propanamide (DM028)**



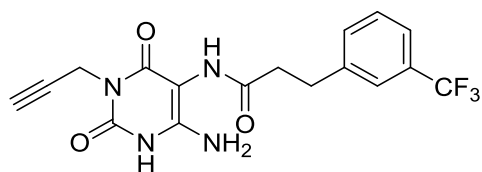
Yield: 90%, brown solid. ^1H NMR (500 MHz, DMSO- d_6) δ 10.57 (s, 1H, N1-H), 8.41 (s, 1H, CONH), 7.22 – 7.16 (m, 1H, H_{arom}), 6.81 (dd, $J = 4.3, 1.9$ Hz, 2H, H_{arom}), 6.75 (dd, $J = 8.3, 2.0$ Hz, 1H, H_{arom}), 5.97 (s, 2H, NH_2), 4.42 (d, $J = 2.4$ Hz, 2H, N3- CH_2), 3.74 (s, 3H, OCH_3), 3.01 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.83 (dd, $J = 9.1, 7.0$ Hz, 2H, CH_2), 2.53 (dd, $J = 9.2, 7.1$ Hz, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO- d_6) δ 171.8 ($\underline{\text{C}}\text{ONH}$), 159.6 ($\underline{\text{C}}_6$), 159.3 (C_{arom}), 150.4 ($\underline{\text{C}}\text{O}$), 149.1 ($\underline{\text{C}}\text{O}$), 143.1 (C_{arom}), 129.3 (C_{arom}), 120.4 (C_{arom}), 113.7 (C_{arom}), 111.4 (C_{arom}), 86.9 (C_5), 80.0 ($\text{C}_{\text{propargyl}}$), 72.3 ($\text{C}_{\text{propargyl}}$), 54.9 (OCH_3), 36.8 (N3- CH_2), 30.9 (CH_2), 28.8 (CH_2). LC-MS (m/z): 343.1 [$\text{M} + \text{H}$] $^+$. Purity (LC-MS): 98.5%.

***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-fluorophenyl)propanamide (DM048)**



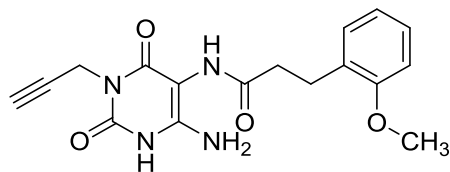
The crude product was used for the next step without further purification. LC-MS (m/z): 331.1 [$\text{M} + \text{H}$] $^+$. Purity (LC-MS): 78.2%.

***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-(trifluoromethyl)phenyl)propanamide (DM052)**



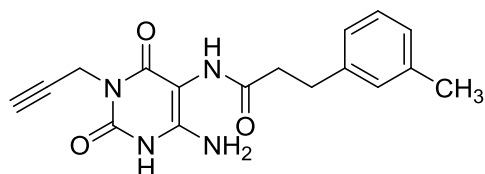
Yield: 72%, off-white solid. ^1H NMR (600 MHz, DMSO- d_6) δ 10.64 (s, 1H, N3- $\underline{\text{H}}$), 8.43 (s, 1H, $\underline{\text{C}}\text{ONH}$), 7.60 - 7.56 (m, 2H, $\underline{\text{H}}_{\text{arom}}$), 7.55 - 7.50 (m, 2H, $\underline{\text{H}}_{\text{arom}}$), 6.04 (s, 2H, $\underline{\text{N}}\underline{\text{H}}_2$), 4.42 - 4.40 (m, 2H, $\underline{\text{N}}\underline{\text{C}}\underline{\text{H}}_2$), 3.00 (s, 1H, $\underline{\text{H}}_{\text{propargyl}}$), 2.95 (t, $J = 7.8$ Hz, 2H, $\underline{\text{C}}\underline{\text{O}}\underline{\text{C}}\underline{\text{H}}_2$), 2.61 - 2.53 (m, 2H, $\underline{\text{C}}\underline{\text{O}}\underline{\text{C}}\underline{\text{H}}_2\underline{\text{C}}\underline{\text{H}}_2$). ^{13}C NMR (151 MHz, DMSO- d_6) δ 171.8 ($\underline{\text{C}}\text{ONH}$), 159.8 ($\underline{\text{C}}_6$), 150.7 ($\underline{\text{C}}_4$ or $\underline{\text{C}}_2$), 149.4 ($\underline{\text{C}}_4$ or $\underline{\text{C}}_2$), 143.2 (C_{arom}), 132.7 (C_{arom}), 129.5 (C_{arom}), 129.2 (q, $^2J_{\text{C},\text{F}} = 31.1$ Hz, 1C, $\underline{\text{C}}\underline{\text{C}}\underline{\text{F}}_3$), 125.0 ($^3J_{\text{C},\text{F}} = 4.0$ Hz, 1C, $\underline{\text{C}}\underline{\text{H}}\underline{\text{C}}\underline{\text{C}}\underline{\text{F}}_3$), 124.5 (q, $^1J_{\text{C},\text{F}} = 273.0$ Hz, 1C, $\underline{\text{C}}\underline{\text{F}}_3$), 122.8 (q, $^3J_{\text{C},\text{F}} = 4.1$ Hz, 1C, $\underline{\text{C}}\underline{\text{H}}\underline{\text{C}}\underline{\text{C}}\underline{\text{F}}_3$), 87.0 (C_5), 80.2 ($\text{C}_{\text{propargyl}}$), 72.5 ($\text{C}_{\text{propargyl}}$), 36.6 ($\underline{\text{C}}\underline{\text{O}}\underline{\text{C}}\underline{\text{H}}_2$), 30.7 ($\underline{\text{N}}\underline{\text{C}}\underline{\text{H}}_2$ or $\underline{\text{C}}\underline{\text{O}}\underline{\text{C}}\underline{\text{H}}_2\underline{\text{C}}\underline{\text{H}}_2$), 29.0 ($\underline{\text{N}}\underline{\text{C}}\underline{\text{H}}_2$ or $\underline{\text{C}}\underline{\text{O}}\underline{\text{C}}\underline{\text{H}}_2\underline{\text{C}}\underline{\text{H}}_2$). LC-MS: positive mode [m/z] = 380.9 ([$\text{M} + \text{H}$] $^+$). ESI-MS purity = 97%.

***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(2-methoxyphenyl)propanamide (DM054)**



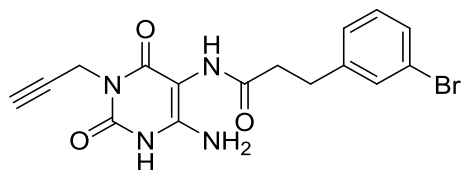
Yield: quantitative, white solid. ^1H NMR (500 MHz, DMSO- d_6) δ 10.58 (s, 1H, N1-H), 8.35 (s, 1H, CONH), 7.18 (ddd, $J = 14.2, 7.5, 1.6$ Hz, 2H, H_{arom}), 6.97 – 6.93 (m, 1H, H_{arom}), 6.87 (td, $J = 7.4, 1.0$ Hz, 1H, H_{arom}), 5.99 (s, 2H, NH_2), 4.42 (d, $J = 2.4$ Hz, 2H, N3- CH_2), 3.80 (s, 3H, OCH_3), 3.01 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.81 (dd, $J = 9.5, 6.8$ Hz, 2H, CH_2), 2.49 – 2.46 (m, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO- d_6) δ 172.1 ($\underline{\text{CONH}}$), 159.6 (C_6), 157.0 (C_{arom}), 150.4 (CO), 149.1 (CO), 129.3 (C_{arom}), 127.2 (C_{arom}), 120.3 (C_{arom}), 110.6 (C_{arom}), 86.9 (C_5), 80.0 ($\text{C}_{\text{propargyl}}$), 72.3 ($\text{C}_{\text{propargyl}}$), 55.2 (OCH_3), 35.1 (N3- CH_2), 28.8 (CH_2), 25.5 (CH_2). LC-MS: positive mode $[\text{m}/\text{z}] = 343.0$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 96.6%.

***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(*m*-tolyl)propanamide (DM090)**



Yield: quantitative, off-white solid. ^1H NMR (500 MHz, DMSO- d_6) δ 10.58 (s, 1H, N1-H), 8.40 (s, 1H, CONH), 7.17 (t, $J = 7.5$ Hz, 1H, H_{arom}), 7.06 (s, 1H, H_{arom}), 7.02 (d, $J = 7.6$ Hz, 1H, H_{arom}), 6.99 (d, $J = 7.4$ Hz, 1H, H_{arom}), 5.96 (s, 2H, NH_2), 4.42 (d, $J = 2.4$ Hz, 2H, N3- CH_2), 3.01 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.82 (dd, $J = 9.2, 6.9$ Hz, 2H, CH_2), 2.54 – 2.50 (m, 2H, CH_2), 2.28 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO- d_6) δ 171.9 ($\underline{\text{CONH}}$), 159.6 (C_6), 150.4 (CO), 149.1 (CO), 141.4 (C_{arom}), 137.3 (C_{arom}), 128.8 (C_{arom}), 128.2 (C_{arom}), 126.4 (C_{arom}), 125.2 (C_{arom}), 86.9 (C_5), 80.0 ($\text{C}_{\text{propargyl}}$), 72.3 ($\text{C}_{\text{propargyl}}$), 36.8 (N3- CH_2), 30.8, (CH_2) 28.8 (CH_2), 21.0 (CH_3). LC-MS: positive mode $[\text{m}/\text{z}] = 327.1$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 97.6%.

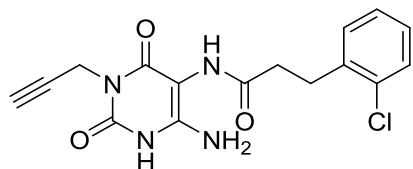
***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-bromophenyl)propanamide (DM089)**



Yield: 98%, brown solid. ^1H NMR (500 MHz, DMSO- d_6) δ 10.59 (s, 1H, N1-H), 8.41 (s, 1H, CONH), 7.47 – 7.43 (m, 1H, H_{arom}), 7.38 (dt, $J = 6.7, 2.1$ Hz, 1H, H_{arom}), 7.28 – 7.23 (m, 2H, H_{arom}), 6.00 (s, 2H, NH_2), 4.42 (d, $J = 2.4$ Hz, 2H, N3- CH_2), 3.01 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.88 – 2.83 (m, 2H, CH_2), 2.54 (dd, $J = 8.9, 7.1$ Hz, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO- d_6) δ 171.6 ($\underline{\text{CONH}}$), 159.6 (C_6), 150.4

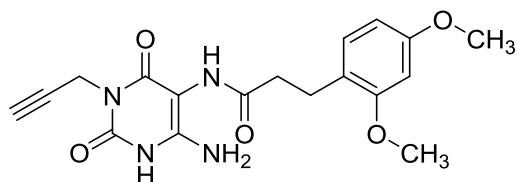
(CO), 149.1 (CO), 144.5 (C_{arom}), 131.0 (C_{arom}), 130.4 (C_{arom}), 128.7 (C_{arom}), 127.3 (C_{arom}), 121.6 (C_{arom}), 86.8 (C5), 80.0 (C_{propargyl}), 72.3 (C_{propargyl}), 36.4 (N3-CH₂), 30.4 (CH₂), 28.8 (CH₂). LC-MS: positive mode [m/z] = 391.1 ([M+H]⁺). ESI-MS purity = 96.2%.

***N*-(6-amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(2-chlorophenyl)propanamide (DM442)**



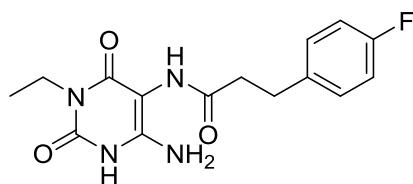
Yield: 82%, white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.61 (s, 1H, N1-H), 8.44 (s, 1H, CONH), 7.42 (dd, *J* = 7.7, 1.4 Hz, 1H, H_{arom}), 7.40 – 7.37 (m, 1H, H_{arom}), 7.29 (td, *J* = 7.3, 1.4 Hz, 1H, H_{arom}), 7.26 – 7.22 (m, 1H, H_{arom}), 6.06 (s, 2H, NH₂), 4.42 (d, *J* = 2.4 Hz, 2H, N3-CH₂), 3.02 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 2.96 (dd, *J* = 9.5, 6.7 Hz, 1H, CH₂), 2.55 (dd, *J* = 9.5, 6.7 Hz, 1H, CH₂). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.5 (CONH), 159.6 (C6), 150.5 (CO), 149.2 (CO), 138.8 (C_{arom}), 132.8 (C_{arom}), 130.4 (C_{arom}), 129.1 (C_{arom}), 127.9 (C_{arom}), 127.3 (C_{arom}), 86.8 (C5), 80.0 (C_{propargyl}), 72.4 (C_{propargyl}), 34.7 (N3-CH₂), 28.8 (CH₂), 28.5 (CH₂). LC-MS: positive mode [m/z] = 347.1 ([M+H]⁺). ESI-MS purity = 98.5%.

***N*-(6-Amino-2,4-dioxo-3-(prop-2-yn-1-yl)-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(2,4-dimethoxyphenyl)propanamide (DM091)**



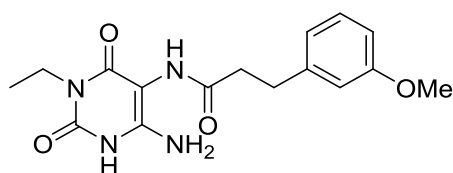
Yield: 88%, offwhite solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.59 (s, 1H, N1-H), 8.34 (s, 1H, CONH), 7.05 (d, *J* = 8.3 Hz, 1H, H_{arom}), 6.52 (d, *J* = 2.3 Hz, 1H, H_{arom}), 6.44 (dd, *J* = 8.2, 2.4 Hz, 1H, H_{arom}), 5.99 (s, 2H, NH₂), 4.42 (d, *J* = 2.2 Hz, 2H, N3-CH₂), 3.78 (s, 3H, OCH₃), 3.73 (s, 3H, OCH₃), 3.02 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 2.73 (dd, *J* = 9.5, 6.8 Hz, 2H, CH₂), 2.44 (dd, *J* = 9.5, 6.9 Hz, 2H, CH₂). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.2 (CONH), 159.7 (C6), 158.9 (C_{arom}), 157.9 (C_{arom}), 150.5 (CO), 149.2 (CO), 129.6 (C_{arom}), 121.4 (C_{arom}), 104.4 (C_{arom}), 98.3 (C_{arom}), 86.9 (C5), 80.0 (C_{propargyl}), 72.4 (C_{propargyl}), 55.3 (OCH₃), 55.1 (OCH₃), 35.4 (N3-CH₂), 28.8 (CH₂), 24.9 (CH₂). LC-MS: positive mode [m/z] = 373.2 ([M+H]⁺). ESI-MS purity: 97.4%.

***N*-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-fluorophenyl)propanamide (DM070)**



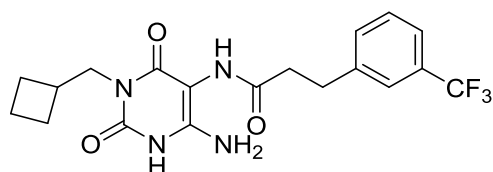
Yield: 98%, brown solid. ^1H NMR (600 MHz, DMSO- d_6) δ 10.37 (s, 1H, N3-H), 8.37 (s, 1H, CONH), 7.30 – 7.23 (m, 2H, H_{arom}), 7.12 – 7.05 (m, 2H, H_{arom}), 5.82 (s, 2H, NH_2), 3.71 (q, $J = 6.9$ Hz, 2H, NCH_2), 2.86 – 2.81 (m, 2H, COCH_2), 2.54 – 2.50 (m, 2H, COCH_2CH_2), 1.03 (t, $J = 7.0$ Hz, 3H, CH_2CH_3). ^{13}C NMR (126 MHz, DMSO- d_6) δ 171.8 (CONH), 160.8 (d, $^1J_{\text{C,F}} = 242$ Hz, 1C, C_{aromF}), 160.5 (C6), 150.0 (C4 or C2), 149.7 (C4 or C2), 137.7 (C_{arom}), 130.1 (d, $^2J_{\text{C,F}} = 7.9$ Hz, 2C, CHCF), 115.1 (C_{arom}), 115.0 (C_{arom}), 87.4 (C5), 37.0 (NCH_2), 34.5 (NCH_2CH or COCH_2CH_2), 30.2 (NCH_2CH or COCH_2CH_2), 13.4 (CH_2CH_3). LC-MS: positive mode $[m/z] = 321.1$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 98%.

***N*-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-methoxyphenyl)propanamide (DM068)**



Yield: 99%, brown solid. ^1H NMR (600 MHz, DMSO- d_6) δ 10.38 (s, 1H, N3-H), 8.39 (s, 1H, CONH), 7.18 (t, $J = 8.0$ Hz, 1H, H_{arom}), 6.79 (s, 2H, H_{arom}), 6.74 (d, $J = 8.1$ Hz, 1H, H_{arom}), 5.83 (s, 2H, NH_2), 3.73 (s, 3H, OCH_3), 3.68 – 3.72 (m, 2H, NCH_2), 2.84 – 2.77 (m, 2H, COCH_2), 2.53 – 2.50 (m, 2H, COCH_2CH_2), 1.03 (t, $J = 6.9$ Hz, 3H, CH_2CH_3). ^{13}C NMR (151 MHz, DMSO- d_6) δ 171.9 (CONH), 160.6 (C6), 159.5 (COCH_3), 150.1 (C4 or C2), 149.7 (C4 or C2), 143.3 (C_{arom}), 129.5 (C_{arom}), 120.6 (C_{arom}), 113.9 (C_{arom}), 111.6 (C_{arom}), 87.4 (C5), 55.0 (OCH_3), 37.0 (NCH_2), 34.5 (COCH_2), 31.2 (COCH_2CH_2), 13.4 (CH_2CH_3). LC-MS: positive mode $[m/z] = 333.0$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 99%.

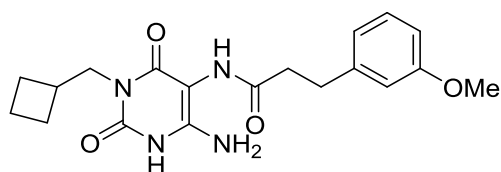
***N*-(6-Amino-3-(cyclobutylmethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-(trifluoromethyl)phenyl)propanamide (DM074)**



Yield: 90%, brown solid. ^1H NMR (600 MHz, DMSO- d_6) δ 10.36 (s, 1H, N3-H), 8.41 (s, 1H, CONH), 7.60 – 7.56 (m, 2H, H_{arom}), 7.54 – 7.50 (m, 2H, H_{arom}), 5.85 (s, 2H, NH_2), 3.73 (d, $J = 7.1$ Hz, 2H, NCH_2), 2.94 (t, $J = 7.8$ Hz, 2H, COCH_2), 2.58 – 2.54 (m, 2H, COCH_2CH_2), 1.90 – 1.85 (m, 2H, $\text{H}_{\text{cyclobutyle}}$), 1.76 (dd, $J = 8.6, 4.8$ Hz, 2H, $\text{H}_{\text{cyclobutyle}}$), 1.73 – 1.69 (m, 2H, $\text{H}_{\text{cyclobutyle}}$). ^{13}C -NMR (151 MHz, DMSO- d_6) δ 171.6 (CONH), 161.0 (C6), 150.3 (C4 or C2), 150.1 (C4 or C2), 143.2 (C_{arom}),

132.7 (C_{arom}), 129.5 (C_{arom}), 129.2 (q, ²J_{C,F} = 31.7 Hz, 1C, CCF₃), 125.0 (q, ³J_{C,F} = 3.8 Hz, 1C, CHCF₃), 123.9 (q, ¹J_{C,F} = 272.9 Hz, 1C, CF₃), 122.8 (q, ³J_{C,F} = 3.8 Hz, 1C, CHCF₃), 87.2 (C₅), 44.1 (NCH₂), 36.7 (COCH₂), 34.2 (NCH₂CH or COCH₂CH₂), 30.7 (NCH₂CH or COCH₂CH₂), 25.6 (2C, C_{cyclobutyle}), 17.9 (C_{cyclobutyle}). LC-MS: positive mode [m/z] = 411.3 ([M+H]⁺). ESI-MS purity = 89%.

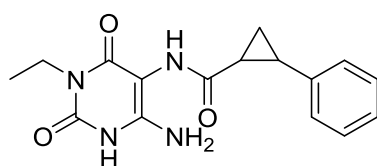
N-(6-Amino-3-(cyclobutylmethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-methoxyphenyl)propanamide (DM067)



Yield: 95%, brown solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.36 (s, 1H, N3-H), 8.38 (s, 1H, CONH), 7.18 (t, *J* = 8.0 Hz, 1H, H_{arom}), 6.82 – 6.77 (m, 2H, H_{arom}), 6.76 – 6.71 (m, 1H, H_{arom}), 5.82 (s, 2H, NH₂), 3.74 (s, 2H, NCH₂), 3.72 (s, 3H, OCH₃), 2.84 – 2.78 (m, 2H, COCH₂), 2.59 – 2.53 (m, 1H, NCH₂CH), 2.52 (s, 2H, COCH₂CH₂), 1.92 – 1.84 (m, 2H, H_{cyclobutyle}), 1.79 – 1.74 (m, 2H, H_{cyclobutyle}), 1.73 – 1.69 (m, 2H, H_{cyclobutyle}).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ 171.9 (CONH), 161.0 (C₆), 159.5 (COCH₃), 150.2 (C₄ or C₂), 150.0 (C₄ or C₂), 143.3 (C_{arom}), 129.5 (C_{arom}), 120.6 (C_{arom}), 113.9 (C_{arom}), 111.6 (C_{arom}), 87.3 (C₅), 55.0 (OCH₃), 44.1 (NCH₂), 37.0 (COCH₂), 34.2 (NCH₂CH or COCH₂CH₂), 31.2 (NCH₂CH or COCH₂CH₂), 25.6 (2C, C_{cyclobutyle}), 17.9 (C_{cyclobutyle}). LC-MS: positive mode [m/z] = 373.3 ([M+H]⁺). ESI-MS purity = 95%.

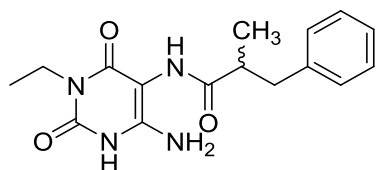
N-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-phenylcyclopropane-1-carboxamide (DM119)



Yield: 89%, white solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.35 (s, 1H, N1-H), 8.68 (s, 1H, CONH), 7.29 (t, *J* = 7.5 Hz, 2H, H_{arom}), 7.19 (d, *J* = 7.7 Hz, 1H, H_{arom}), 7.14 (d, *J* = 7.9 Hz, 2H, H_{arom}), 5.90 (s, 2H, NH₂), 3.72 (q, *J* = 6.9 Hz, 2H, N3-CH₂),

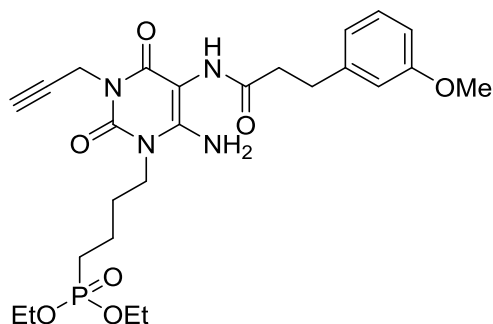
2.28 (dt, *J* = 9.5, 6.0 Hz, 1H, CH), 2.09 (dt, *J* = 8.8, 4.7 Hz, 1H, CH), 1.37 (dt, *J* = 9.0, 4.5 Hz, 1H, CH), 1.26 – 1.20 (m, 1H, CH), 1.04 (t, *J* = 6.9 Hz, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 171.5 (CONH), 160.4 (C₆), 149.9 (CO), 149.5 (CO), 141.2 (C_{arom}), 128.2 (2C, C_{arom}), 125.9 (3C, C_{arom}), 87.4 (C₅), 34.4 (N3-CH₂), 25.6 (C_{cyclopropyle}), 24.3 (C_{cyclopropyle}), 16.1 (C_{cyclopropyle}), 13.2 (CH₃). LC-MS: positive mode [m/z] = 315.2 ([M+H]⁺). ESI-MS purity: 95.9%.

***N*-(6-Amino-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-3-phenylpropanamide (DM120)**



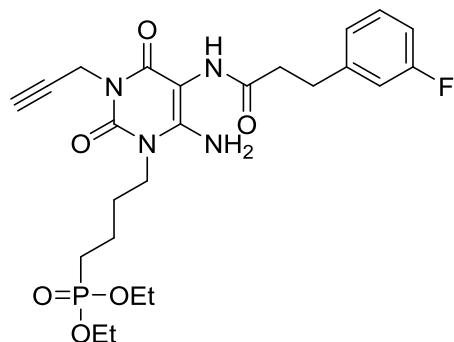
Yield: quantitative, white solid. ^1H NMR (500 MHz, DMSO- d_6) δ 10.37 (s, 1H, N1-H), 8.42 (s, 1H, CONH), 7.28 (t, $J = 7.4$ Hz, 2H, H_{arom}), 7.25 – 7.21 (m, 2H, H_{arom}), 7.18 (t, $J = 7.2$ Hz, 1H, H_{arom}), 5.51 (s, 2H, N3-NH $_2$), 3.72 (q, $J = 6.9$ Hz, 2H, CH $_2$), 2.98 (dd, $J = 13.4, 6.2$ Hz, 1H, CH), 2.78 – 2.68 (m, 1H, CH), 2.57 – 2.51 (m, 1H, CH), 1.04 (t, $J = 7.0$ Hz, 3H, CH $_3$), 1.00 (d, $J = 6.8$ Hz, 3H, CH $_3$). ^{13}C NMR (126 MHz, DMSO- d_6) δ 175.4 (CONH), 160.3 (C $_6$), 149.6 (CO), 149.5 (CO), 140.1 (C $_{\text{arom}}$), 128.9 (2C, C $_{\text{arom}}$), 128.1 (2C, C $_{\text{arom}}$), 125.9 (C $_{\text{arom}}$), 87.5 (C $_5$), 41.0 (CCH $_3$), 34.4 (N3-CH $_2$), 16.8 (CH $_3$), 13.2 (CH $_3$). LC-MS: positive mode $[m/z] = 317.0$ ($[\text{M}+\text{H}]^+$). ESI-MS purity: 98.3%.

Diethyl (4-(6-amino-5-(3-(3-methoxyphenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM031)



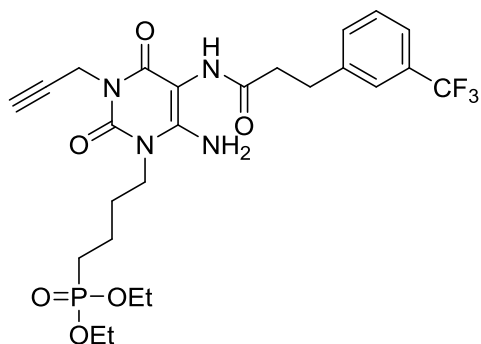
Yield: 42%, colorless oil. ^1H -NMR (600 MHz, DMSO- d_6) δ 8.39 (s, 1H, CONH), 6.83 – 6.78 (m, 2H, H_{arom}), 6.75 (td, $J = 8.1, 3.4$ Hz, 1H, H_{arom}), 6.62 – 6.66 (m, 1H, H_{arom}), 6.64 (s, 2H, NH $_2$), 4.47 (d, $J = 2.2$ Hz, 2H, N3-CH $_2$), 4.01 – 3.94 (m, 4H, 2 x OCH $_2$), 3.88 – 3.82 (m, 2H, N1-CH $_2$), 3.73 (s, 3H, OCH $_3$), 3.01 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.85 – 2.81 (m, 2H, COCH $_2$), 2.56 – 2.51 (m, 2H, COCH $_2$ CH $_2$), 1.80 – 1.73 (m, 2H, C $_{\text{alkyl}}$), 1.62 (dt, $J = 15.1, 7.7$ Hz, 2H, C $_{\text{alkyl}}$), 1.57 – 1.48 (m, 2H, C $_{\text{alkyl}}$), 1.22 (t, $J = 7.0$ Hz, 6H, CH $_2$ CH $_3$). ^{13}C -NMR (151 MHz, DMSO- d_6) δ 172.3 (CONH), 162.5 (OCH $_3$), 159.5 (C $_6$), 151.9 (C $_4$ or C $_2$), 149.8 (C $_4$ or C $_2$), 143.4 (C $_{\text{arom}}$), 129.5 (C $_{\text{arom}}$), 120.5 (C $_{\text{arom}}$), 113.9 (C $_{\text{arom}}$), 111.6 (C $_{\text{arom}}$), 87.4 (C $_5$), 80.1 (C $_{\text{propargyl}}$), 72.6 (C $_{\text{propargyl}}$), 61.0 (d, $^2J_{\text{C,P}} = 6.6$ Hz, 2C, POCH $_2$), 55.0 (OCH $_3$), 42.3 (N1-CH $_2$), 37.0 (N3-CH $_2$), 30.0 (COCH $_2$), 28.2 (d, $^2J_{\text{C,P}} = 15$ Hz, 1C, PCH $_2$ CH $_2$), 24.2 (d, $^1J_{\text{C,P}} = 134.2$ Hz, 1C, PCH $_2$), 19.3 (C $_{\text{alkyl}}$), 16.4 (d, $^3J_{\text{C,P}} = 5$ Hz, 2C, POCH $_2$ CH $_3$). ^{31}P -NMR (243 MHz, DMSO- d_6) δ 32.7. LC-MS: positive mode $[m/z] = 535.0$ ($[\text{M}+\text{H}]^+$). ESI-MS purity: 85.2%.

Diethyl (4-(6-amino-5-(3-(3-fluorophenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM050)



Compound **DM050** was used for the next step without further purification. LC-MS: positive mode $[m/z]=523.2$ ($[M+H]^+$). ESI-MS purity: 83.5%.

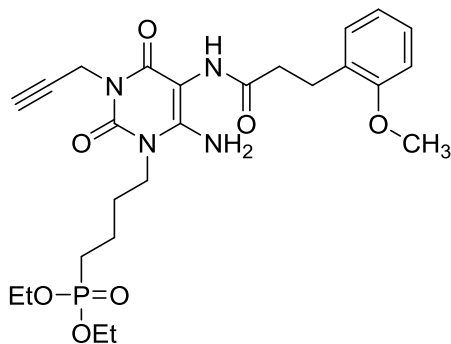
Diethyl (4-(6-amino-2,4-dioxo-3-(prop-2-yn-1-yl)-5-(3-(3-(trifluoromethyl)phenyl)prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM056) Yield: 51%,



colorless oil. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 8.41 (s, 1H, CONH), 7.61 – 7.57 (m, 2H, H_{arom}), 7.55 – 7.51 (m, 2H, H_{arom}), 6.68 (s, 2H, NH_2), 4.47 (d, $J=2.2$ Hz, 2H, N3-CH_2), 3.99 – 3.95 (m, 4H, 2 x OCH_2), 3.87 – 3.83 (m, 2H, N1-CH_2), 3.00 (t, $J=2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.96 (d, $J=7.7$ Hz, 2H, COCH_2), 2.60 – 2.57 (m, 2H, COCH_2CH_2), 1.79 –

1.74 (m, 2H, H_{alkyl}), 1.64 – 1.60 (m, 2H, H_{alkyl}), 1.52 (td, $J=12.6, 7.8$ Hz, 2H, H_{alkyl}), 1.21 (d, $J=6.9$ Hz, 6H, CH_2CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ 172.0 (CONH), 158.2 (C6), 151.9 (C4 or C2), 149.8 (C4 or C2), 143.3 (C_{arom}), 132.7 (C_{arom}), 129.5 (C_{arom}), 129.2 (q, $^2J_{\text{C,F}}=30.8$ Hz, 1C, CCF_3), 125.0 (q, $^3J_{\text{C,F}}=3.5$ Hz, 1C, C_{arom}), 124.4 (q, $^1J_{\text{C,F}}=272.2$ Hz, 1C, CF_3), 122.8 (q, $^3J_{\text{C,F}}=3.3$ Hz, 1C, C_{arom}), 87.3 (C5), 80.1 ($\text{C}_{\text{propargyl}}$), 72.6 ($\text{C}_{\text{propargyl}}$), 61.0 (d, $^2J_{\text{C,P}}=6.7$ Hz, 2C, POCH_2), 42.3 (N1-CH_2), 36.7 (N3-CH_2), 35.9 (COCH_2), 30.9 (COCH_2CH_2), 28.3 (d, $^2J_{\text{C,P}}=15.2$ Hz, 1C, PCH_2CH_2), 24.2 (d, $^1J_{\text{C,P}}=138.7$ Hz, 1C, PCH_2), 19.3 (d, $^3J_{\text{C,P}}=4.2$ Hz, 1C, $\text{P}(\text{CH}_2)_2\text{CH}_2$), 16.4 (d, $^3J_{\text{C,P}}=5$ Hz, 2C, POCH_2CH_3). $^{31}\text{P-NMR}$ (243 MHz, $\text{DMSO-}d_6$) δ 32.5. LC-MS: positive mode $[m/z]=573.0$ ($[M+H]^+$). ESI-MS purity: 91.2%.

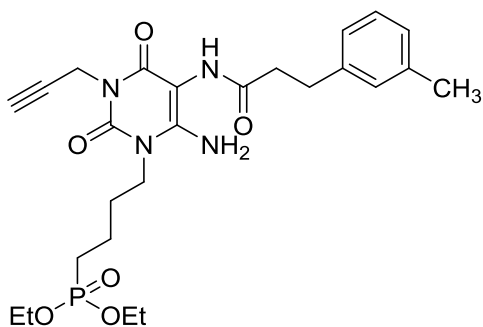
Diethyl (4-(6-amino-5-(3-(2-methoxyphenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM066)



Yield: 37%, colorless oil. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 8.34 (s, 1H, CONH), 7.19 – 7.15 (m, 2H, H_{arom}), 6.95 (d, $J = 8.1$ Hz, 1H, H_{arom}), 6.86 (t, $J = 7.4$ Hz, 1H, H_{arom}), 6.65 (s, 2H, NH_2), 3.98 – 3.95 (m, 6H, 2 x OCH_2 , N3-CH_2), 3.87 – 3.83 (m, 2H, N1-CH_2), 3.79 (s, 3H, OCH_3), 3.00 (t, $J = 2.3$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.82 – 2.79 (m, 2H, COCH_2), 2.48-2.52 (m, 2H, COCH_2CH_2) 1.79 – 1.73

(m, 2H, H_{alkyl}), 1.59-1.63 (m, 2H, H_{alkyl}), 1.50-1.54 (m, 2H, H_{alkyl}), 1.21 (t, $J = 6.7$ Hz, 6H, CH_2CH_3). $^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ 172.6 (CONH), 158.3 (C6), 157.2 (COCH_3), 151.9 (C4 or C2), 149.8 (C4 or C2), 129.5 (C_{arom}), 129.4 (C_{arom}), 127.4 (C_{arom}), 120.4 (C_{arom}), 110.7 (C_{arom}), 87.4 (C5), 80.1 ($\text{C}_{\text{propargyl}}$), 72.6 ($\text{C}_{\text{propargyl}}$), 61.1 (d, $^2J_{\text{C,P}} = 6.6$ Hz, 2C, POCH_2), 55.4 (OCH_3), 42.3 (N1-CH_2), 35.3 (N3-CH_2), 30.0 (COCH_2), 28.3 (d, $^2J_{\text{C,P}} = 15.4$ Hz, 1C, PCH_2CH_2), 25.6 (COCH_2CH_2), 24.2 (d, $^1J_{\text{C,P}} = 137.8$ Hz, 1C, PCH_2), 19.3 (d, $^3J_{\text{C,P}} = 4.1$ Hz, 1C, $\text{P}(\text{CH}_2)_2\text{CH}_2$), 16.4 (d, $^3J_{\text{C,P}} = 4.9$ Hz, 2C, POCH_2CH_3). $^{31}\text{P-NMR}$ (243 MHz, $\text{DMSO-}d_6$) δ 32.8. LC-MS: positive mode $[\text{m/z}] = 535.2$ ($[\text{M}+\text{H}]^+$). ESI-MS purity: 92.7%.

Diethyl (4-(6-amino-2,4-dioxo-3-(prop-2-yn-1-yl)-5-(3-(m-tolyl)propanamido)-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM098)

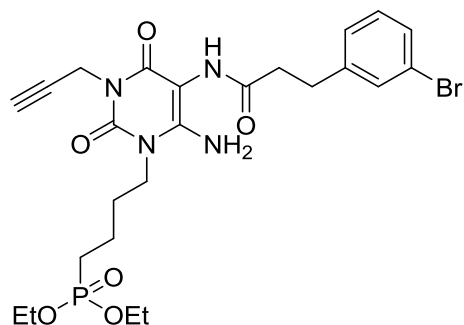


Yield: 70% (colorless oil); $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 8.39 (s, 1H, CONH), 7.16 (t, $J = 7.5$ Hz, 1H, H_{arom}), 7.06 (s, 1H, H_{arom}), 7.02 (d, $J = 7.6$ Hz, 1H, H_{arom}), 6.99 (d, $J = 7.5$ Hz, 1H, H_{arom}), 6.62 (s, 2H, NH_2), 4.47 (d, $J = 2.3$ Hz, 2H, N3-CH_2), 4.01 – 3.95 (m, 4H, 2 x OCH_2), 3.88 – 3.82 (m, 2H, N1-CH_2), 3.00

(t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 2.85 – 2.77 (m, 2H, CH_2CH_2), 2.54 – 2.51 (m, 2H, COCH_2CH_2), 2.28 (s, 3H, CH_3), 1.80 – 1.72 (m, 2H, H_{alkyl}), 1.62 (dt, $J = 15.1, 7.6$ Hz, 2H, H_{alkyl}), 1.52 (dp, $J = 19.9, 7.5$ Hz, 2H, H_{alkyl}), 1.22 (t, $J = 7.1$ Hz, 6H, 2 x OCH_2CH_3). $^{13}\text{C-NMR}$ (126 MHz, $\text{DMSO-}d_6$) δ 172.3 (CONH), 158.2 (C6), 151.9 (C4 or C2), 149.8 (C4 or C2), 141.6 (C_{arom}), 137.5 (C_{arom}), 129.0 (C_{arom}), 128.4 (C_{arom}), 126.6 (C_{arom}), 125.3 (C_{arom}), 87.4 (C5), 80.1 ($\text{C}_{\text{propargyl}}$), 72.6 ($\text{C}_{\text{propargyl}}$), 61.0 (d, $^2J_{\text{C,P}} = 6.2$ Hz, 2C, POCH_2), 42.3 (N1-CH_2), 37.1 (C_{alkyl}), 30.9 (C_{alkyl}), 30.0 (C_{alkyl}), 28.3 (d, $^2J_{\text{C,P}} = 15.1$ Hz, PCH_2CH_2), 24.2 (d, $^1J_{\text{C,P}} = 136.4$ Hz, PCH_2), 21.2 (C_{alkyl}), 19.3 (d, $^3J_{\text{C,P}} = 5.5$ Hz, $\text{P}(\text{CH}_2)_2\text{CH}_2$), 16.5 (C_{alkyl}), 16.4 (d, $^3J_{\text{C,P}} = 5.3$ Hz, 2C,

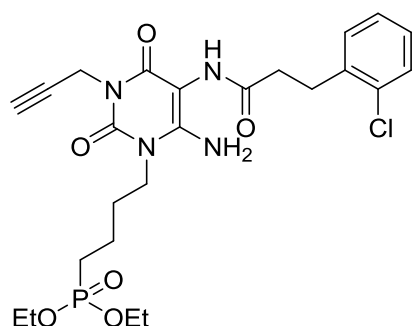
POCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆) δ 32.7. LC-MS: positive mode [m/z]= 519.2 ([M+H]⁺). ESI-MS purity: 83.2%.

Diethyl (4-(6-amino-5-(3-(3-bromophenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2*H*)-yl)butyl)phosphonate (DM097)



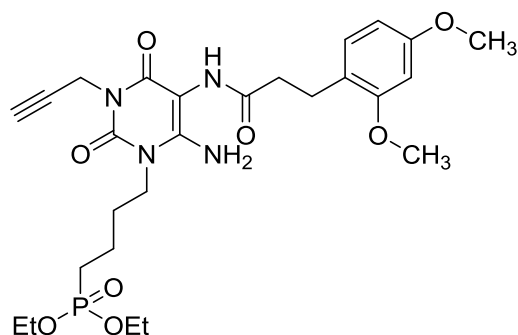
Compound **DM050** was used for the next step without further purification. LC-MS: positive mode [m/z]= 583.1 ([M+H]⁺). ESI-MS purity: 74.7%.

Diethyl (4-(6-amino-5-(3-(2-chlorophenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2*H*)-yl)butyl)phosphonate (DM444)



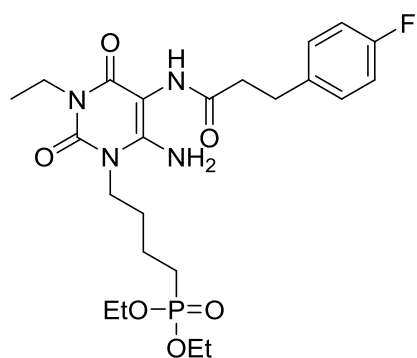
Yield: 80% (colorless oil); ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.43 (s, 1H, CONH), 7.42 (dd, *J* = 7.8, 1.5 Hz, 1H, H_{arom}), 7.39 (dd, *J* = 7.7, 1.8 Hz, 1H, H_{arom}), 7.31 – 7.26 (m, 1H, H_{arom}), 7.26 – 7.22 (m, 1H, H_{arom}), 6.71 (s, 2H, NH₂), 4.48 (d, *J* = 2.4 Hz, 2H, N3-CH₂), 4.02 – 3.94 (m, 6H, 2 x OCH₂, N1-CH₂), 3.02 (t, *J* = 2.4 Hz, 1H, H_{propargyl}), 2.99 – 2.93 (m, 2H, CH₂), 2.59 – 2.55 (m, 2H, CH₂), 1.79 – 1.74 (m, 2H, CH₂), 1.69 – 1.59 (m, 2H, CH₂), 1.58 – 1.49 (m, 2H, CH₂), 1.23 (t, *J* = 7.0, 2.0 Hz, 6H, 2 x OCH₂CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.8 (CONH), 158.1 (C₆), 151.8 (C₄ or C₂), 149.6 (C₄ or C₂), 138.9 (C_{arom}), 132.8 (C_{arom}), 130.4 (C_{arom}), 129.1 (C_{arom}), 127.9 (C_{arom}), 127.3 (C_{arom}), 87.1 (C₅), 79.9 (C_{propargyl}), 72.4 (C_{propargyl}), 60.9 (d, ²*J*_{C,P} = 6.4 Hz, 2C, 2 x POCH₂), 42.1 (N1-CH₂), 35.7 (C_{alkyl}), 34.7 (C_{alkyl}), 30.7 (C_{alkyl}), 28.1 (d, ²*J*_{C,P} = 15.6 Hz, PCH₂CH₂), 24.0 (d, ¹*J*_{C,P} = 138.5 Hz, PCH₂), 19.1 (d, ³*J*_{C,P} = 4.9 Hz, P(CH₂)₂CH₂), 16.3 (d, ³*J*_{C,P} = 5.6 Hz, 2C, 2 x POCH₂CH₃). ³¹P NMR (243 MHz, DMSO-*d*₆) δ 32.6.

Diethyl (4-(6-amino-5-(3-(2,4-dimethoxyphenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM103)



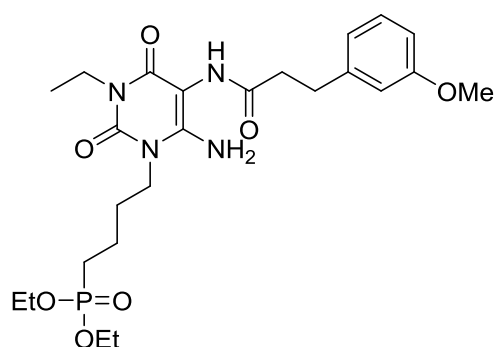
Compound **DM103** was used for the next step without further purification. LC-MS: positive mode [m/z]= 565.1 ([M+H]⁺). ESI-MS purity: 77.1%.

Diethyl (4-(6-amino-3-ethyl-5-(3-(4-fluorophenyl)propanamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM072)



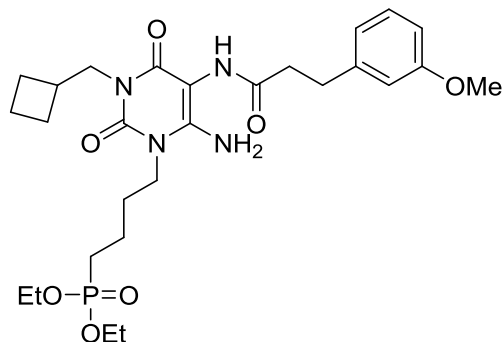
Compound **DM072** was used for the next step without further purification. LC-MS: positive mode [m/z]= 513.2 ([M+H]⁺). ESI-MS purity: 67.0%.

Diethyl (4-(6-amino-3-ethyl-5-(3-(3-methoxyphenyl)propanamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM076)



Compound **DM076** was used for the next step without further purification. LC-MS: positive mode [m/z]= 525.2 ([M+H]⁺). ESI-MS purity: 68.8%.

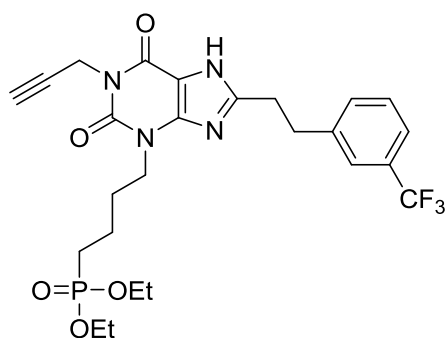
Diethyl (4-(6-amino-3-(cyclobutylmethyl)-5-(3-(3-methoxyphenyl)propanamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM075)



Yield: 41%, yellowish oil. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 8.36 (s, 1H, CONH), 7.18 (t, $J = 8.0$ Hz, 1H, H_{arom}), 6.83 – 6.78 (m, 2H, H_{arom}), 6.76 – 6.72 (m, 1H, H_{arom}), 6.47 (s, 2H, NH_2), 4.00 – 3.92 (m, 4H, $\text{P}(\text{OCH}_2)_2$), 3.84 (t, $J = 7.5$ Hz, 2H, N1-CH_2), 3.80 (d, $J = 7.2$ Hz, 2H, N3-CH_2), 3.73 (s, 3H, OCH_3), 2.84 – 2.79 (m, 2H, CH_2), 2.59 – 2.51 (m, 3H, CH_2 , CH),

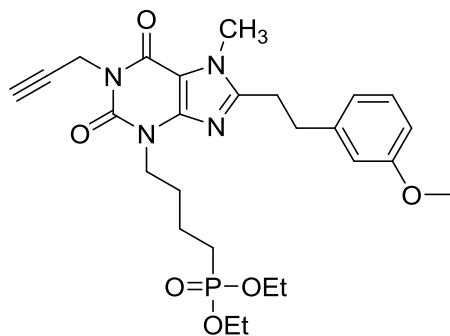
1.91 – 1.83 (m, 2H, CH_2), 1.79 – 1.68 (m, 6H, CH_2), 1.63 – 1.56 (m, 2H, CH_2), 1.53 – 1.45 (m, 2H, CH_2), 1.22 (t, $J = 7.0$ Hz, 6H, $\text{P}(\text{OCH}_2\text{CH}_3)_2$). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 172.2 (CONH), 159.5 ($\text{C}_{\text{arom-OCH}_3}$), 159.4 (C6), 151.4 (CO), 150.7 (CO), 143.4 (C_{arom}), 129.4 (C_{arom}), 120.5 (C_{arom}), 113.9 (C_{arom}), 111.6 (C_{arom}), 87.6 (C5), 61.0 (d, $^2J_{\text{C,P}} = 6.6$ Hz, 2C, POCH_2), 55.0 (OCH_3), 45.0 (N3-CH_2), 42.0 (N1-CH_2), 37.0 (CH_2), 34.1 (CH), 31.1 (CH_2), 28.3 (d, $^2J_{\text{C,P}} = 15.8$ Hz, PCH_2CH_2), 25.7 (CH_2), 24.2 (d, $^1J_{\text{C,P}} = 139.0$ Hz, PCH_2), 19.3 (d, $^3J_{\text{C,P}} = 5.1$ Hz, $\text{P}(\text{CH}_2)_2\text{CH}_2$), 17.9 (CH_2), 16.4 (d, $^3J_{\text{C,P}} = 5.6$ Hz, 2C, POCH_2CH_3). ^{31}P -NMR (243 MHz, $\text{DMSO-}d_6$) δ 32.7. LC-MS: positive mode $[m/z] = 565.3$ ($[\text{M}+\text{H}]^+$). ESI-MS purity: 92.7%.

Diethyl (4-(2,6-dioxo-1-(prop-2-yn-1-yl)-8-(3-(trifluoromethyl)phenethyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM216)



Compound **DM216** was used for the next step without further purification. LC-MS: positive mode $[m/z] = 555.3$ ($[\text{M}+\text{H}]^+$). ESI-MS purity: 82.8%.

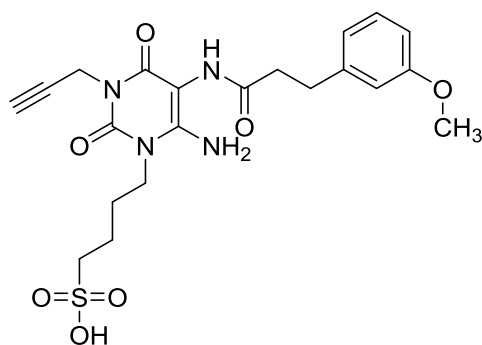
Diethyl (4-(8-(3-methoxyphenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM253)



Yield: 97%, colorless oil. $^1\text{H NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 7.18 (t, $J = 7.8$ Hz, 1H, H_{arom}), 6.82 – 6.78 (m, 2H, H_{arom}), 6.78 – 6.74 (m, 1H, H_{arom}), 4.58 (d, $J = 2.4$ Hz, 2H, N1- CH_2), 3.99 (t, $J = 6.9$ Hz, 2H, N3- CH_2), 3.97 – 3.90 (m, 4H, PO CH_2), 3.72 (s, 3H, OCH $_3$), 3.70 (s, 3H, N7- CH_3), 3.06 – 3.05 (m, 1H, $\text{H}_{\text{propargyl}}$), 3.05 – 3.02 (m, 2H, CH_2), 3.00 – 2.95 (m, 2H, CH_2), 1.82 – 1.72 (m, 4H,

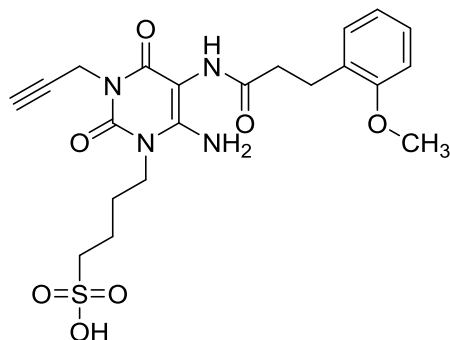
CH_2CH_2), 1.51 – 1.42 (m, 2H, CH_2), 1.18 (t, $J = 7.0$ Hz, 6H, PO CH_2CH_3). $^{13}\text{C NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ 159.5 ($\text{C}_{\text{arom-OCH}_3}$), 154.3 (C8), 153.4 (CO), 150.0 (CO), 147.6 (C6), 142.1 (C_{arom}), 129.5 (C_{arom}), 120.8 (C_{arom}), 114.2 (C_{arom}), 111.9 (C_{arom}), 106.4 (C5), 79.8 ($\text{C}_{\text{propargyl}}$), 72.8 ($\text{C}_{\text{propargyl}}$), 60.9 (d, $^2J_{\text{C,P}} = 6.4$ Hz, 2C, PO CH_2), 55.1 (OCH $_3$), 42.2 (N3- CH_2), 32.8 (CH_2), 31.4 (N7- CH_3), 30.0 (N1- CH_2), 28.3 (d, $^2J_{\text{C,P}} = 16.0$ Hz, 1C, P CH_2CH_2), 27.9 (CH_2), 24.2 (d, $^1J_{\text{C,P}} = 139.3$ Hz, 1C, P CH_2), 19.4 (d, $^3J_{\text{C,P}} = 4.9$ Hz, 1C, P(CH_2) $_2\text{CH}_2$), 16.4 (d, $^3J_{\text{C,P}} = 6.1$ Hz, PO CH_2CH_3). $^{31}\text{P-NMR}$ (243 MHz, $\text{DMSO-}d_6$) δ 32.8. LC-MS: positive mode $[\text{m/z}] = 531.0$ ($[\text{M}+\text{H}]^+$). ESI-MS purity: 96.0%.

4-(6-Amino-5-(3-(3-methoxyphenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butane-1-sulfonic acid (DM277)



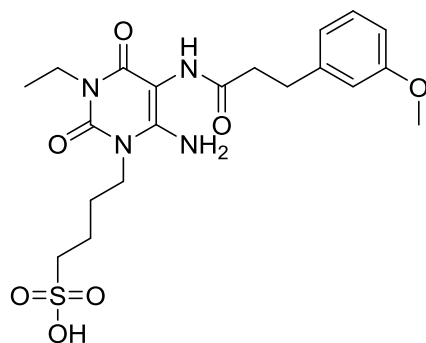
Compound **DM277** was used for the next step without further purification.

4-(6-Amino-5-(3-(2-methoxyphenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)butane-1-sulfonic acid (DM257)



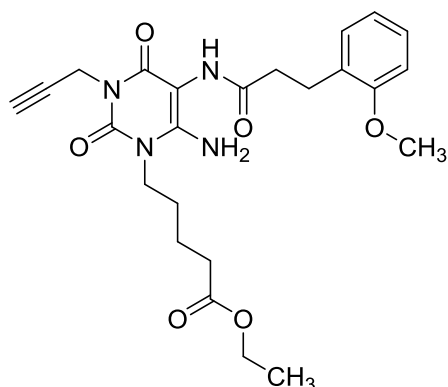
Compound **DM257** was used for the next step without further purification.

4-(6-Amino-3-ethyl-5-(3-(3-methoxyphenyl)propanamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)butane-1-sulfonic acid (DM131)



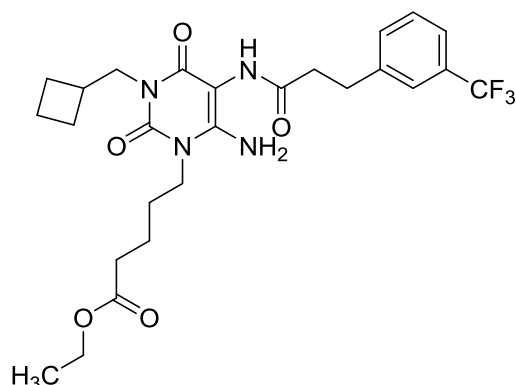
Compound **DM131** was used for the next step without further purification.

Ethyl 5-(6-amino-5-(3-(2-methoxyphenyl)propanamido)-2,4-dioxo-3-(prop-2-yn-1-yl)-3,4-dihydropyrimidin-1(2H)-yl)pentanoate (DM256)



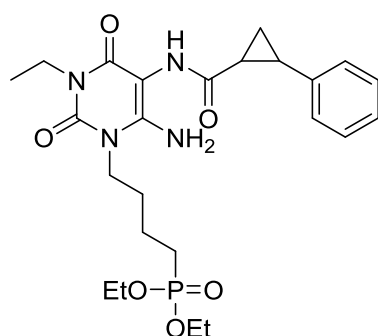
Compound **DM256** was used for the next step without further purification.

**Ethyl 5-(6-amino-3-(cyclobutylmethyl)-2,4-dioxo-5-(3-(3-(trifluoromethyl)phenyl)prop-
anamido)-3,4-dihydropyrimidin-1(2H)-yl)pentanoate (DM079)**



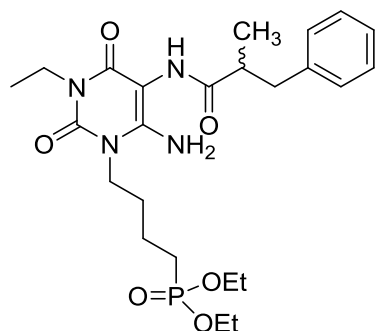
Compound **DM079** was used for the next step without further purification.

**Diethyl (4-(6-amino-3-ethyl-2,4-dioxo-5-(2-phenylcyclopropane-1-carboxamido)-3,4-
dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM127)**



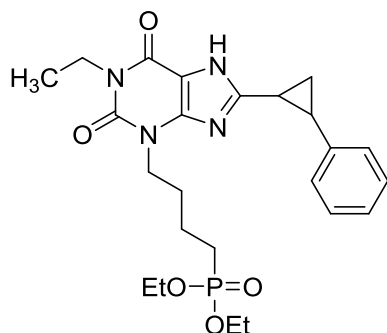
Compound **DM127** was used for the next step without further purification.

**Diethyl (4-(6-amino-3-ethyl-5-(2-methyl-3-phenylpropanamido)-2,4-dioxo-3,4-
dihydropyrimidin-1(2H)-yl)butyl)phosphonate (DM129)**



Compound **DM129** was used for the next step without further purification.

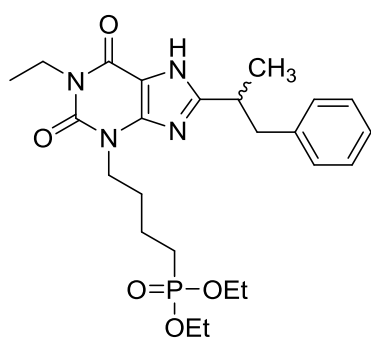
Diethyl (4-(1-ethyl-2,6-dioxo-8-(2-phenylcyclopropyl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM191)



Yield: 38% over two steps, off-white solid; m.p. 124-126 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ [ppm] = 13.10 (s, 1H, N7-H), 7.31 – 7.26 (m, 2H, H_{arom}), 7.21 – 7.16 (m, 3H, H_{arom}), 3.99 – 3.95 (m, 2H, N1-CH₂ or N3-CH₂), 3.95 – 3.87 (m, 6H, 2 x OCH₂ and N1-CH₂ or N3-CH₂), 2.25 – 2.20 (m, 1H, CH_{cyclopropyle}), 1.84 – 1.67 (m, 5H, CH_{cyclopropyle} and 2 x H_{alkyl}), 1.62 – 1.54 (m, 1H, CH_{cyclopropyle}), 1.48 – 1.37 (m, 2H, H_{alkyl}),

1.16 (td, *J* = 7.0, 2.7 Hz, 6H, 2 x OCH₂CH₃), 1.10 (t, *J* = 7.0 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO): δ [ppm] = 154.7 (C_{xanthine}), 153.5 (C_{xanthine}), 150.6 (C_{xanthine}), 148.1 (C_{xanthine}), 140.7 (C_{xanthine}), 128.6 (C_{arom}), 126.3 (C_{arom}), 125.9 (C_{arom}), 106.1 (C₅), 60.9 (d, ²*J*_{C,P} = 6.1 Hz, POCH₂), 42.2 (C_{alkyl}), 35.7 (C_{alkyl}), 28.3 (d, ²*J*_{C,P} = 16.1 Hz, 1C, PCH₂CH₂), 27.1 (C_{cyclopropyle}), 24.1 (d, ¹*J*_{C,P} = 139.0 Hz, 1C, PCH₂), 21.4 (C_{cyclopropyle}), 19.4 (d, ³*J*_{C,P} = 5.1 Hz, 1C, P(CH₂)₂CH₂), 17.5 (C_{cyclopropyl}), 16.4 (d, ³*J*_{C,P} = 6.2 Hz, POCH₂CH₃), 13.3 (CH₃). ³¹P NMR (243 MHz, DMSO): δ [ppm] = 32.9. LC-MS: positive mode [m/z] = 490.2 ([M+H]⁺). ESI-MS purity: 93.5%.

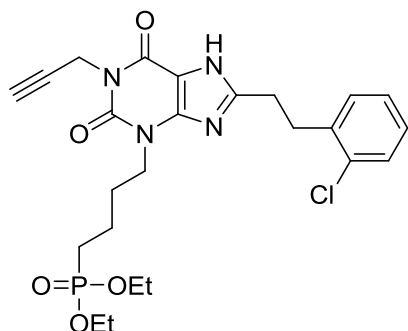
Diethyl (4-(1-ethyl-2,6-dioxo-8-(1-phenylpropan-2-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM196)



Yield: 11% over 2 steps, off-white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 13.12 (s, 1H, N7-H), 7.26 – 7.22 (m, 2H, H_{arom}), 7.18 – 7.14 (m, 1H, H_{arom}), 7.12 (dd, *J* = 7.9, 1.4 Hz, 2H, H_{arom}), 4.01 – 3.88 (m, 8H, 4 x CH₂), 3.23 – 3.16 (m, 1H, CH), 3.07 (dd, *J* = 13.4, 7.7 Hz, 1H, CH(CH₂)), 2.84 (dd, *J* = 13.4, 7.3 Hz, 1H, CH(CH₂)), 1.82 – 1.72 (m, 4H, CH₂CH₂), 1.50 – 1.41 (m, 2H, CH₂), 1.24 (d, *J* = 7.0 Hz, 3H, CHCH₃), 1.18 (t, *J* = 7.0 Hz,

6H, 2 x -OCH₂CH₃), 1.10 (t, *J* = 7.0 Hz, 3H, -NCH₂CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 157.7 (C₈), 153.6 (C₄), 150.4 (CO), 147.6 (CO), 139.4 (C_{arom}), 128.8 (2C, C_{arom}), 128.2 (2C, C_{arom}), 126.1 (C_{arom}), 106.0 (C₅), 60.7 (d, ²*J*_{C,P} = 6.4 Hz, P(OCH₂)₂), 42.1 (CH₂), 41.1 (CH₂), 35.6 (CH₂), 35.6 (CH), 28.2 (d, ²*J*_{C,P} = 15.5 Hz, 1C, PCH₂CH₂), 24.1 (d, ¹*J*_{C,P} = 139.3 Hz, 1C, PCH₂), 19.2 (d, ³*J*_{C,P} = 4.6 Hz, POCH₂CH₃), 19.0 (CH₂), 16.2 (d, ³*J*_{C,P} = 5.8 Hz, POCH₂CH₃), 13.1 (NCH₂CH₃). ³¹P-NMR (243 MHz, DMSO-*d*₆): δ [ppm] = 32.6. LC-MS: positive mode [m/z] = 491.2 ([M+H]⁺). ESI-MS purity: 84.1%.

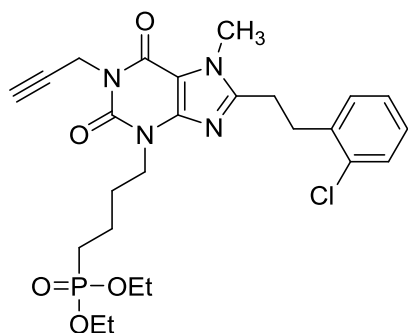
Diethyl (4-(8-(2-chlorophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM447)



Yield: 37%, colorless oil; $^1\text{H NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 13.35 (s, 1H, N7-H), 7.44 – 7.41 (m, 1H, H_{arom}), 7.29 (dd, $J = 7.1, 2.4$ Hz, 1H, H_{arom}), 7.25 (m, 2H, H_{arom}), 4.59 (d, $J = 2.4$ Hz, 2H, CH_2), 4.01 – 3.97 (m, 2H, CH_2), 3.97 – 3.92 (m, 4H, 2 x OCH_2), 3.18 – 3.11 (m, 2H, CH_2), 3.06 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 3.01 (dd, $J = 8.5, 6.8$ Hz, 2H, CH_2), 1.82 – 1.73 (m, 4H, 2 x CH_2), 1.51 – 1.43 (m, 2H, CH_2), 1.19 (t, $J = 7.0$ Hz,

6H, 2 x OCH_2CH_3). $^{13}\text{C NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ 153.5 ($\text{C}_{\text{xanthine}}$), 152.9 ($\text{C}_{\text{xanthine}}$), 150.0 ($\text{C}_{\text{xanthine}}$), 148.1 ($\text{C}_{\text{xanthine}}$), 137.7 (C_{arom}), 132.9 (C_{arom}), 130.6 (C_{arom}), 129.2 (C_{arom}), 128.2 (C_{arom}), 127.3 (C_{arom}), 105.9 (C_5), 79.7 ($\text{C}_{\text{propargyl}}$), 72.7 ($\text{C}_{\text{propargyl}}$), 60.7 (d, $^2J_{\text{C,P}} = 6.4$ Hz, 2 x OCH_2), 42.4 (CH_2), 39.9 (CH_2), 31.1 (CH_2), 30.0 (CH_2), 28.2 (CH_2), 28.1 (CH_2), 24.0 (d, $^1J_{\text{C,P}} = 138.7$ Hz, 1C, PCH_2), 19.2 (d, $^3J_{\text{C,P}} = 4.8$ Hz, POCH_2CH_3), 16.2 (d, $^3J_{\text{C,P}} = 5.4$ Hz, 2 x OCH_2CH_3). $^{31}\text{P NMR}$ (243 MHz, $\text{DMSO-}d_6$) δ 32.8.

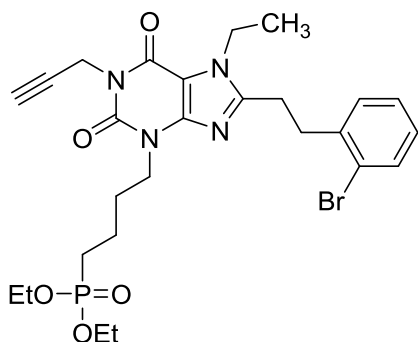
Diethyl (4-(8-(2-chlorophenethyl)-7-methyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM450)



Yield: 98%, white solid; $^1\text{H NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 7.45 – 7.41 (m, 1H, H_{arom}), 7.37 – 7.34 (m, 1H, H_{arom}), 7.30 – 7.23 (m, 2H, H_{arom}), 4.59 (d, $J = 2.5$ Hz, 2H, N1- CH_2), 4.00 – 3.92 (m, 6H, 2 x OCH_2 and N3- CH_2), 3.75 (s, 3H, N7- CH_3), 3.17 (d, $J = 5.2$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 3.13 (t, $J = 7.2$ Hz, 2H, CH_2), 3.07 (dd, $J = 5.4, 2.9$ Hz, 2H, CH_2), 1.82 – 1.70 (m, 4H, 2 x CH_2), 1.51 – 1.41 (m, 2H, CH_2), 1.19 (t, $J = 7.0$ Hz,

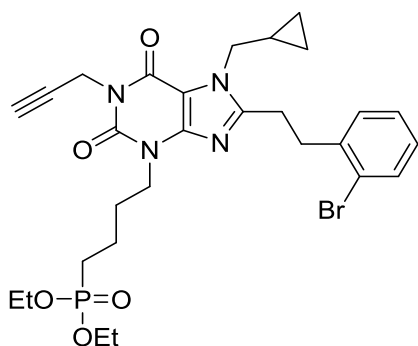
6H, 2 x OCH_2CH_3). $^{13}\text{C NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ 153.6 ($\text{C}_{\text{xanthine}}$), 153.3 ($\text{C}_{\text{xanthine}}$), 149.8 ($\text{C}_{\text{xanthine}}$), 147.4 ($\text{C}_{\text{xanthine}}$), 137.6 (C_{arom}), 133.0 (C_{arom}), 130.9 (C_{arom}), 129.2 (C_{arom}), 128.3 (C_{arom}), 127.3 (C_{arom}), 106.3 (C_5), 79.6 ($\text{C}_{\text{propargyl}}$), 72.7 ($\text{C}_{\text{propargyl}}$), 60.7 (d, $^2J_{\text{C,P}} = 6.5$ Hz, 2 x OCH_2), 42.1 (CH_2), 31.3 (CH_2), 30.4 (CH_2), 29.9 (CH_2), 28.1 (d, $^2J_{\text{C,P}} = 16.0$ Hz, 1C, PCH_2CH_2), 26.1 (CH_2), 24.0 (d, $^1J_{\text{C,P}} = 138.9$ Hz, 1C, PCH_2), 19.2 (d, $^3J_{\text{C,P}} = 4.6$ Hz, POCH_2CH_3), 16.2 (d, $^3J_{\text{C,P}} = 5.6$ Hz, 2 x OCH_2CH_3). $^{31}\text{P NMR}$ (243 MHz, $\text{DMSO-}d_6$) δ 32.8.

Synthesis of diethyl (4-(8-(2-bromophenethyl)-7-ethyl-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM351)



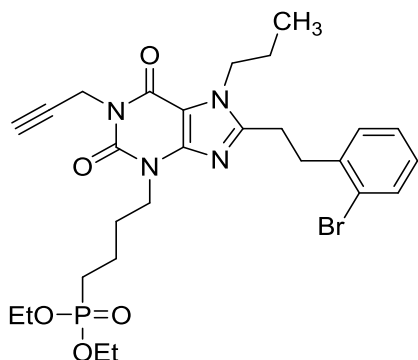
Yield: 86% (colorless oil); $^1\text{H NMR}$ (600 MHz, DMSO- d_6) δ 7.60 (dd, $J = 8.0, 1.2$ Hz, 1H, H_{arom}), 7.37 (dd, $J = 7.7, 1.7$ Hz, 1H, H_{arom}), 7.31 (td, $J = 7.4, 1.2$ Hz, 1H, H_{arom}), 7.17 (td, $J = 7.6, 1.8$ Hz, 1H, H_{arom}), 4.59 (d, $J = 2.5$ Hz, 2H, N1- CH_2), 4.20 (q, $J = 7.1$ Hz, 2H, N7- CH_2CH_3), 4.01 – 3.98 (m, 2H, N3- CH_2), 3.97 – 3.91 (m, 4H, $\text{P}(\text{OCH}_2\text{CH}_3)_2$), 3.18 – 3.14 (m, 2H, - CH_2), 3.09 – 3.05 (m, 3H, - CH_2 and - CH), 1.82 – 1.73 (m, 4H, - CH_2CH_2), 1.52 – 1.43 (m, 2H, - CH_2), 1.23 (t, $J = 7.1$ Hz, 3H, N7- CH_2CH_3), 1.19 (t, $J = 7.0$ Hz, 6H, $\text{P}(\text{OCH}_2\text{CH}_3)_2$). $^{13}\text{C NMR}$ (151 MHz, DMSO- d_6) δ 152.9 ($\text{C}_{\text{xanthine}}$), 152.7 ($\text{C}_{\text{xanthine}}$), 149.8 ($\text{C}_{\text{xanthine}}$), 147.8 ($\text{C}_{\text{xanthine}}$), 139.3 (C_{arom}), 132.5 (C_{arom}), 131.0 (C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 123.7 (C_{arom}), 105.5 (C_5), 79.7 ($\text{C}_{\text{propargyl}}$), 72.7 ($\text{C}_{\text{propargyl}}$), 60.8 (d, $^2J_{\text{C,P}} = 6.1$ Hz, $\text{P}(\text{OCH}_2)_2$), 42.1 (N- CH_2), 40.1 (N7- CH_2), 33.3 (N- CH_2), 30.0 (- CH_2), 28.1 (d, $^2J_{\text{C,P}} = 16.3$ Hz, 1C, PCH_2CH_2), 26.1 (- CH_2), 24.1 (d, $^1J_{\text{C,P}} = 138.8$ Hz, 1C, PCH_2), 19.3 (d, $^3J_{\text{C,P}} = 4.8$ Hz, $\text{PCH}_2\text{CH}_2\text{CH}_2$), 16.3 (d, $^3J_{\text{C,P}} = 5.7$ Hz, POCH_2CH_3), 15.9 (N7- CH_2CH_3). $^{31}\text{P-NMR}$ (243 MHz, DMSO- d_6): δ [ppm] = 32.8. LC-MS: positive mode $[m/z] = 593.2$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 99.0%.

Synthesis of diethyl (4-(8-(2-bromophenethyl)-7-(cyclopropylmethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM352)



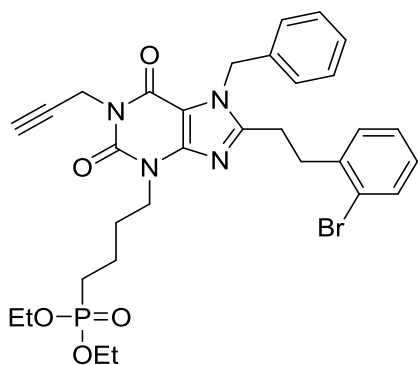
Compound **DM352** was used for the next step without further purification. LC-MS: positive mode $[m/z] = 619.2$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 89.4%; yellowish oil.

Synthesis of diethyl (4-(8-(2-bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-7-propyl-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM353)



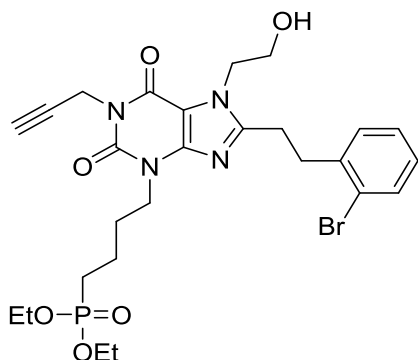
Compound **DM353** was used for the next step without further purification. LC-MS: positive mode $[m/z] = 607.0$ ($[M+H]^+$). ESI-MS purity = 88.5%; colorless oil.

Synthesis of diethyl (4-(7-benzyl-8-(2-bromophenethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM360)



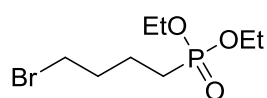
Yield: 87% (white solid); ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 7.55 (dd, $J = 8.0, 1.1$ Hz, 1H, H_{arom}), 7.35 – 7.31 (m, 2H, H_{arom}), 7.30 – 7.25 (m, 3H, H_{arom}), 7.17 – 7.11 (m, 3H, H_{arom}), 5.51 (s, 2H, N7- CH_2), 4.59 (d, $J = 2.5$ Hz, 2H, N1- CH_2), 4.02 (t, $J = 6.9$ Hz, 2H, N3- CH_2), 3.98 – 3.90 (m, 4H, $\text{P}(\text{OCH}_2\text{CH}_3)_2$), 3.08 – 3.04 (m, 3H, - CH_2 and $\text{H}_{\text{propargyl}}$), 3.02 – 2.98 (m, 2H, - CH_2), 1.83 – 1.74 (m, 4H, - $\text{CH}_2\text{-CH}_2$), 1.54 – 1.45 (m, 2H, - CH_2), 1.18 (t, $J = 7.0$ Hz, 6H, $\text{P}(\text{OCH}_2\text{CH}_3)_2$). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 153.5 ($\text{C}_{\text{xanthine}}$), 153.3 ($\text{C}_{\text{xanthine}}$), 149.8 ($\text{C}_{\text{xanthine}}$), 147.7 ($\text{C}_{\text{xanthine}}$), 139.2 (C_{arom}), 136.4 (C_{arom}), 132.5 (C_{arom}), 130.8 (C_{arom}), 128.8 (2C, C_{arom}), 128.6 (C_{arom}), 127.9 (C_{arom}), 127.7 (C_{arom}), 126.6 (2C, C_{arom}), 123.7 (C_{arom}), 106.0 (C_5), 79.6 ($\text{C}_{\text{propargyl}}$), 72.8 ($\text{C}_{\text{propargyl}}$), 60.8 (d, $^2J_{\text{C,P}} = 6.1$ Hz, $\text{P}(\text{OCH}_2)_2$), 47.2 (N7- CH_2), 42.2 (N- CH_2), 32.9 (N- CH_2), 30.0 (- CH_2), 28.2 (d, $^2J_{\text{C,P}} = 16.3$ Hz, 1C, PCH_2CH_2), 26.3 (- CH_2), 24.1 (d, $^1J_{\text{C,P}} = 138.5$ Hz, 1C, PCH_2), 19.3 (d, $^3J_{\text{C,P}} = 4.6$ Hz, $\text{PCH}_2\text{CH}_2\text{CH}_2$), 16.3 (d, $^3J_{\text{C,P}} = 5.7$ Hz, POCH_2CH_3). ^{31}P -NMR (243 MHz, $\text{DMSO-}d_6$): δ [ppm] = 32.8. LC-MS: positive mode $[m/z] = 657.2$ ($[M+H]^+$). ESI-MS purity = 99.4%.

Synthesis of diethyl (4-(8-(2-bromophenethyl)-7-(2-hydroxyethyl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,6,7-tetrahydro-3H-purin-3-yl)butyl)phosphonate (DM361)



Yield: 65% (off-white solid); ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 7.60 (dd, $J = 8.0, 1.2$ Hz, 1H, H_{arom}), 7.38 (dd, $J = 7.7, 1.7$ Hz, 1H, H_{arom}), 7.34 – 7.29 (m, 1H, H_{arom}), 7.17 (td, $J = 7.6, 1.8$ Hz, 1H, H_{arom}), 4.96 (t, $J = 5.4$ Hz, 1H, -OH), 4.59 (d, $J = 2.4$ Hz, 2H, N1- CH_2), 4.20 (t, $J = 5.3$ Hz, 2H, N7- CH_2), 4.02 – 3.91 (m, 6H, $\text{P}(\text{OCH}_2)_2$ and $-\text{CH}_2$), 3.67 (q, $J = 5.3$ Hz, 2H, $-\text{CH}_2\text{OH}$), 3.18 – 3.14 (m, 2H, $-\text{CH}_2$), 3.13 – 3.09 (m, 2H, $-\text{CH}_2$), 3.07 (t, $J = 2.4$ Hz, 1H, $\text{H}_{\text{propargyl}}$), 1.83 – 1.72 (m, 4H, $-\text{CH}_2\text{CH}_2$), 1.53 – 1.44 (m, 2H, $-\text{CH}_2$), 1.19 (t, $J = 7.0$ Hz, 6H, $\text{P}(\text{OCH}_2\text{CH}_3)_2$). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 154.3 ($\text{C}_{\text{xanthine}}$), 153.0 ($\text{C}_{\text{xanthine}}$), 149.8 ($\text{C}_{\text{xanthine}}$), 147.7 ($\text{C}_{\text{xanthine}}$), 139.6 (C_{arom}), 132.5 (C_{arom}), 130.9 (C_{arom}), 128.5 (C_{arom}), 127.9 (C_{arom}), 123.7 (C_{arom}), 105.7 (C_5), 79.7 ($\text{C}_{\text{propargyl}}$) 72.7 ($\text{C}_{\text{propargyl}}$), 60.8 (d, $^2J_{\text{C,P}} = 6.1$ Hz, $\text{P}(\text{OCH}_2)_2$), 60.1 ($-\text{CH}_2\text{OH}$), 47.3 (N- CH_2), 42.1 (N- CH_2), 33.1 ($-\text{CH}_2$), 29.9 ($-\text{CH}_2$), 28.2 (d, $^2J_{\text{C,P}} = 16.1$ Hz, 1C, PCH_2CH_2), 26.5 ($-\text{CH}_2$), 24.1 (d, $^1J_{\text{C,P}} = 138.3$ Hz, 1C, PCH_2), 19.3 (d, $^3J_{\text{C,P}} = 4.7$ Hz, $\text{PCH}_2\text{CH}_2\text{CH}_2$), 16.3 (d, $^3J_{\text{C,P}} = 5.7$ Hz, POCH_2CH_3). ^{31}P -NMR (243 MHz, $\text{DMSO-}d_6$): δ [ppm] = 32.8. LC-MS: positive mode $[\text{m/z}] = 609.2$ ($[\text{M}+\text{H}]^+$). ESI-MS purity = 77.2%.

Diethyl (4-bromobutyl)phosphonate (DM014)

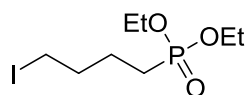


1,4-Dibromobutane (10.9 ml, 92.62 mmol, 1 equiv.) in $\text{P}(\text{OEt})_3$ (4.01 ml, 23.16 mmol, 0.25 equiv.) stirred under reflux for 30 min. After cooling to rt, excess of 1,4-dibromobutane and $\text{P}(\text{OEt})_3$ were removed under reduced pressure at 70 °C. The oily colorless residue was purified by flash column chromatography with dichloromethane/methanol (9.5:0.5, colored with AgNO_3 solution) to yield the desired product **DM014** (5.23 g, 21%) as colorless oil.

^1H -NMR (600 MHz, CDCl_3): δ [ppm] = 4.12 – 4.03 (m, 4H, 2 x OCH_2), 3.38 (t, $J = 6.6$ Hz, 2H, BrCH_2), 1.96 – 1.90 (m, 2H, C_{alkyl}), 1.77 – 1.68 (m, 4H, C_{alkyl}), 1.30 (t, $J = 7.1$ Hz, 6H, 2 x CH_2CH_3). ^{13}C -NMR (151 MHz, CDCl_3): δ [ppm] = 61.5 (d, $^2J_{\text{C,P}} = 6.3$ Hz, 2C, POCH_2), 33.1

(d, $^2J_{C,P} = 15.0$ Hz, 1C, PCH₂CH₂) 32.7 (Br-CH₂), 24.7 (d, $^1J_{C,P} = 142.1$ Hz, 1C, PCH₂), 21.2 (d, $^3J_{C,P} = 6.2$ Hz, 1C, PCH₂CH₂CH₂), 16.4 (d, $^3J_{C,P} = 6.5$ Hz, 2C, POCH₂CH₃). ^{31}P -NMR (243 MHz, CDCl₃): δ [ppm] = 31.73. LC-MS: positive mode [m/z] = 272.8 ([M+H]⁺).

Diethyl (4-iodobutyl)phosphonate (DM033)



For the Finkelstein reaction of **DM014** (8.2 g, 30.02 mmol, 1 equiv.) and NaI (9 g, 60.05 mmol, 2 equiv.) was dissolved in acetone (30 ml) and stirred for 1 h at 65 °C. After removing the solvent under reduced pressure, water (50 ml) and dichloromethane (50 ml) were added and the phases were separated. The water layer was extracted three times with dichloromethane (20 ml) and the combined organic phases filtered over celite and dried over MgSO₄. The solvent was removed *in vacuo* to yield **DM033** (9.13 g, 28.52 mmol, 95%) as an yellow oil.

^1H -NMR (600 MHz, Chloroform-*d*): δ [ppm] = 4.13 – 4.01 (m, 4H, 2 x OCH₂), 3.15 (t, $J = 6.9$ Hz, 2H, I-CH₂), 1.88 (p, $J = 6.9$ Hz, 2H, C_{alkyl}), 1.73 – 1.67 (m, 4H, C_{alkyl}), 1.29 (t, $J = 7.0$ Hz, 6H, 2 x CH₂CH₃). ^{13}C -NMR (151 MHz, CDCl₃): δ [ppm] = 61.6 (d, $^2J_{C,P} = 6.7$ Hz, 2C, POCH₂), 33.8 (d, $^2J_{C,P} = 15.2$ Hz, 1C, PCH₂CH₂), 24.6 (d, $^1J_{C,P} = 140.9$ Hz, 1C, PCH₂), 25.1 (d, $^3J_{C,P} = 5.4$ Hz, 1C, PCH₂CH₂CH₂) 16.5 (d, $^3J_{C,P} = 4.7$ Hz, OCH₂CH₃), 5.5 (ICH₂). ^{31}P -NMR (243 MHz, CDCl₃): δ [ppm] = 31.7. LC-MS: positive mode [m/z] = 320.9 ([M+H]⁺). ESI-MS purity = 96%.

2. ^1H -, ^{13}C - and ^{31}P -NMR spectra of final compounds

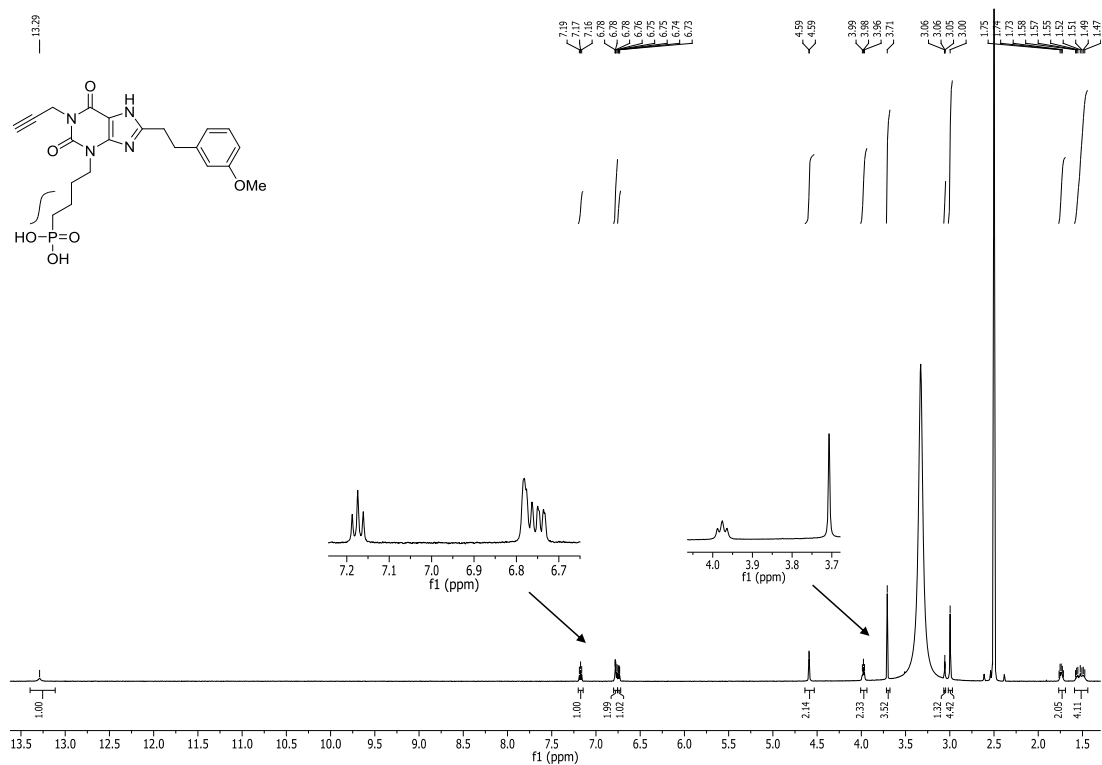


Figure S1: ^1H -NMR of compound **B-2**

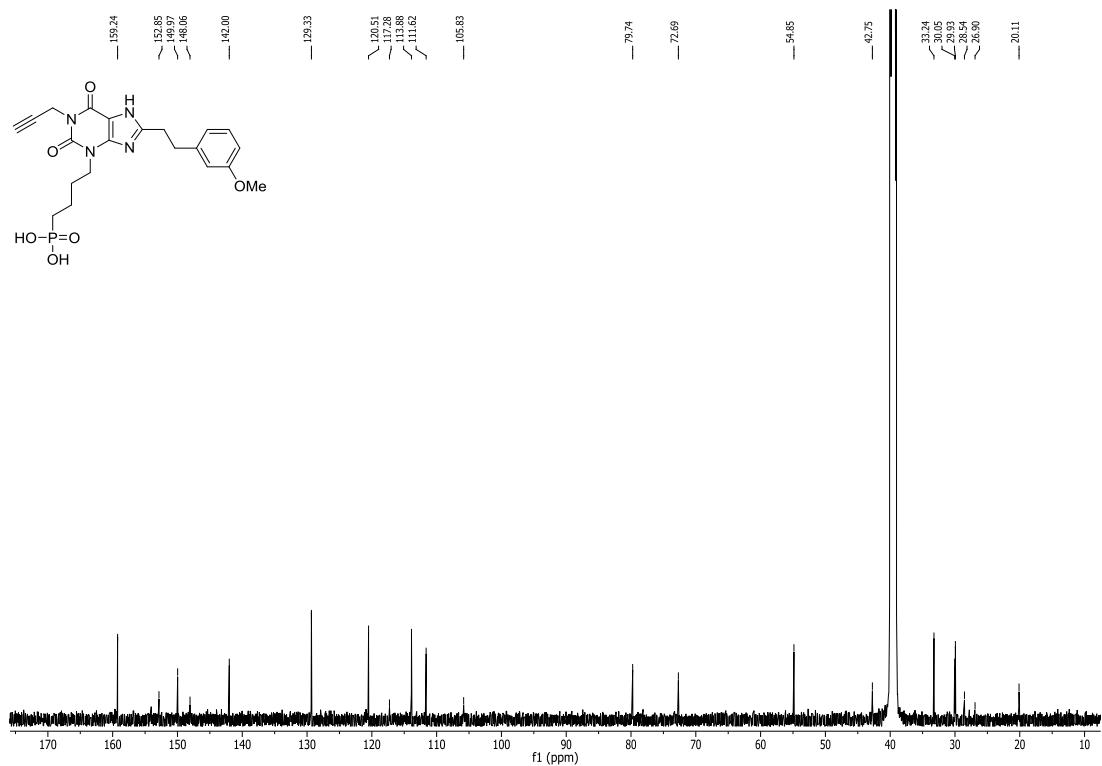


Figure S2: ^{13}C -NMR of compound **B-2**

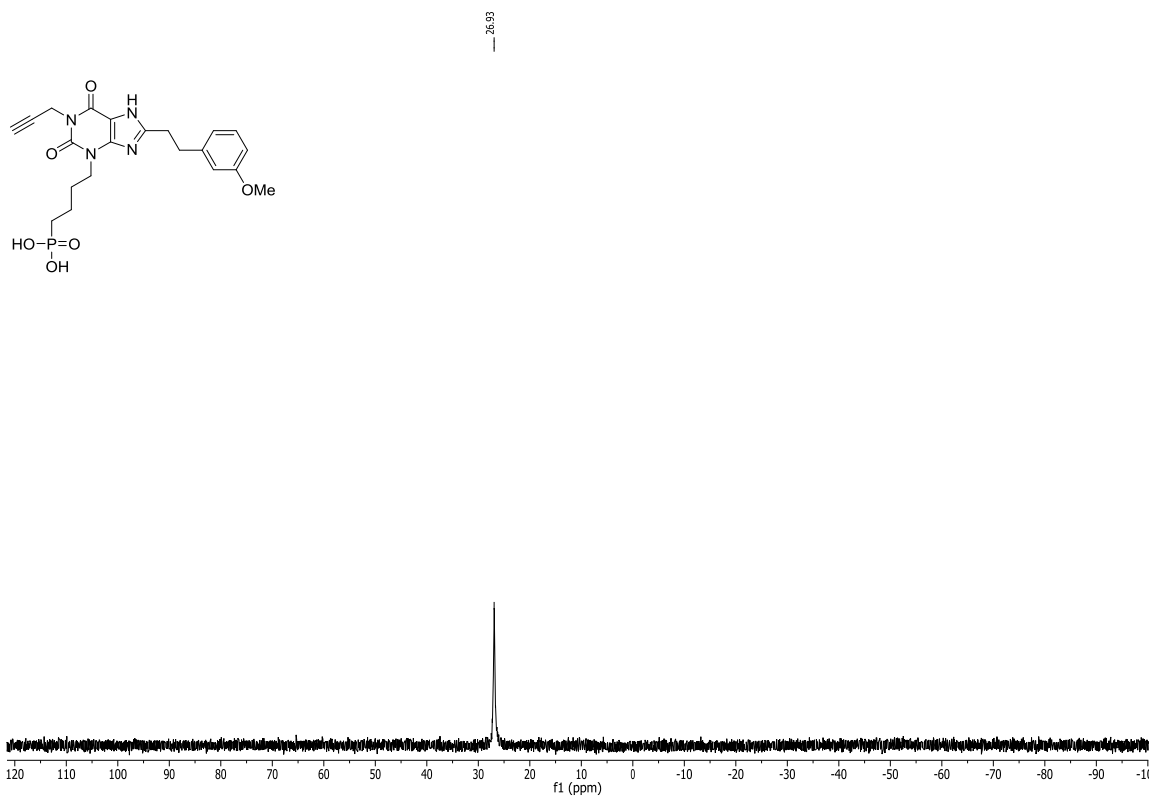


Figure S3: ^{31}P -NMR of compound B-2

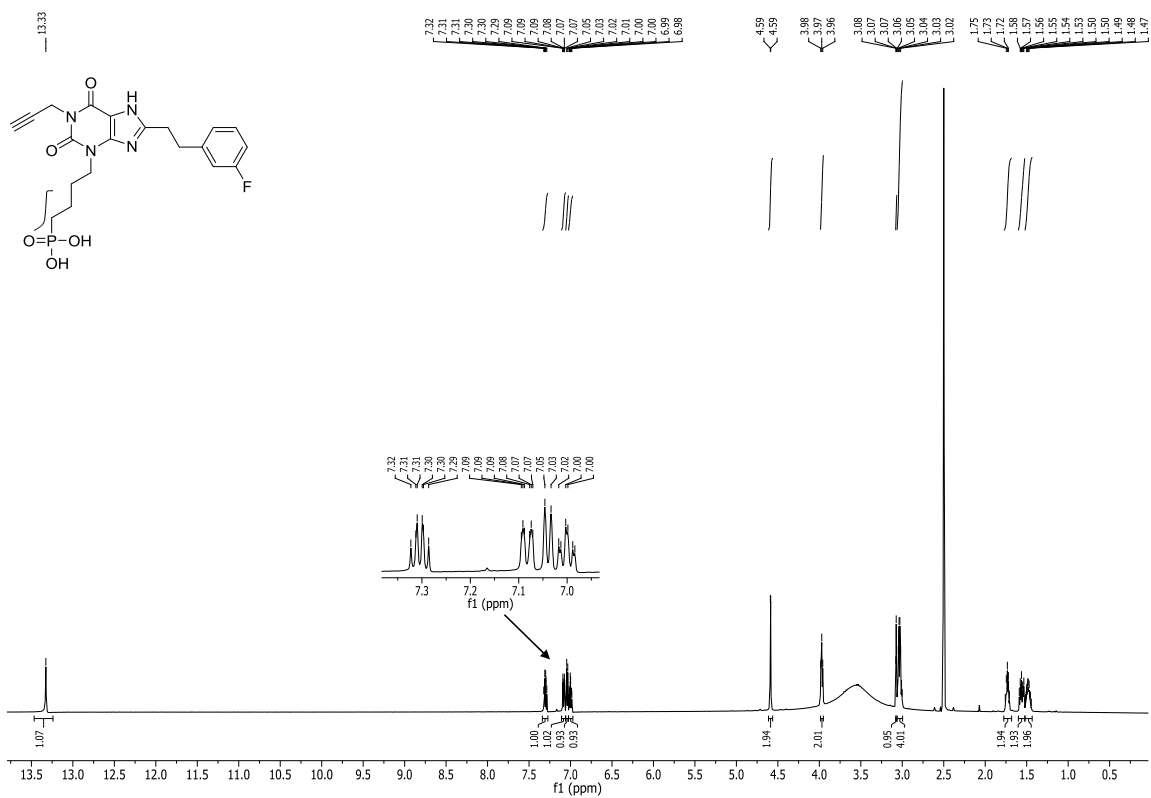


Figure S4: ^1H -NMR of compound B-3

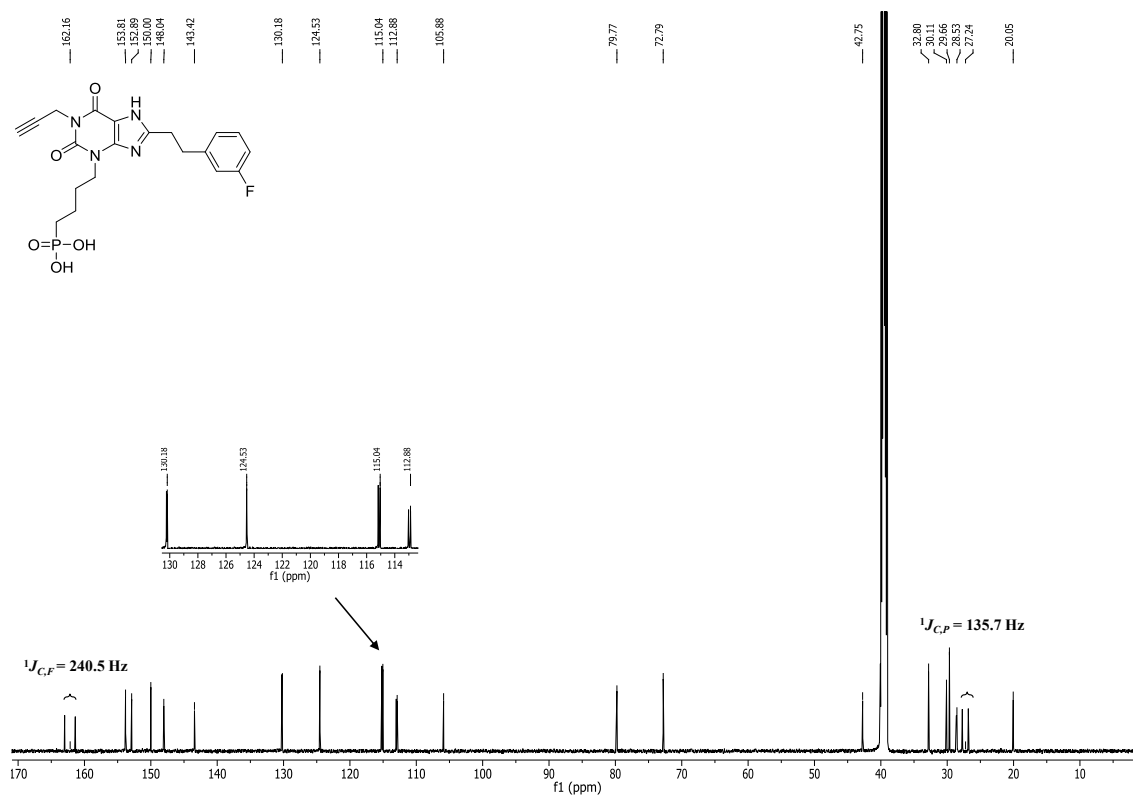


Figure S5: ^{13}C -NMR of compound **B-3**

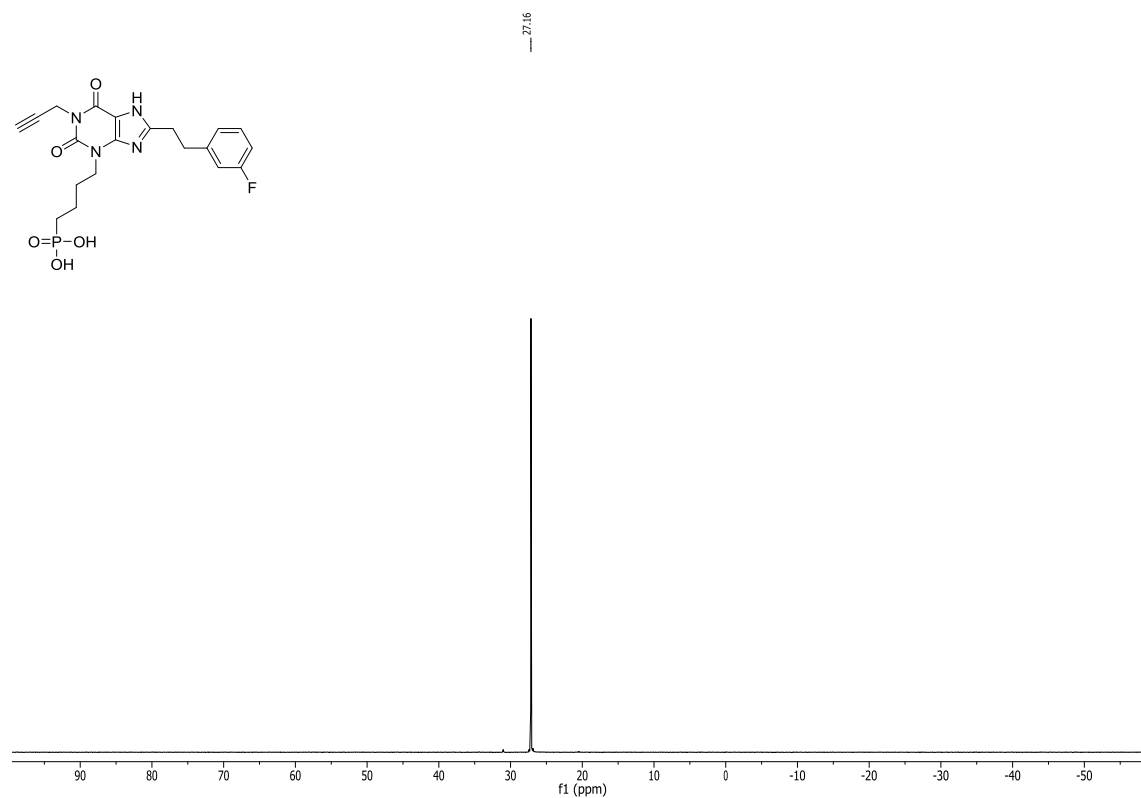


Figure S6: ^{31}P -NMR of compound **B-3**

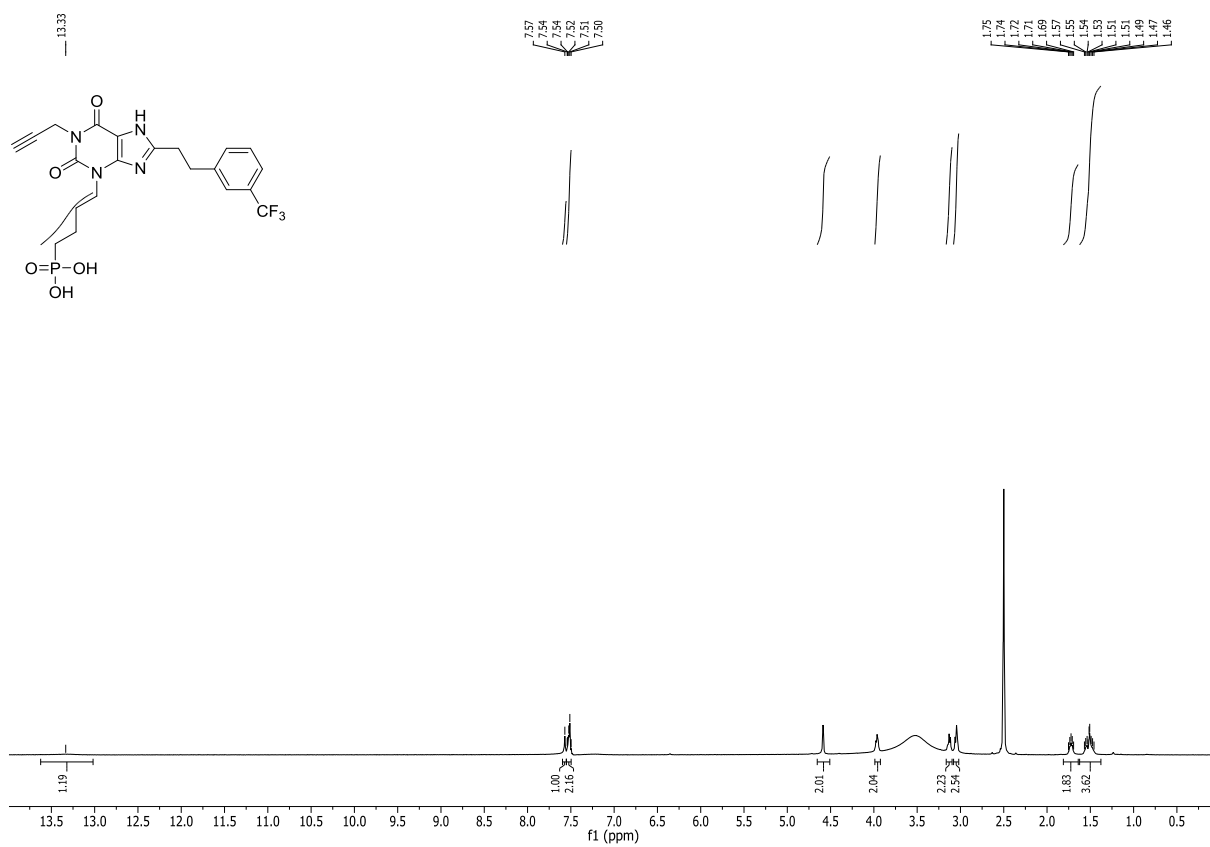


Figure S7: $^1\text{H-NMR}$ of compound **B-6**

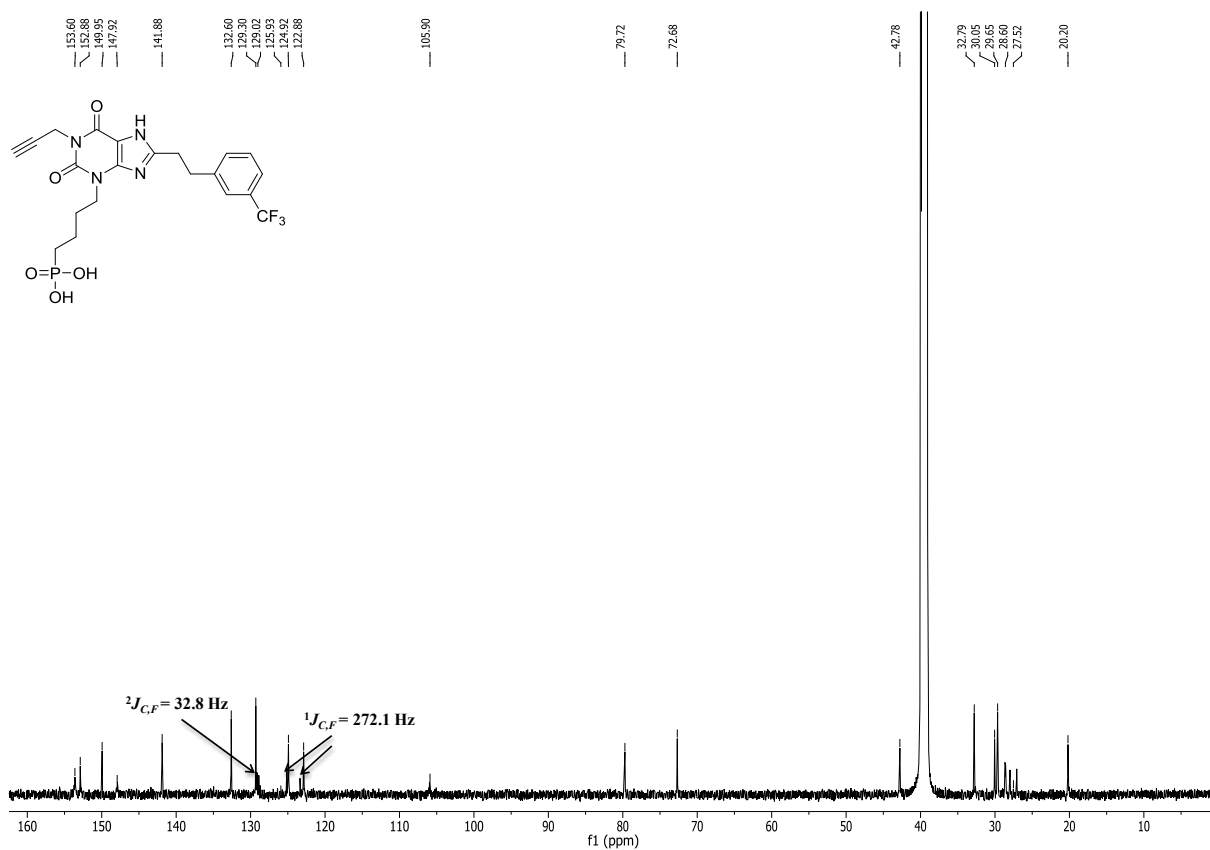


Figure S8: ^{13}C -NMR of compound **B-6**

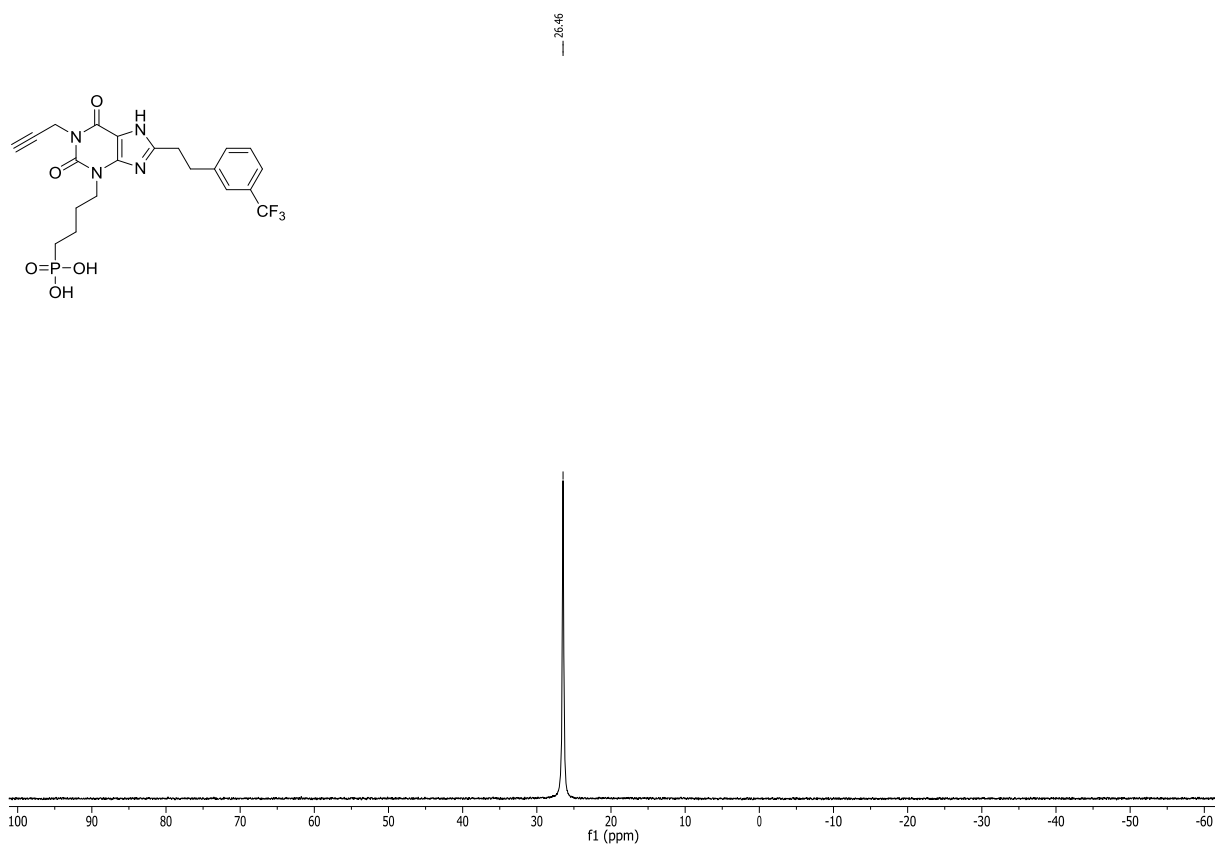


Figure S10: ^{31}P -NMR of compound **B-6**

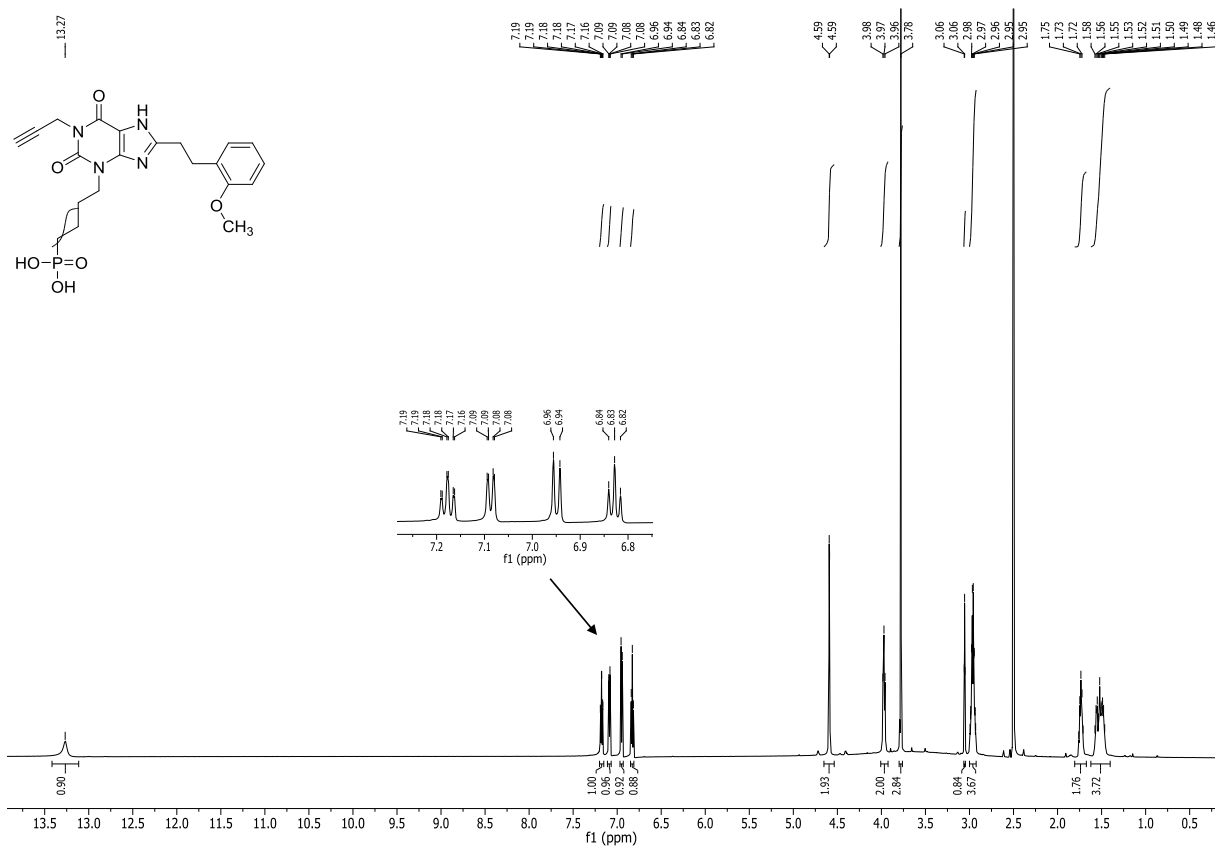


Figure S11: $^1\text{H-NMR}$ of compound **B-7**

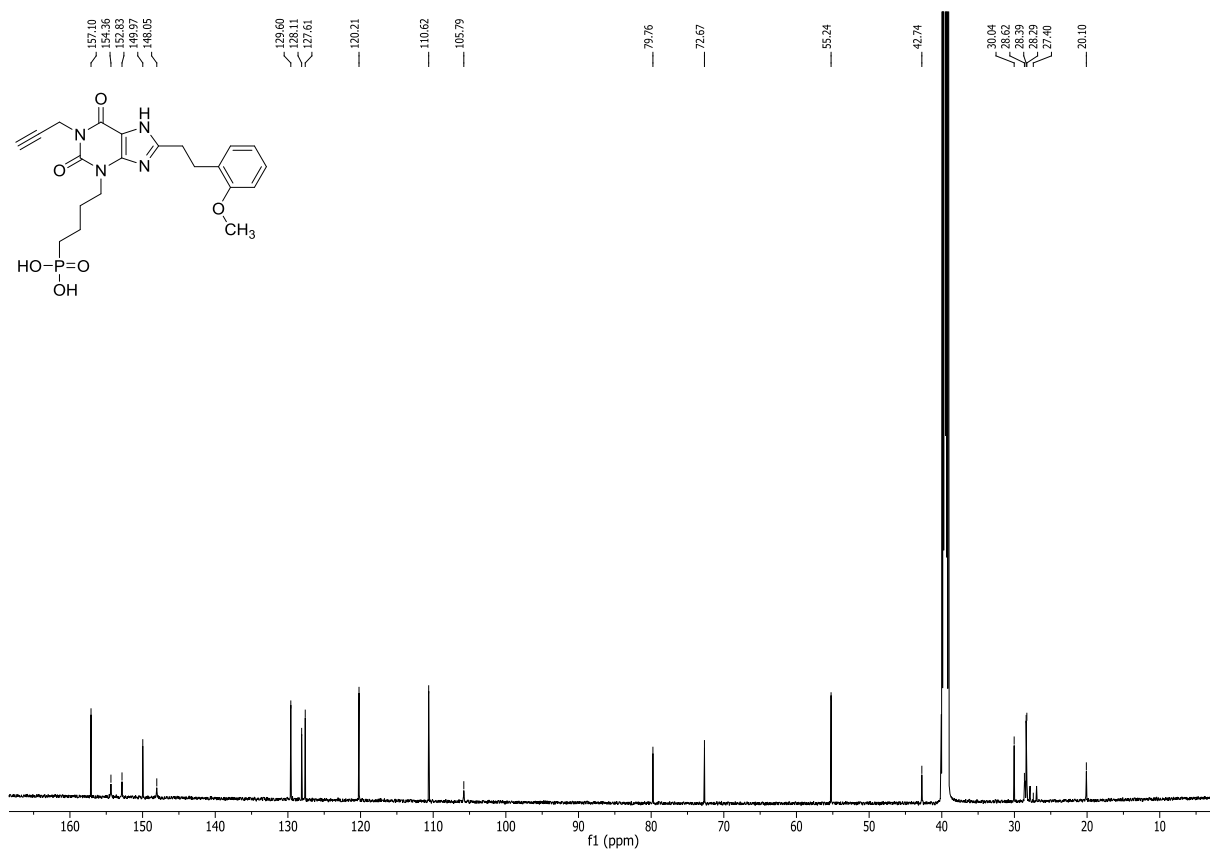


Figure S12: ^{13}C -NMR of compound **B-7**

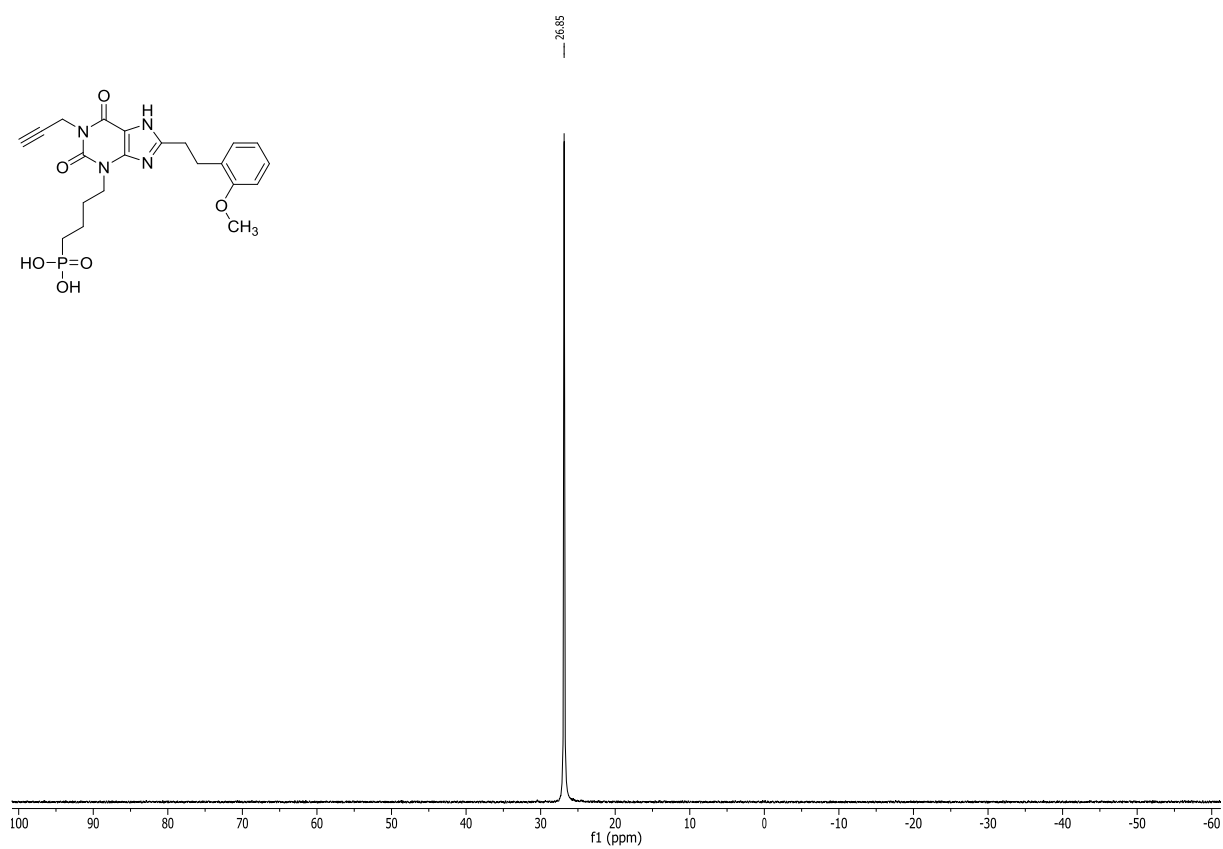


Figure S12: ^{31}P -NMR of compound **B-7**

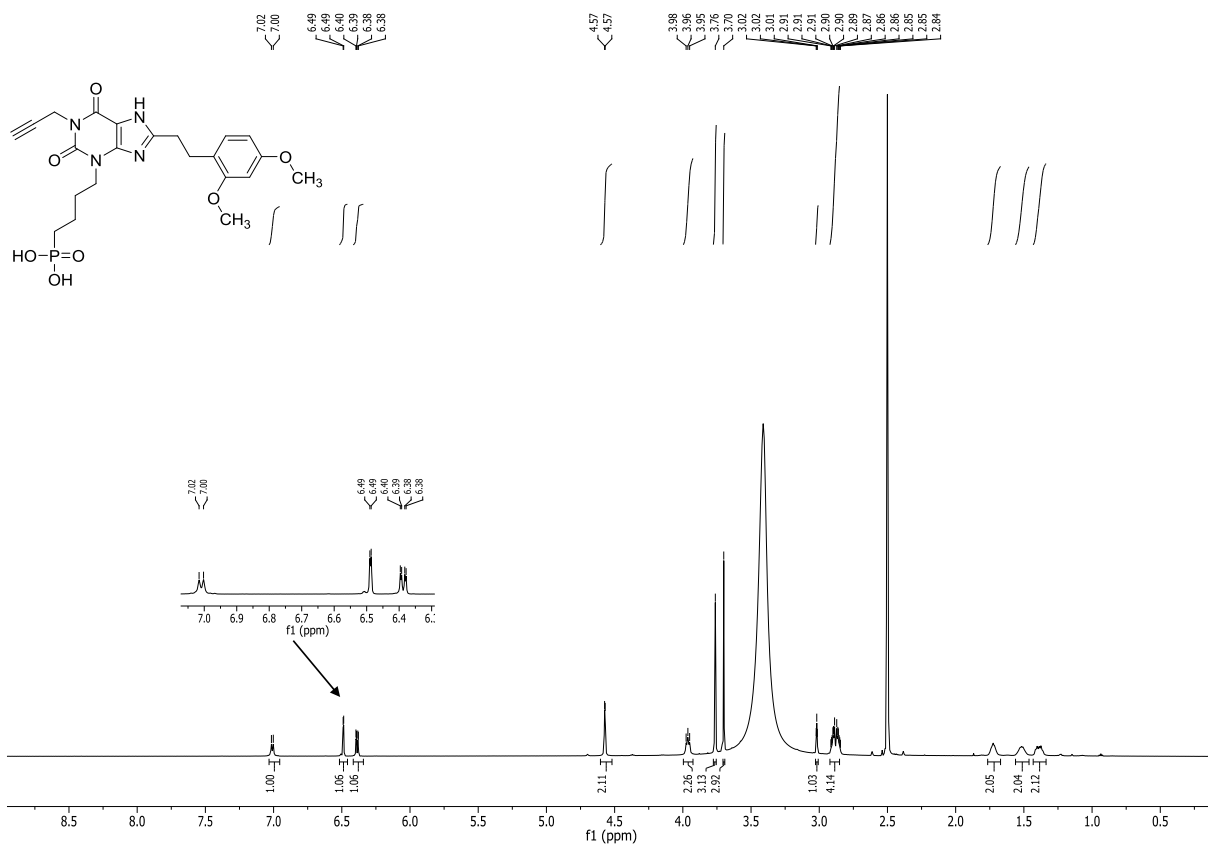


Figure S13: $^1\text{H-NMR}$ of compound **B-10**

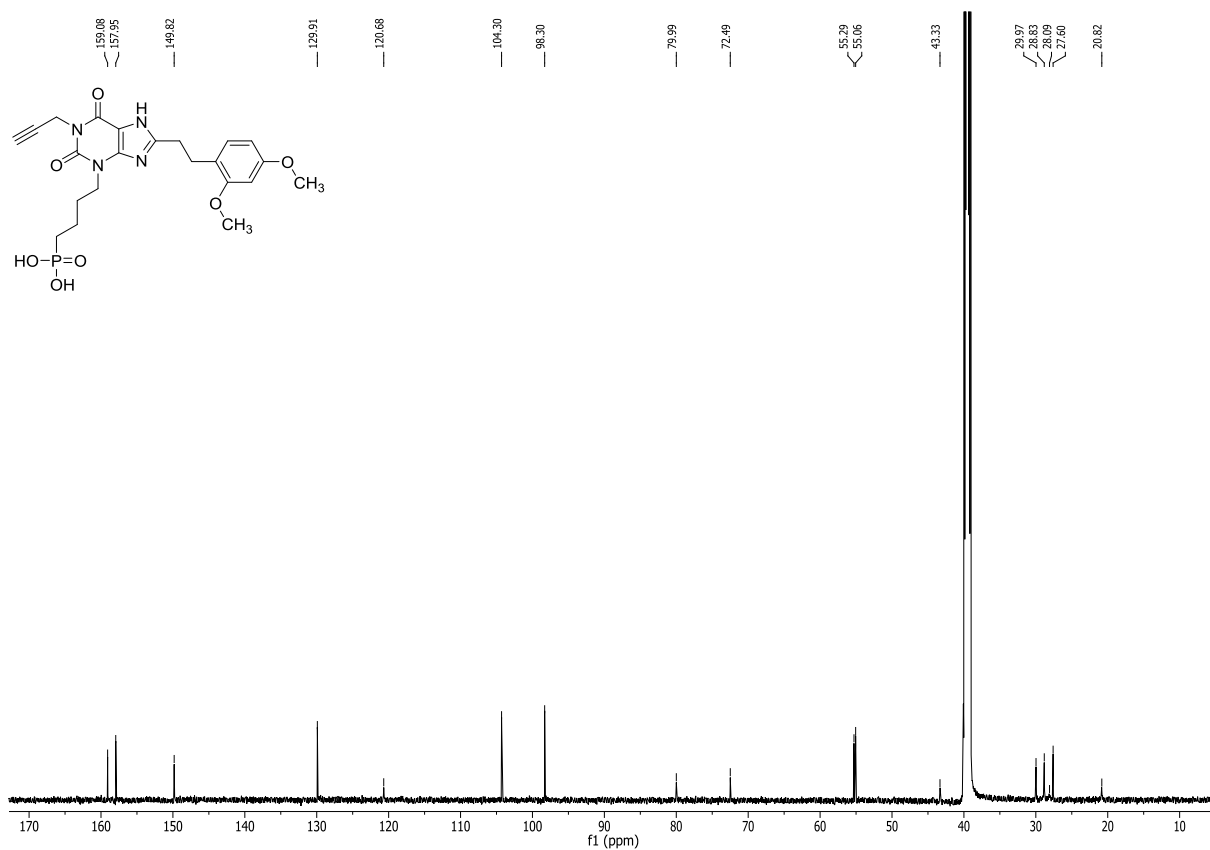


Figure S14: ^{13}C -NMR of compound **B-10**

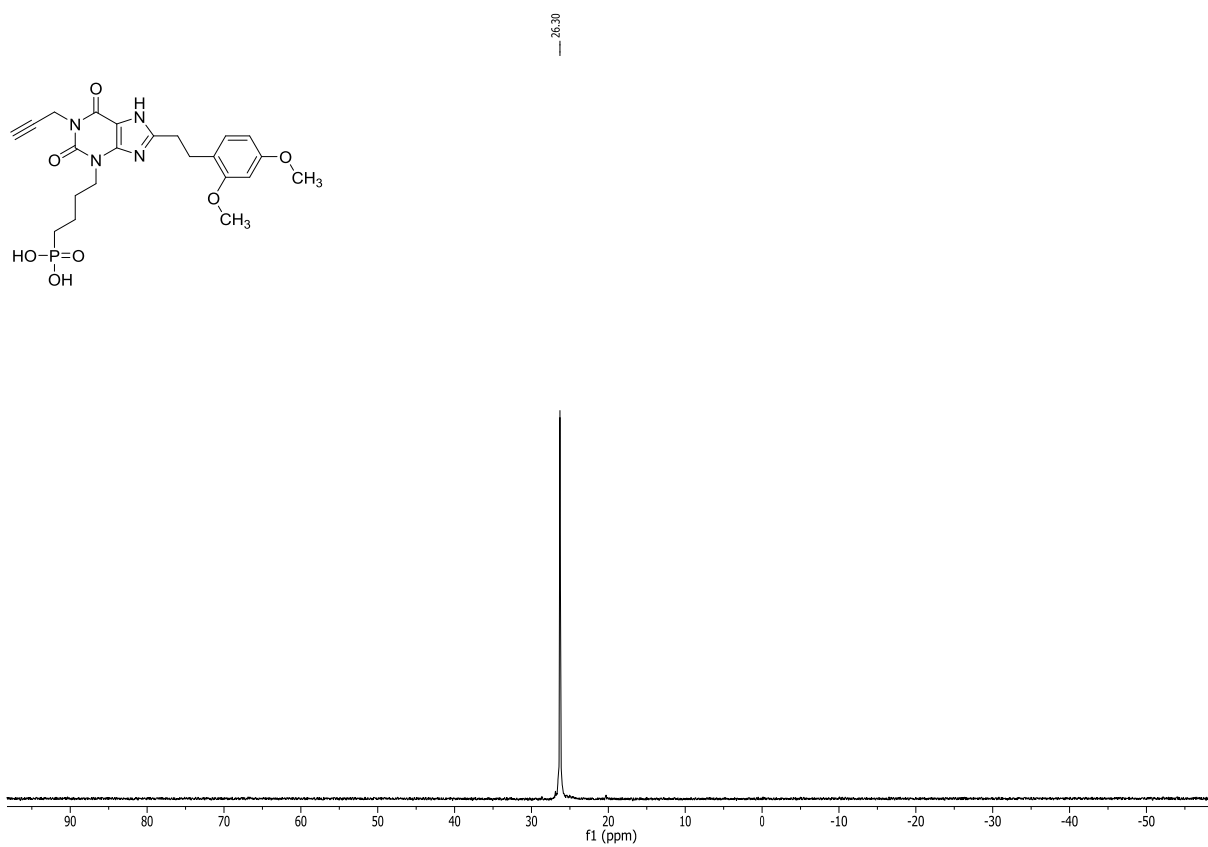


Figure S15: ^{31}P -NMR of compound **B-10**

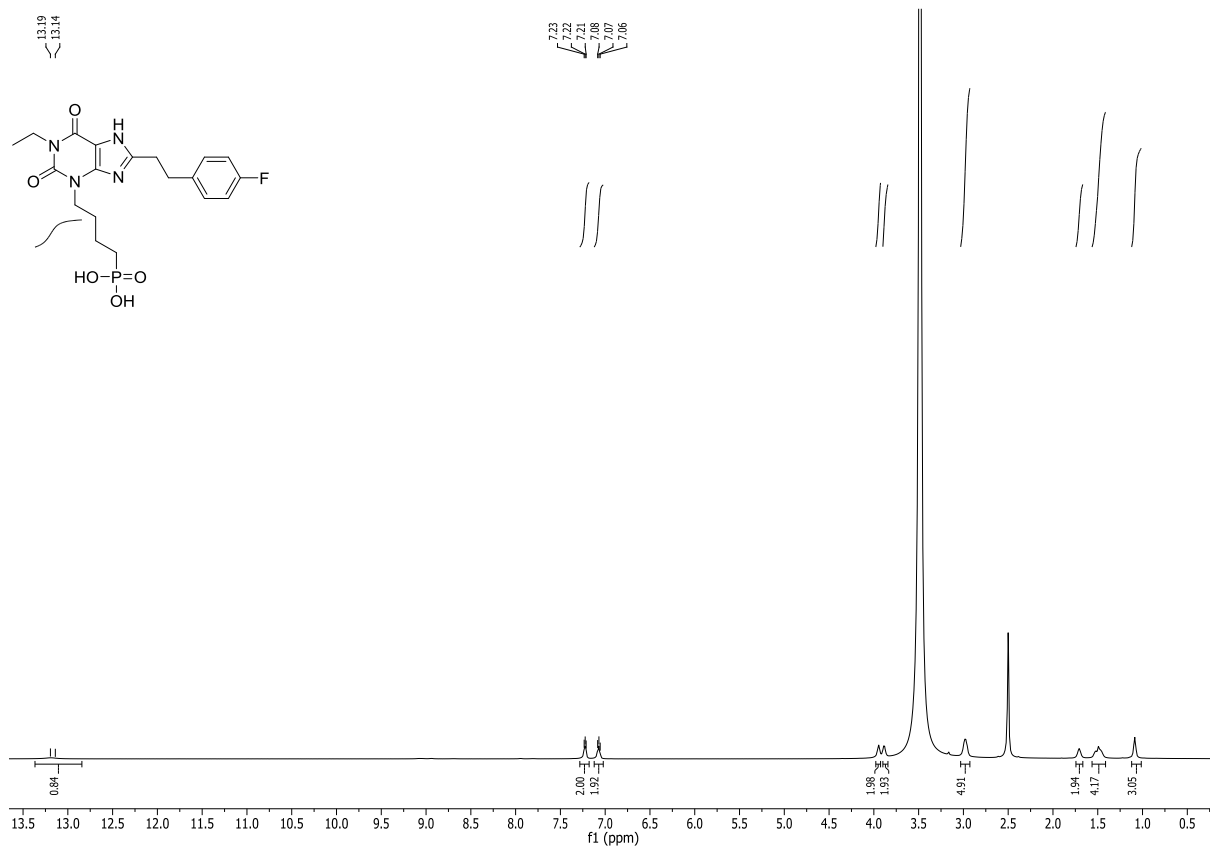


Figure S16: ¹H-NMR of compound **B-14**

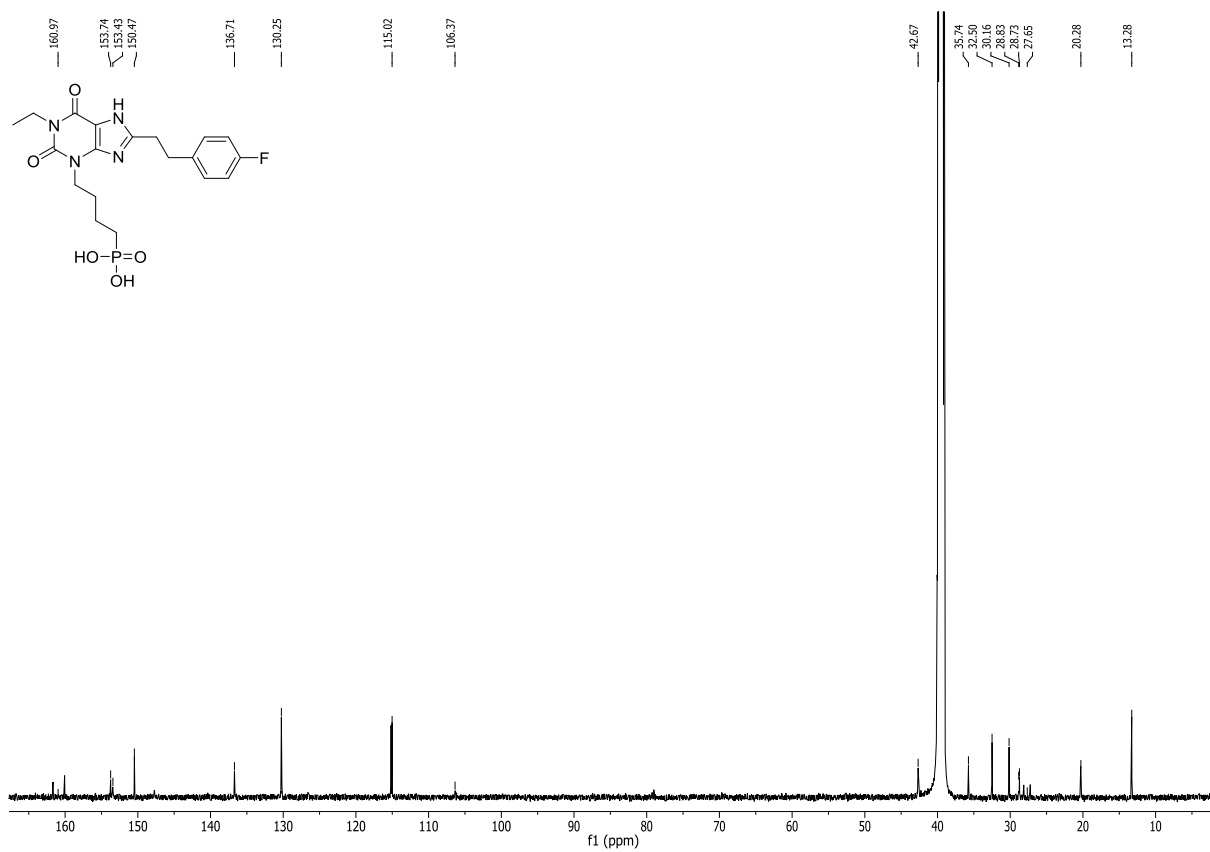


Figure S17: ¹³C-NMR of compound B-14

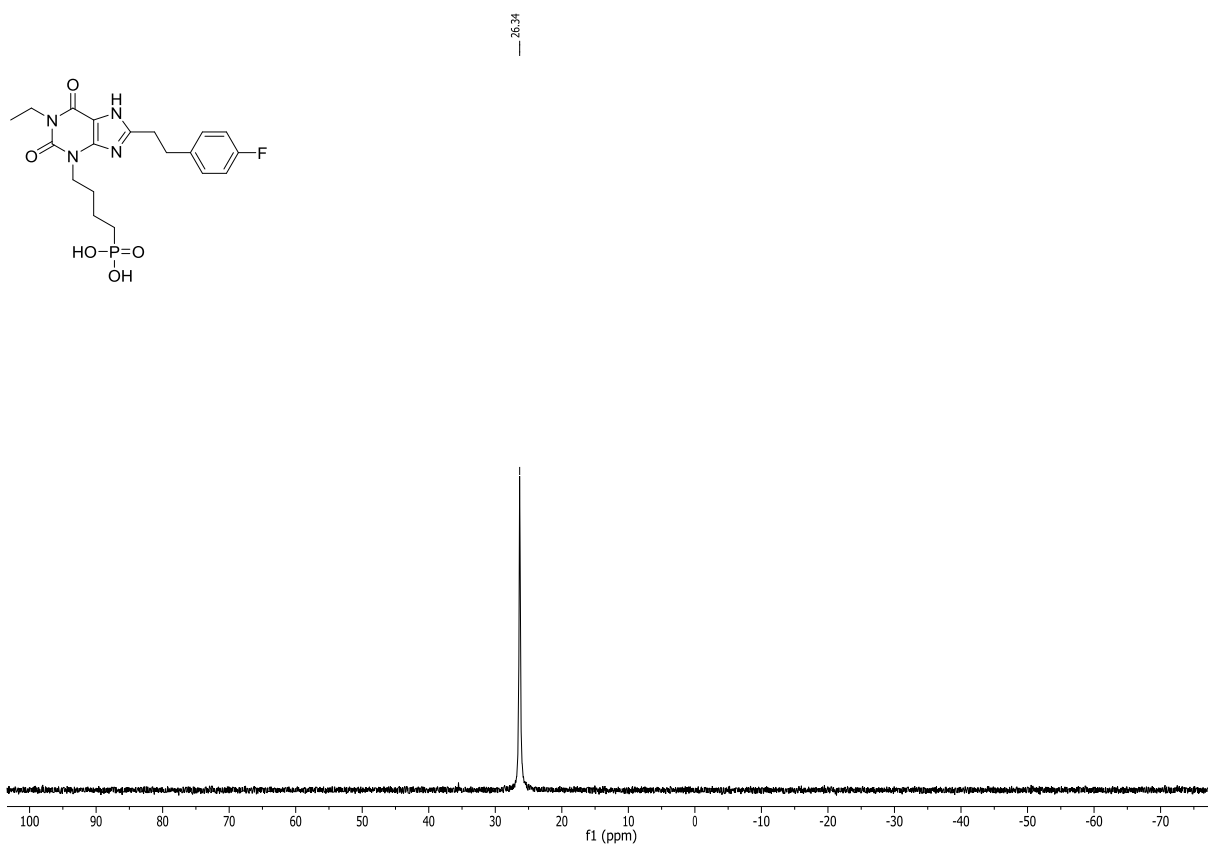


Figure S18: ^{31}P -NMR of compound B-14

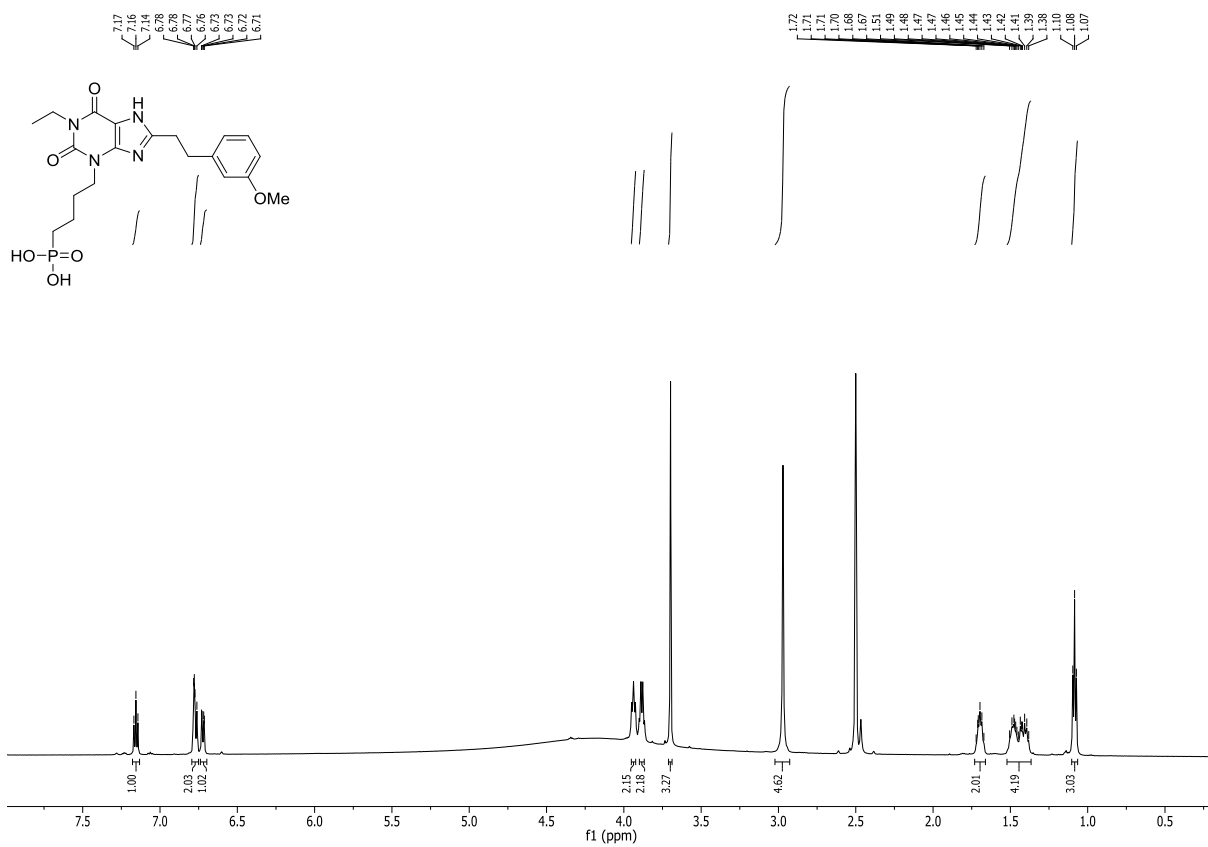


Figure S19: $^1\text{H-NMR}$ of compound **B-12**

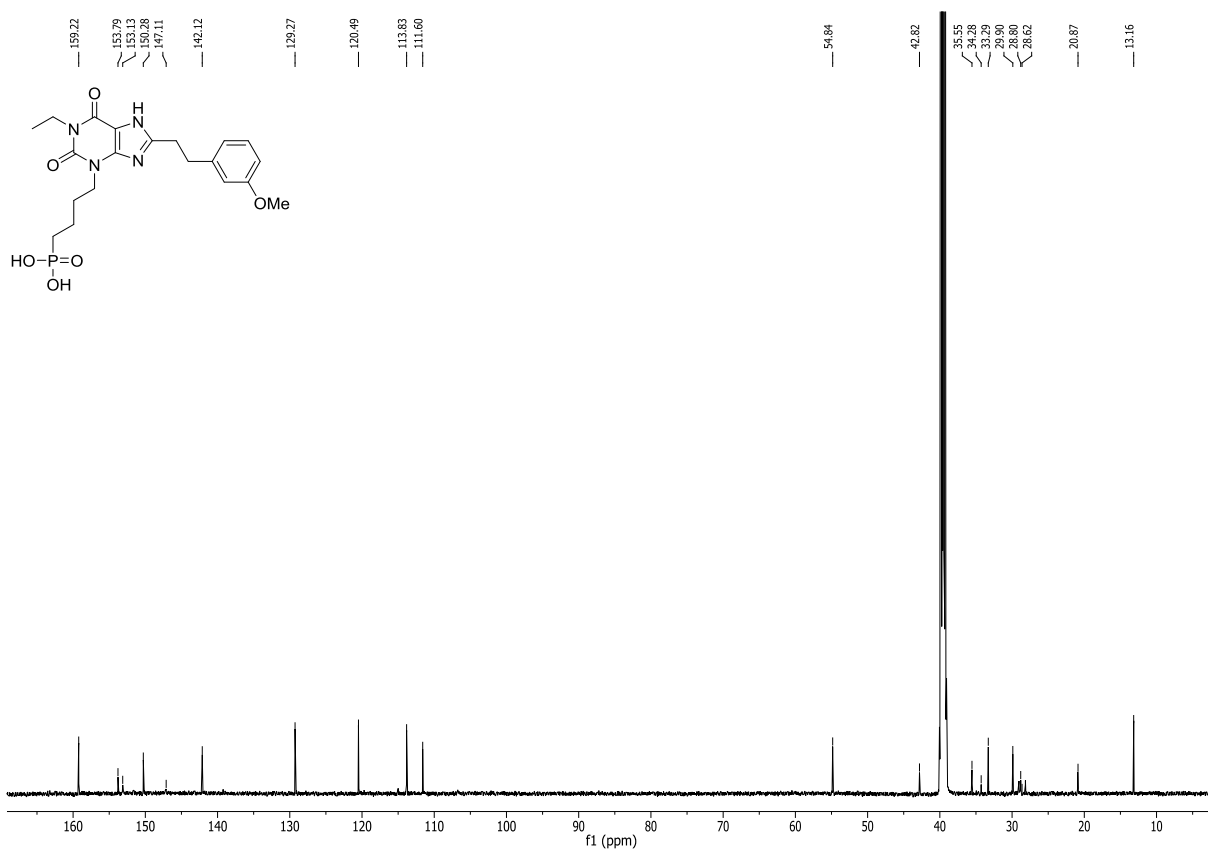


Figure S20: ¹³C-NMR of compound B-12

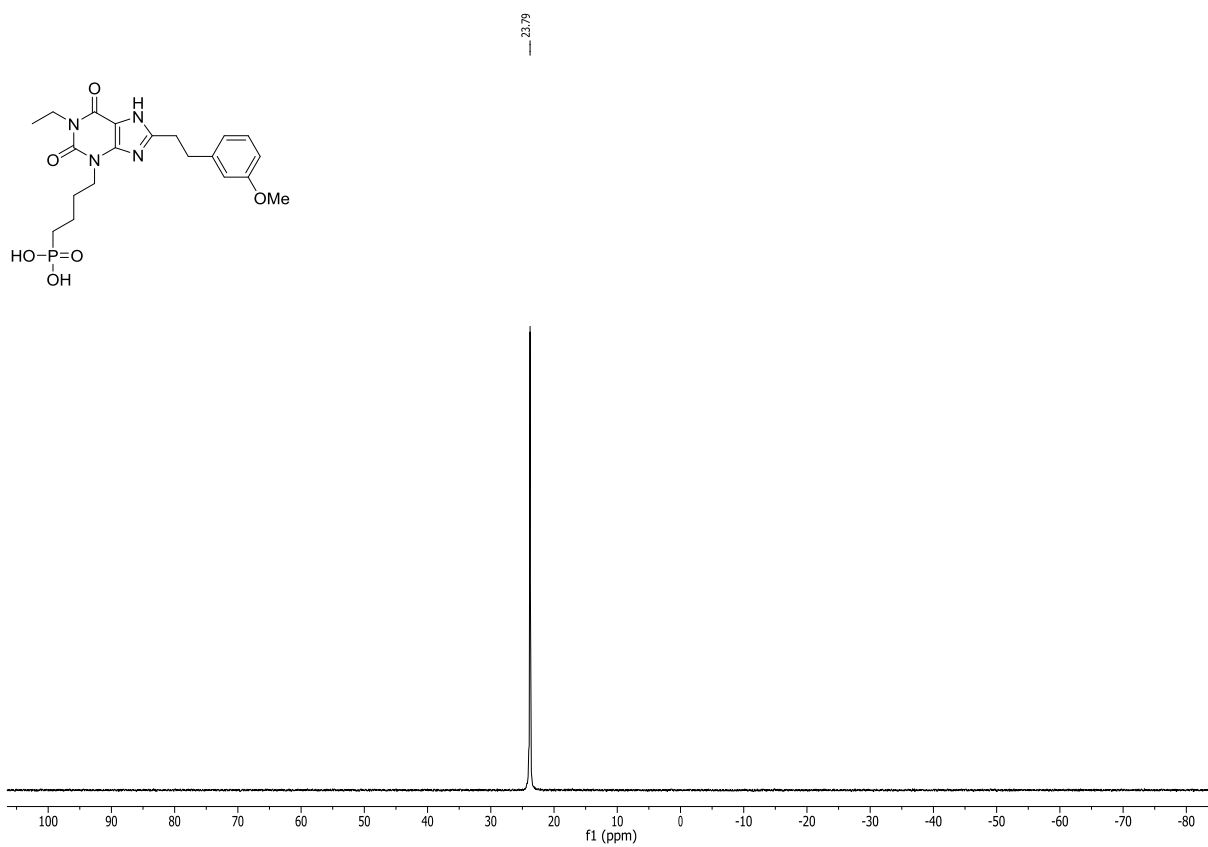


Figure S21: ^{31}P -NMR of compound **B-12**

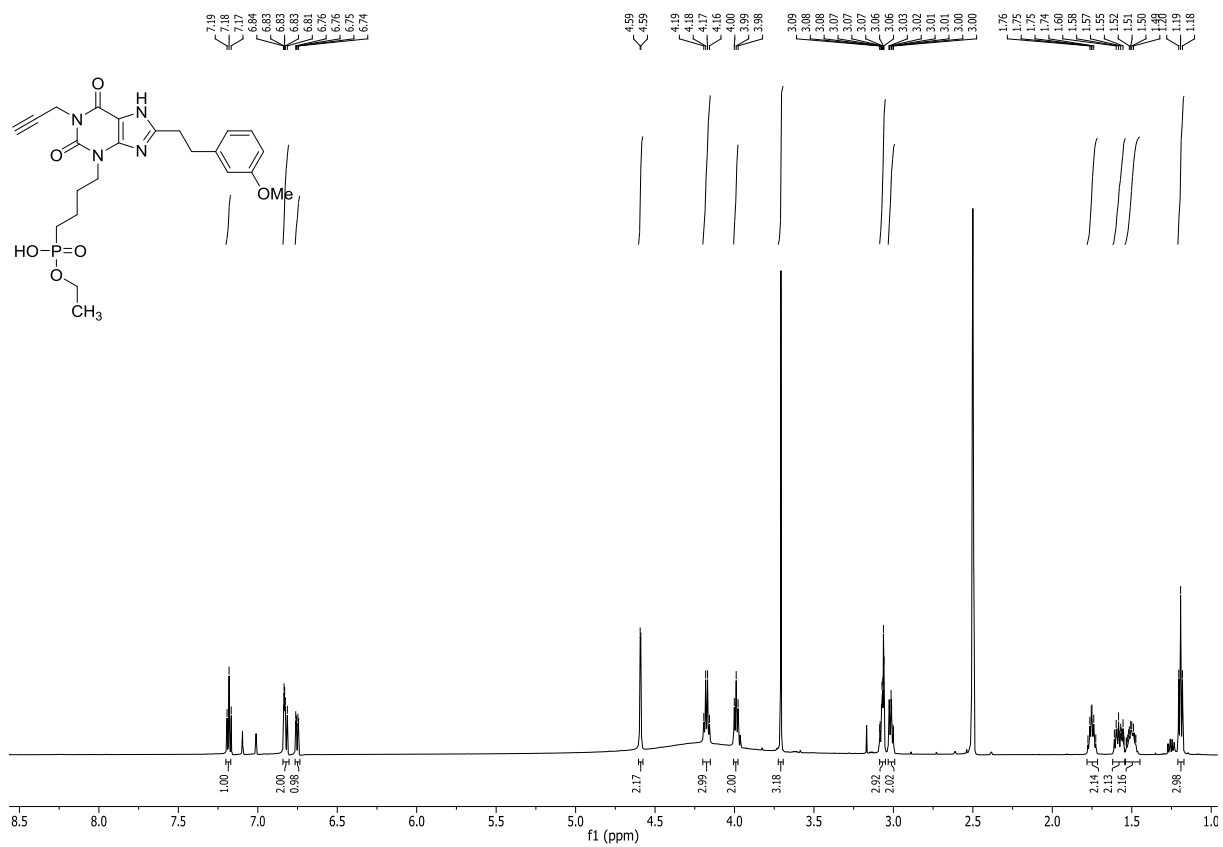


Figure S22: ¹H-NMR of compound D-3

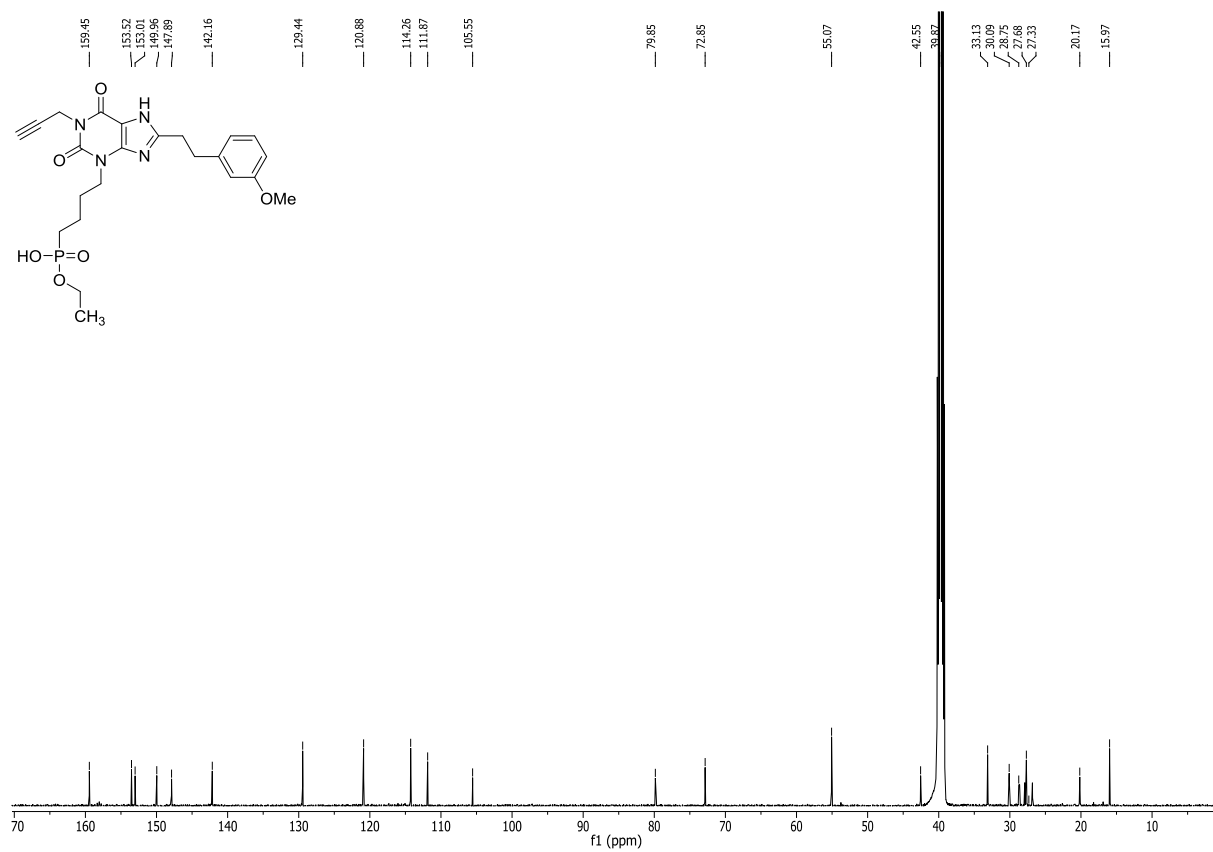


Figure S23: ^{13}C -NMR of compound **D-3**

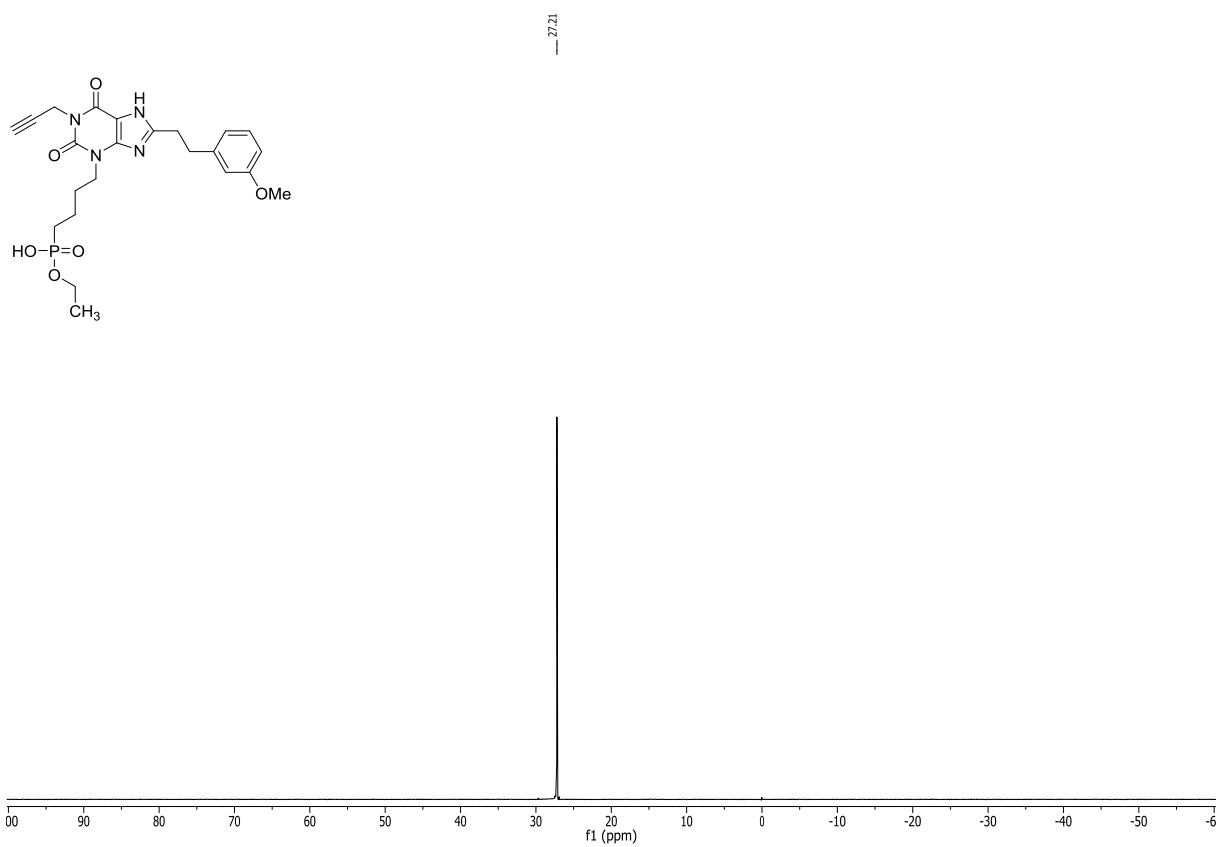


Figure S24: ^{31}P -NMR of compound **D-3**

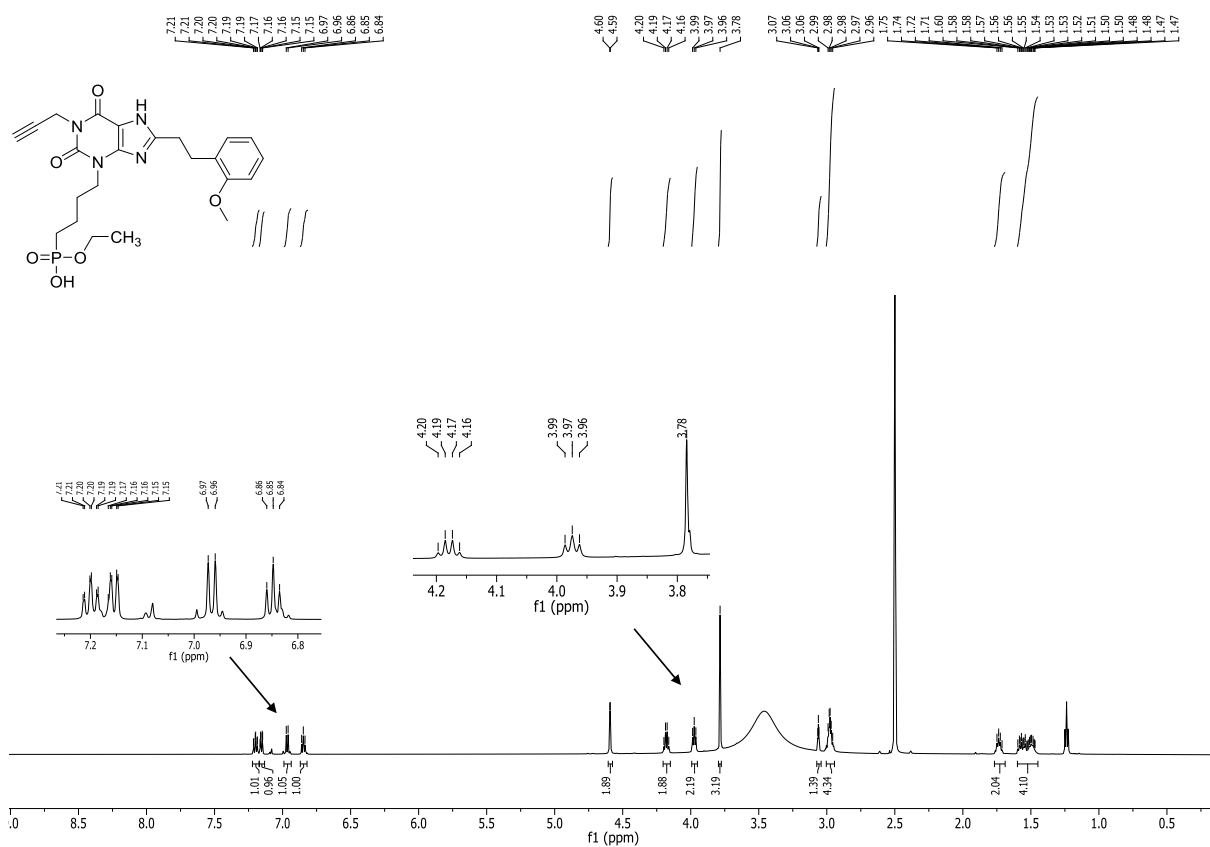


Figure S25: ¹H-NMR of compound D-2

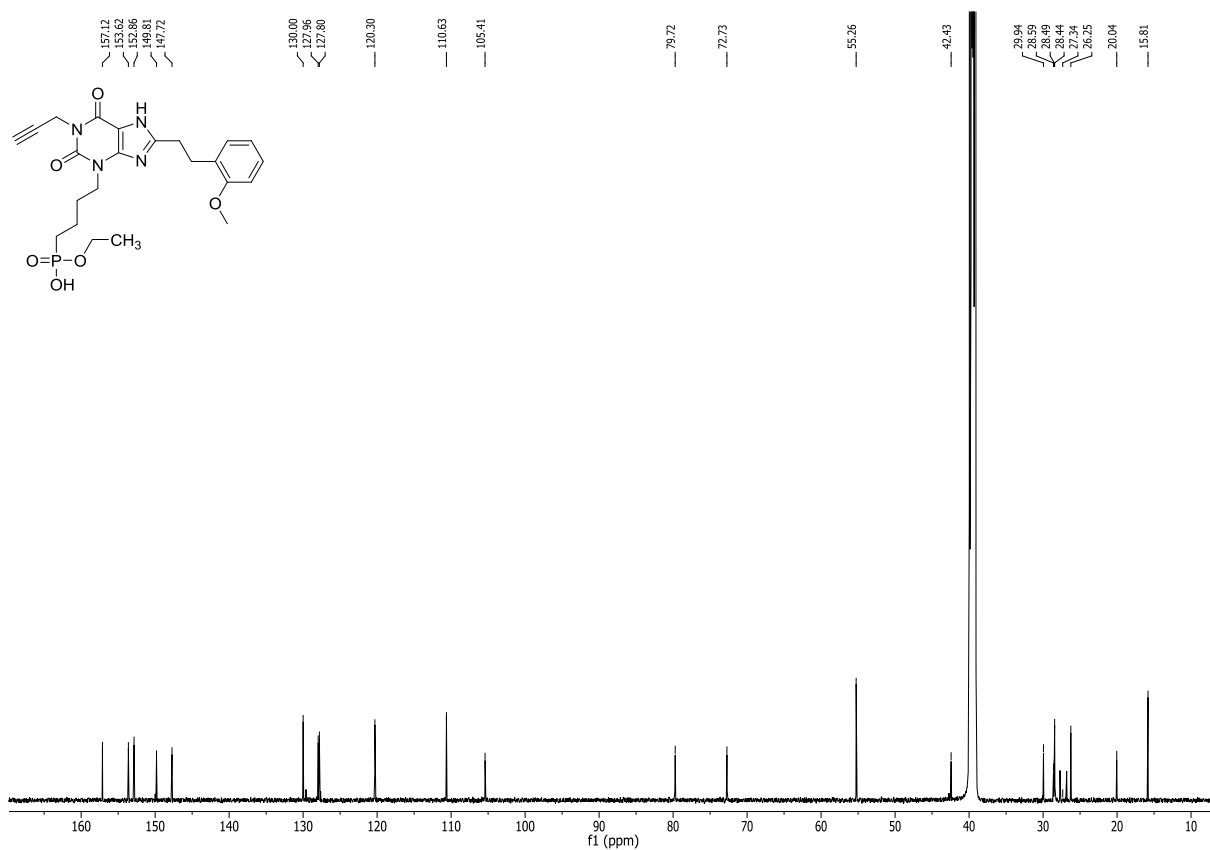


Figure S26: ^{13}C -NMR of compound **D-2**

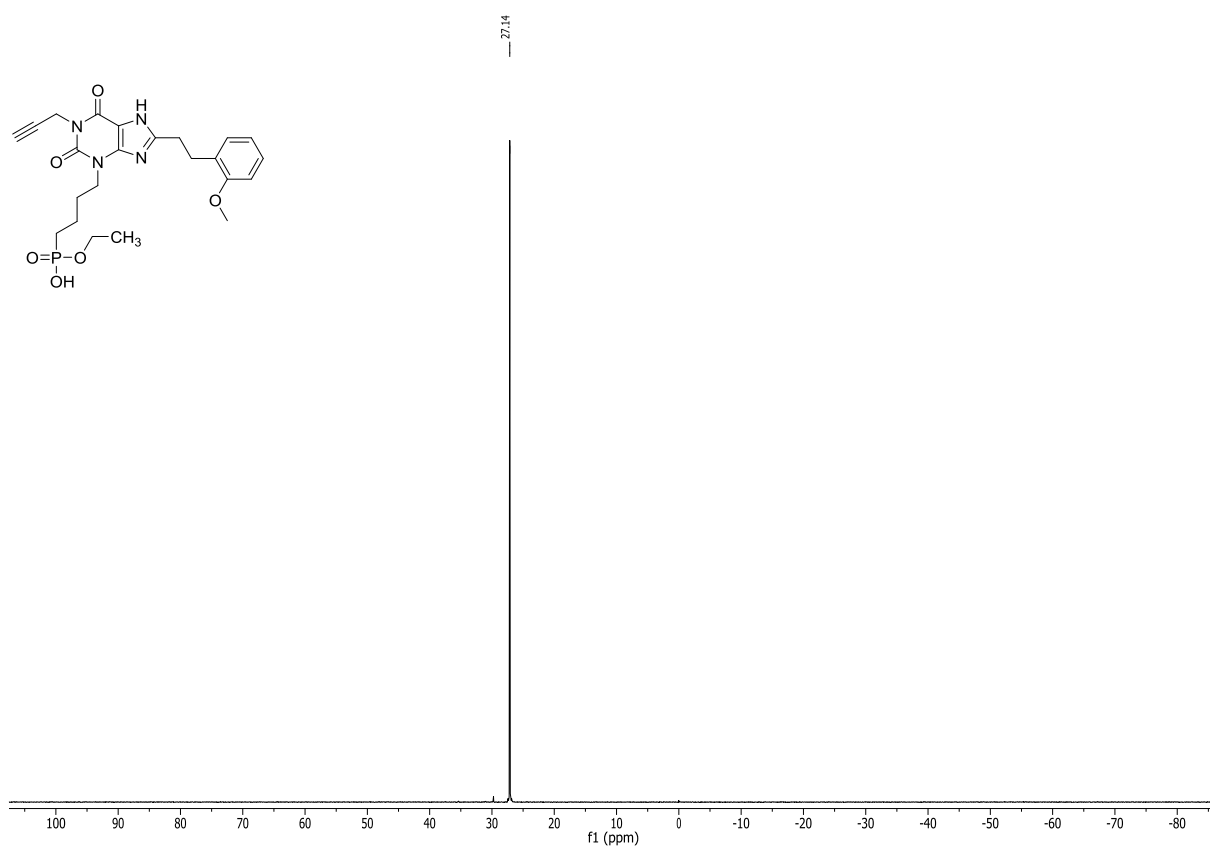


Figure S27: ^{31}P -NMR of compound **D-2**

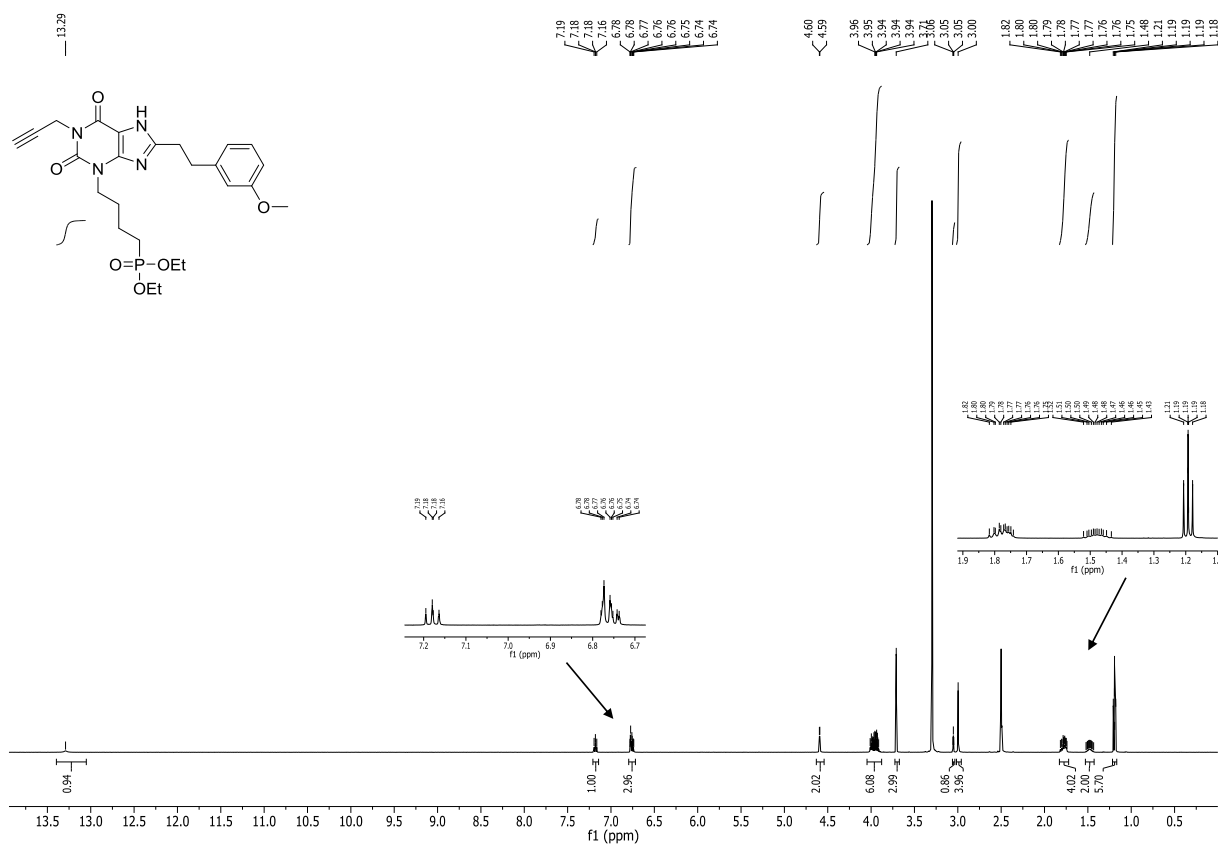


Figure S28: $^1\text{H-NMR}$ of compound C-6

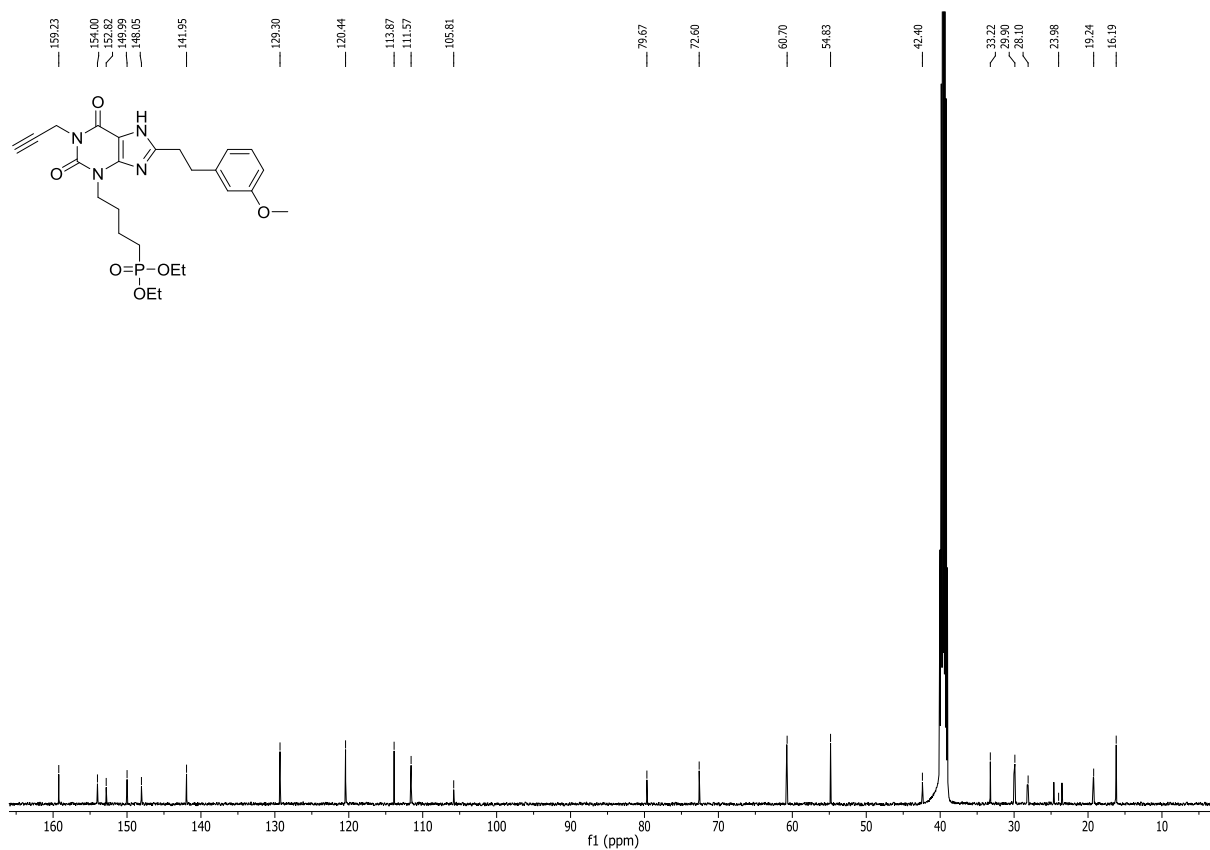


Figure S29: ^{13}C -NMR of compound C-6

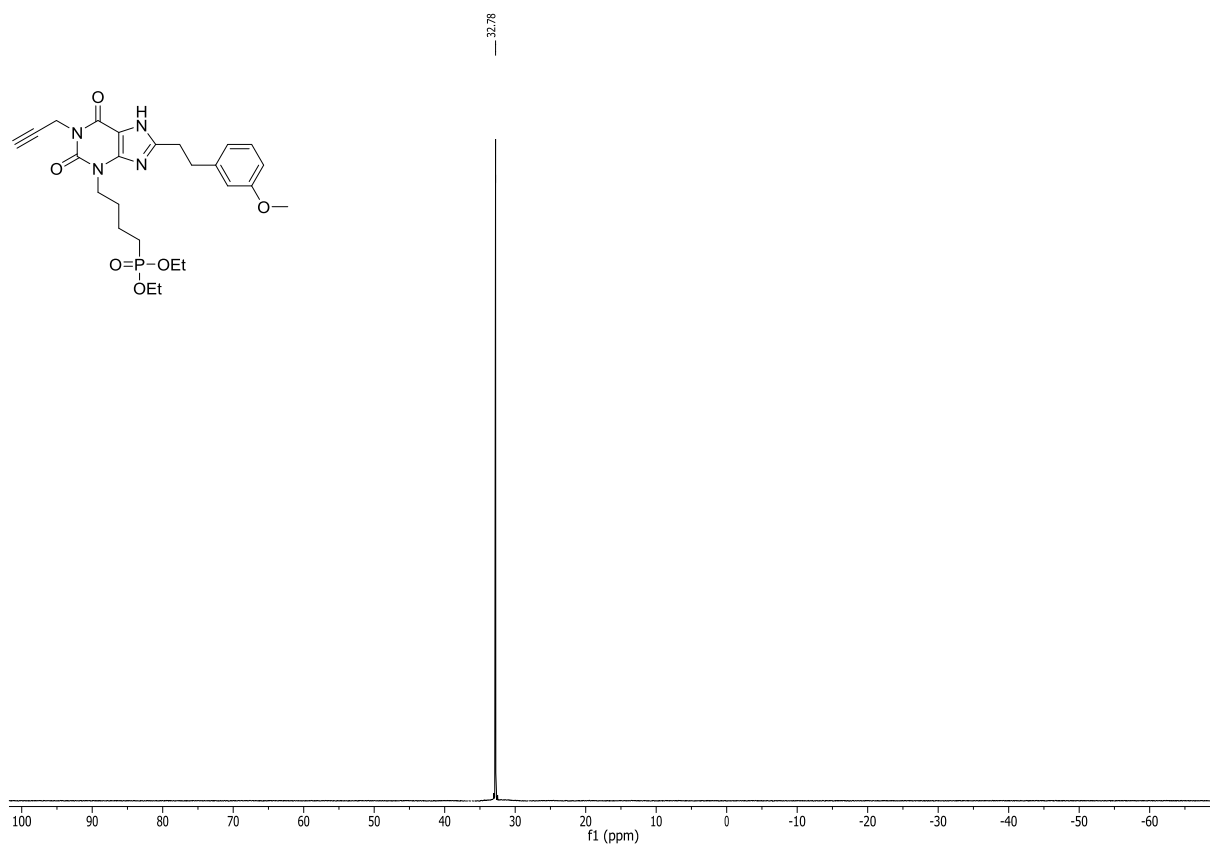


Figure S30: ^{31}P -NMR of compound C-6

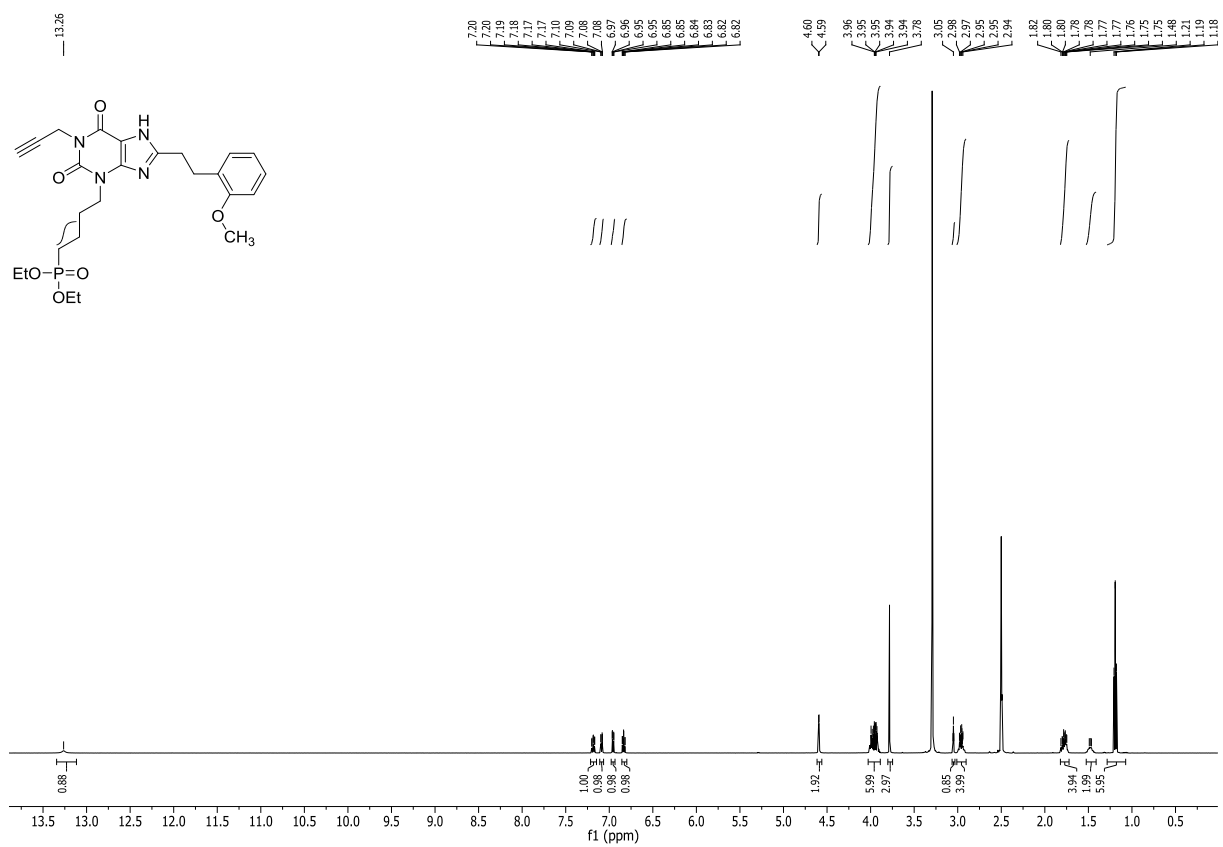


Figure S31: ¹H-NMR of compound C-1

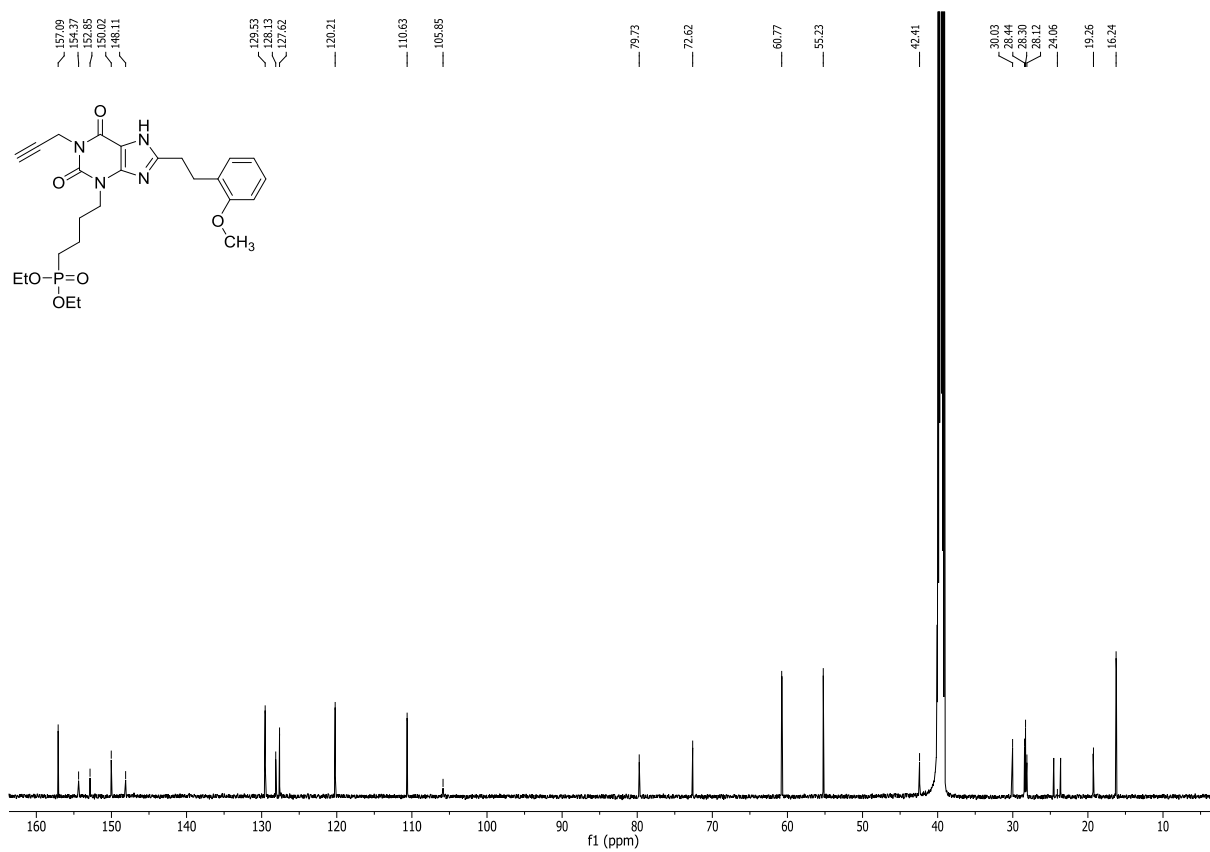


Figure S32: ¹³C-NMR of compound C-1

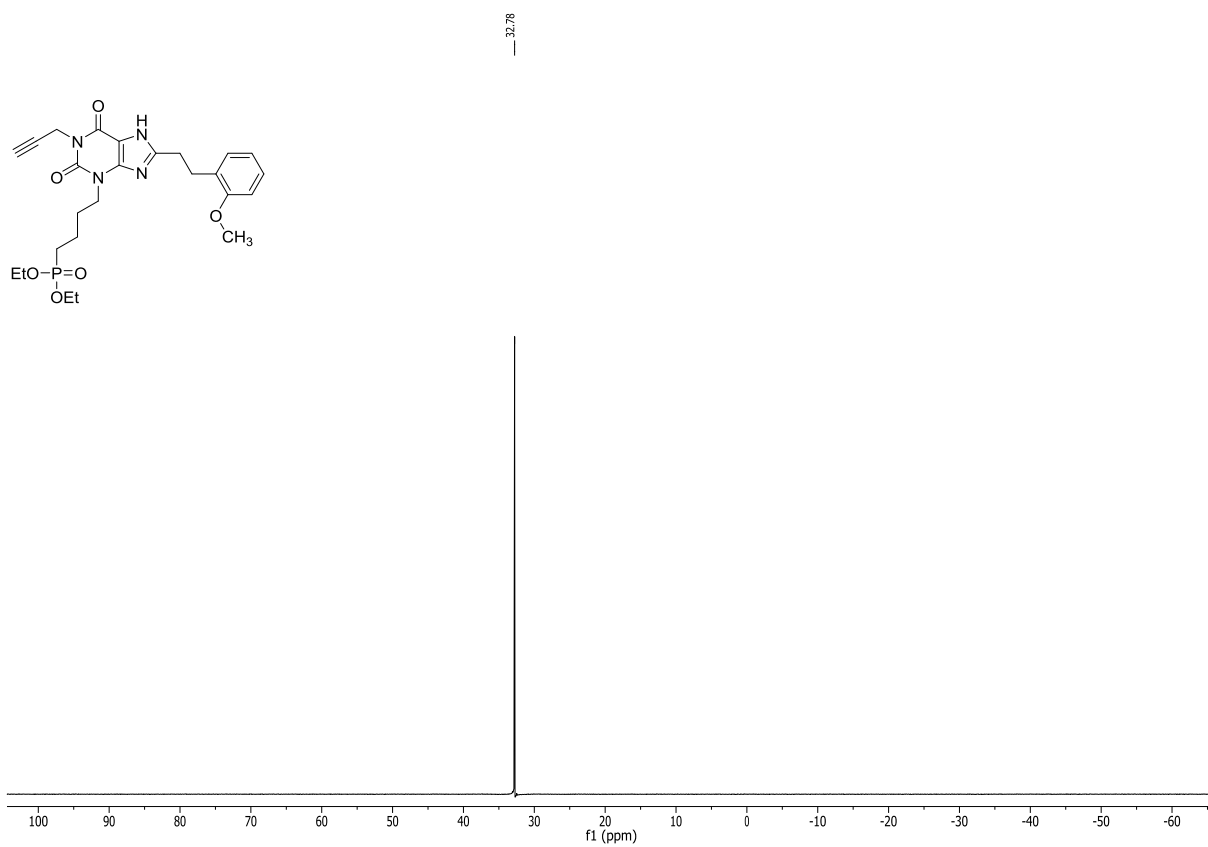


Figure S33: ^{31}P -NMR of compound C-1

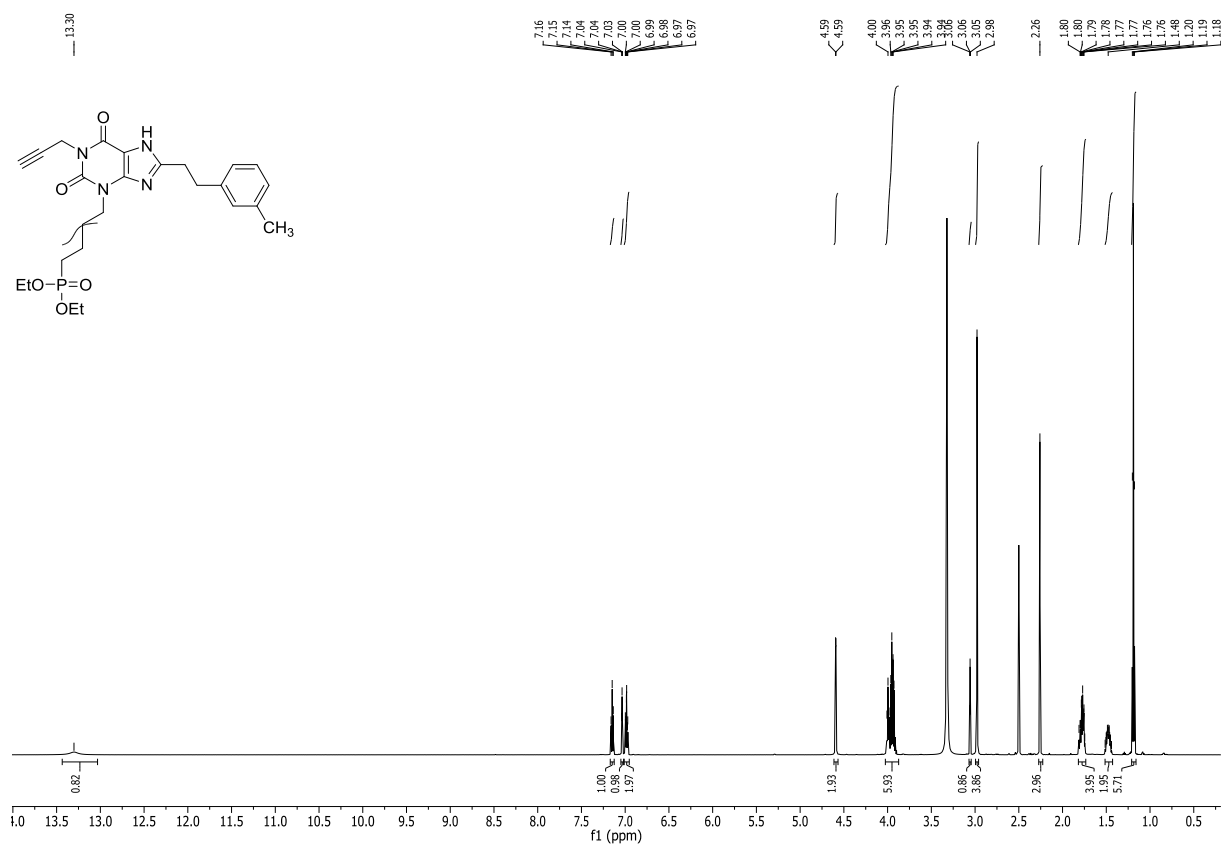


Figure S34: $^1\text{H-NMR}$ of compound C-4

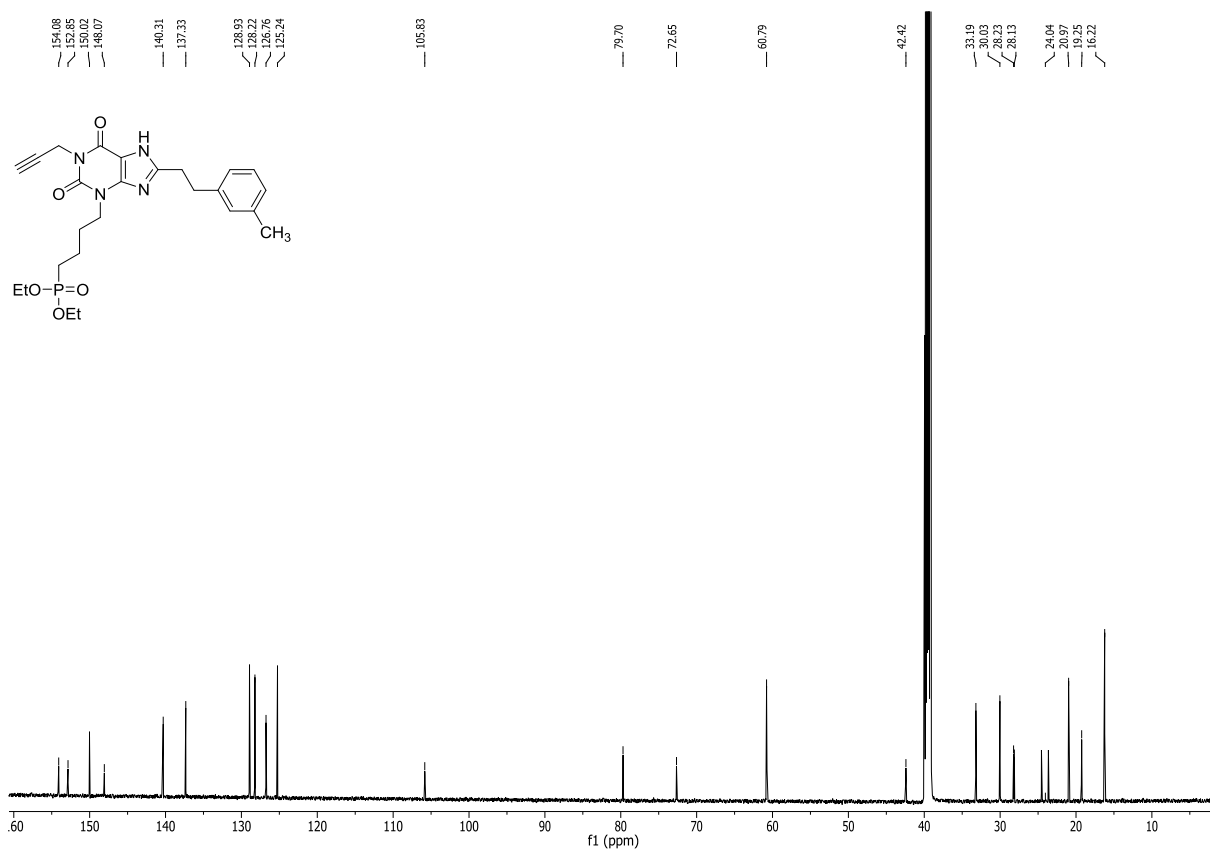


Figure S35: ¹³C-NMR of compound C-4

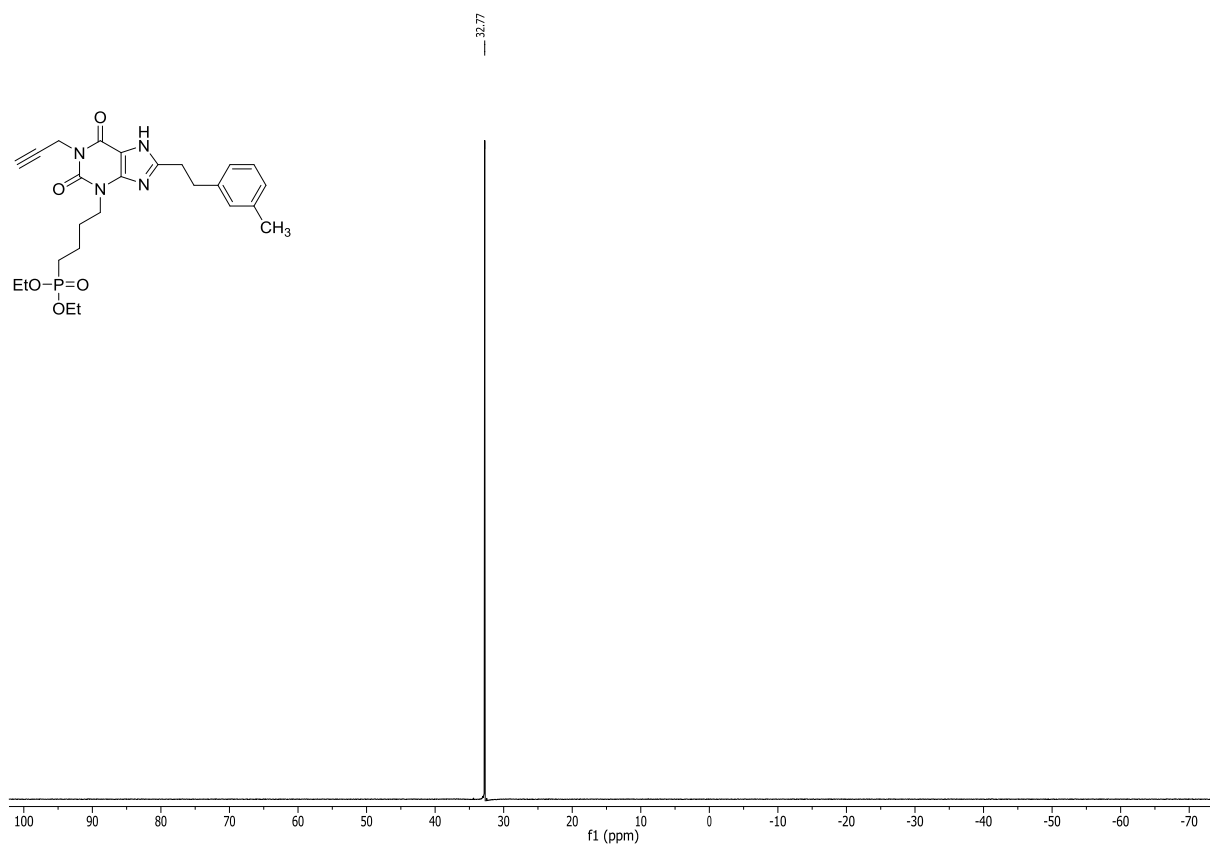


Figure S36: ^{31}P -NMR of compound C-4

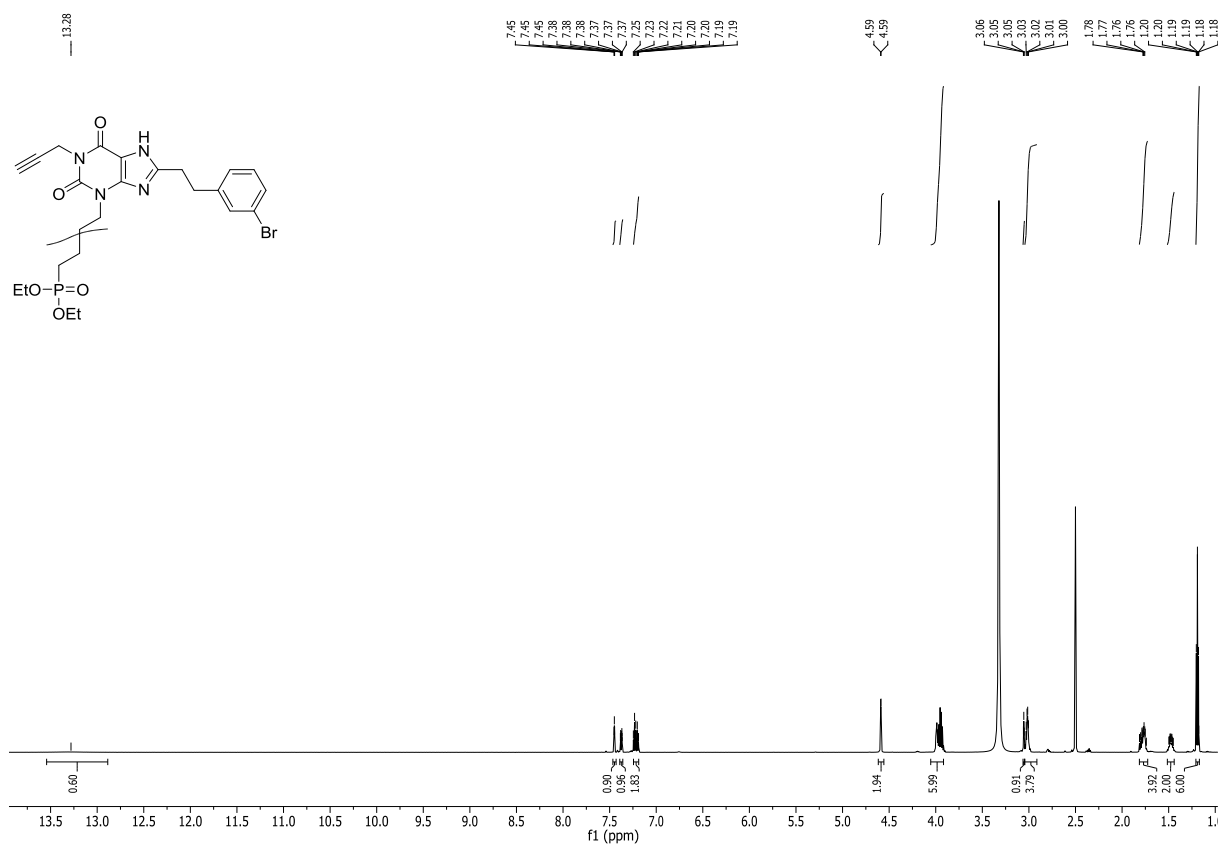


Figure S37: ¹H-NMR of compound C-3

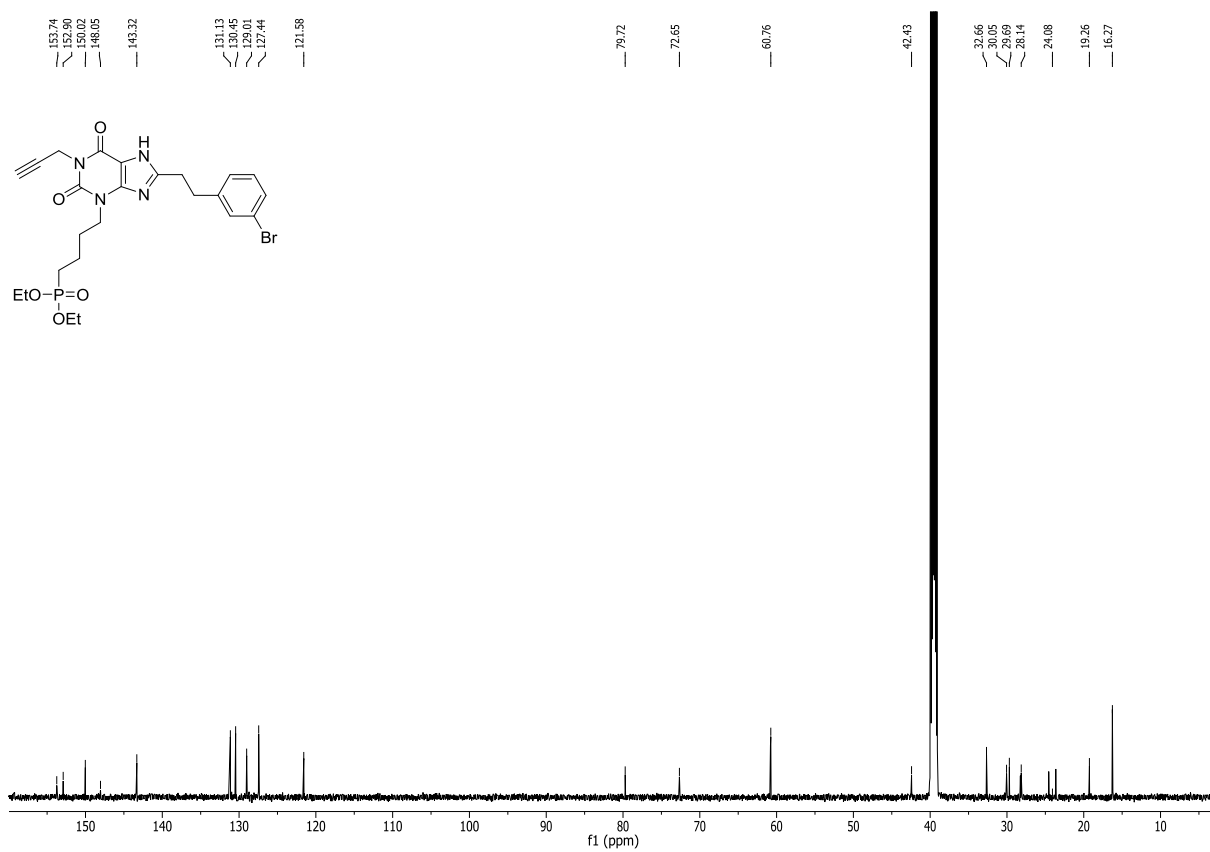


Figure S38: $^{13}\text{C-NMR}$ of compound C-3

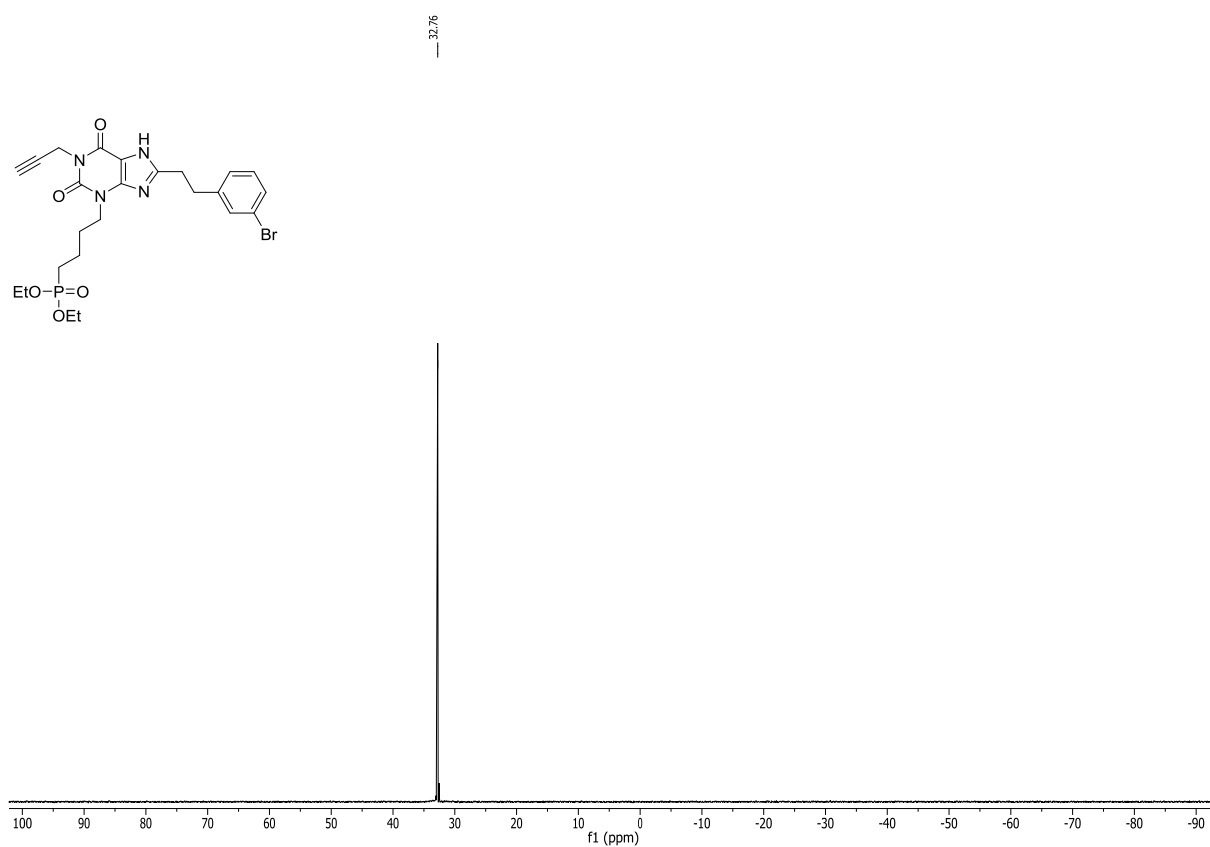


Figure S39: ^{31}P -NMR of compound C-3

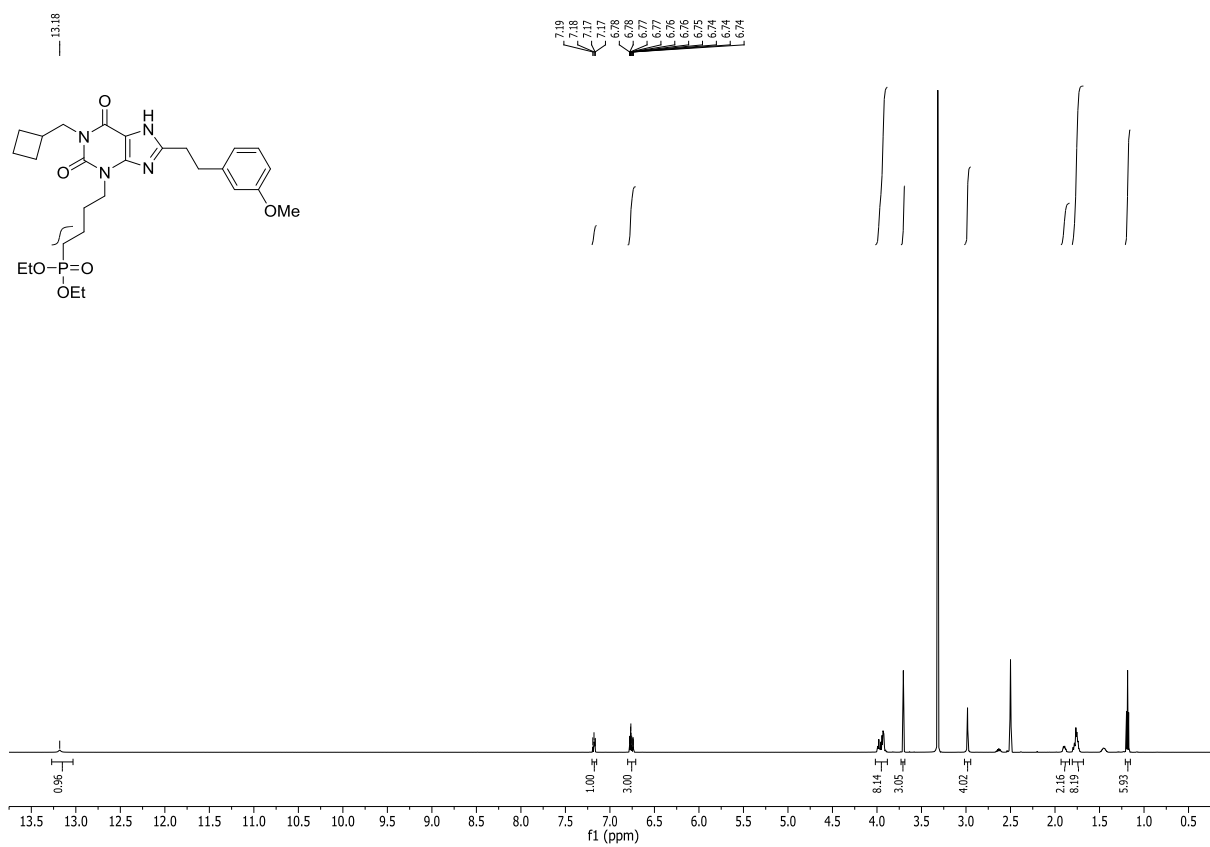


Figure S40: ¹H-NMR of compound C-7

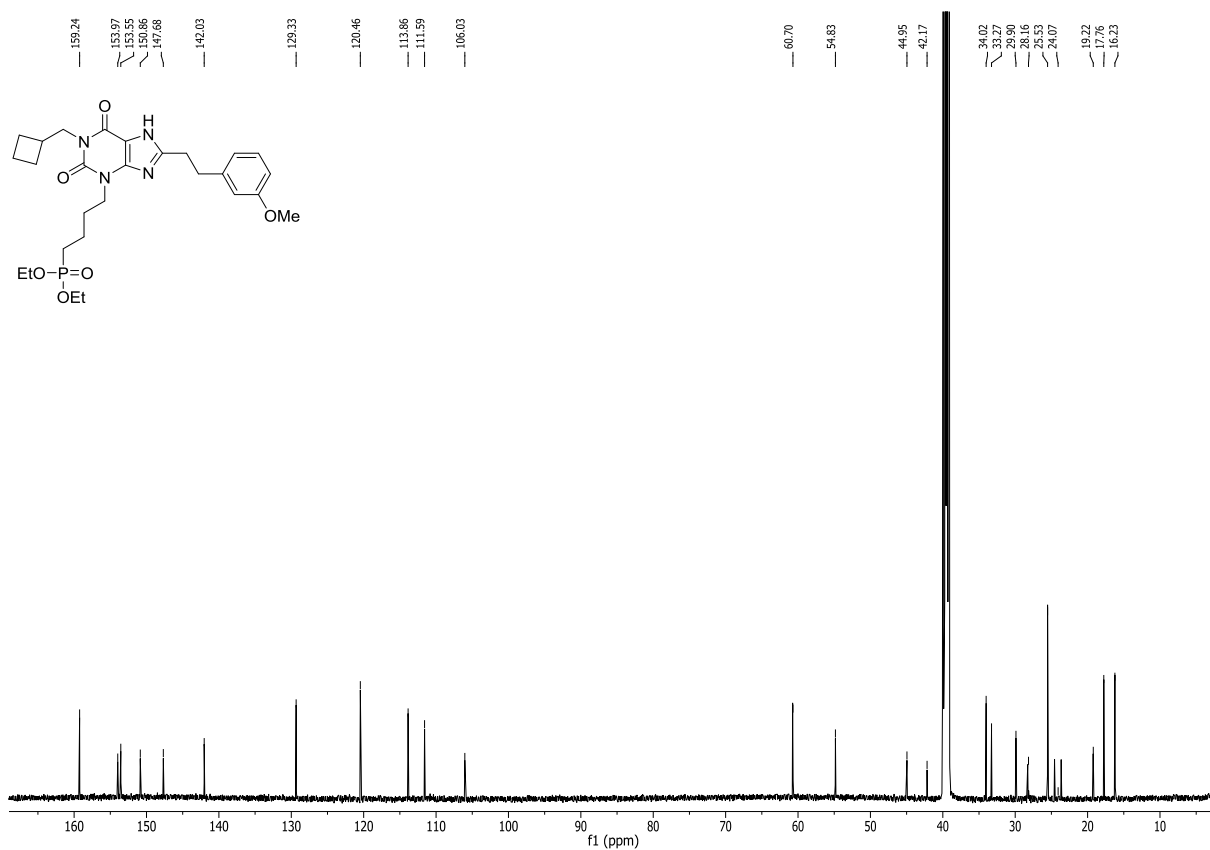


Figure S41: ¹³C-NMR of compound C-7

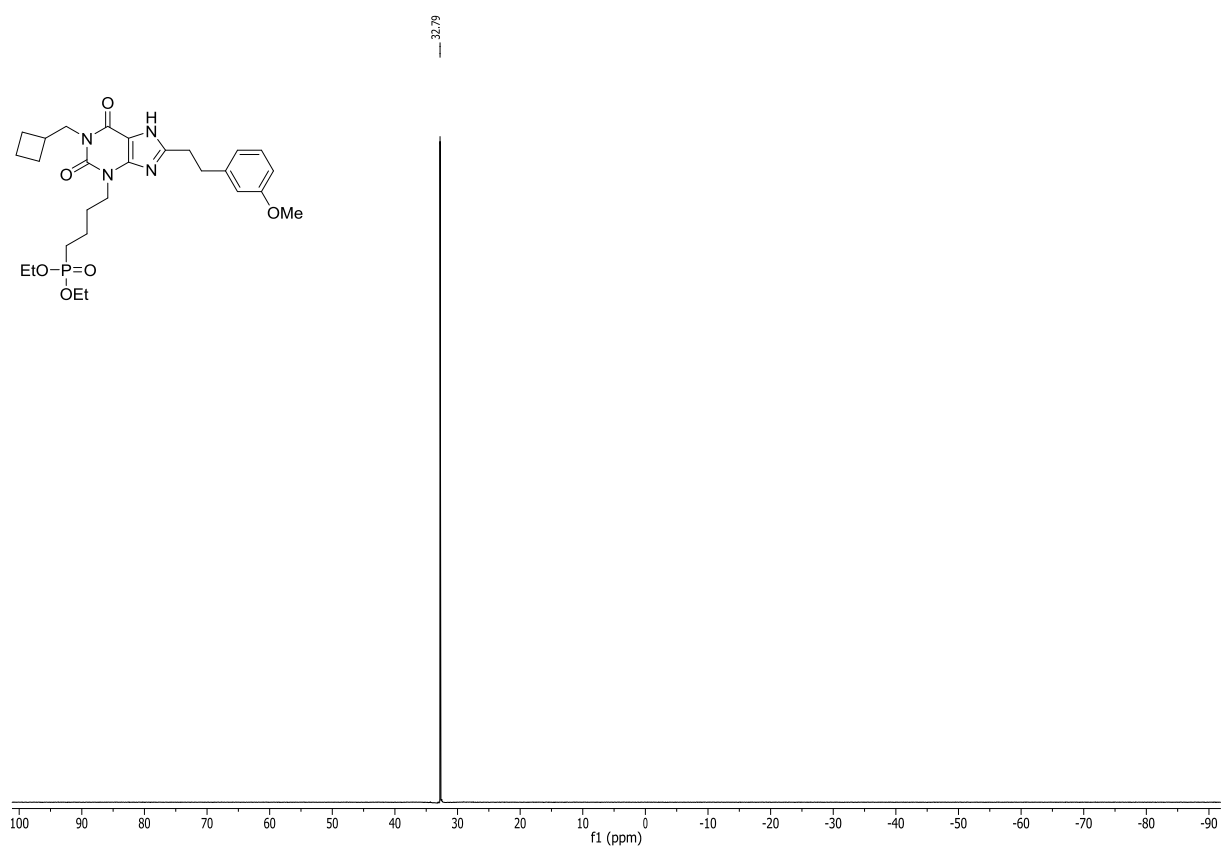


Figure S42: ^{31}P -NMR of compound C-7

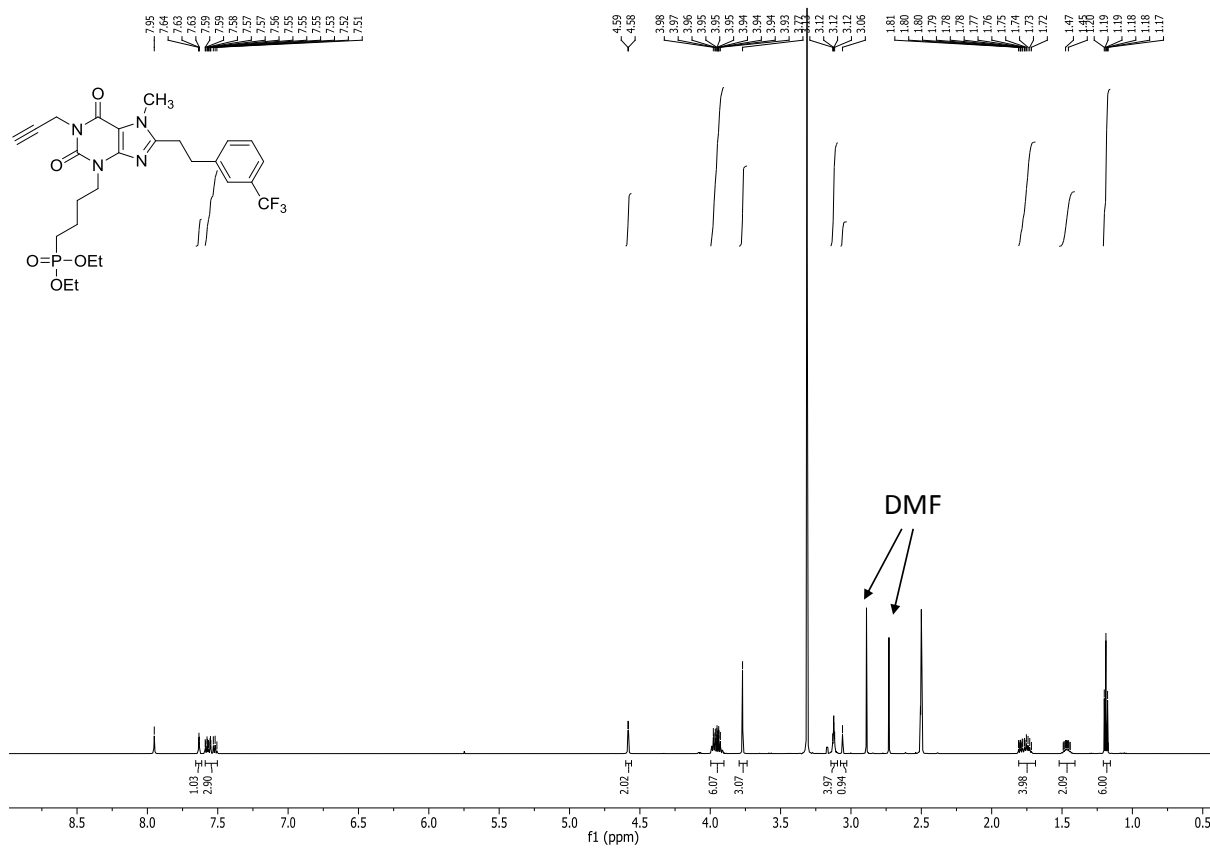


Figure S43: ¹H-NMR of compound C-5

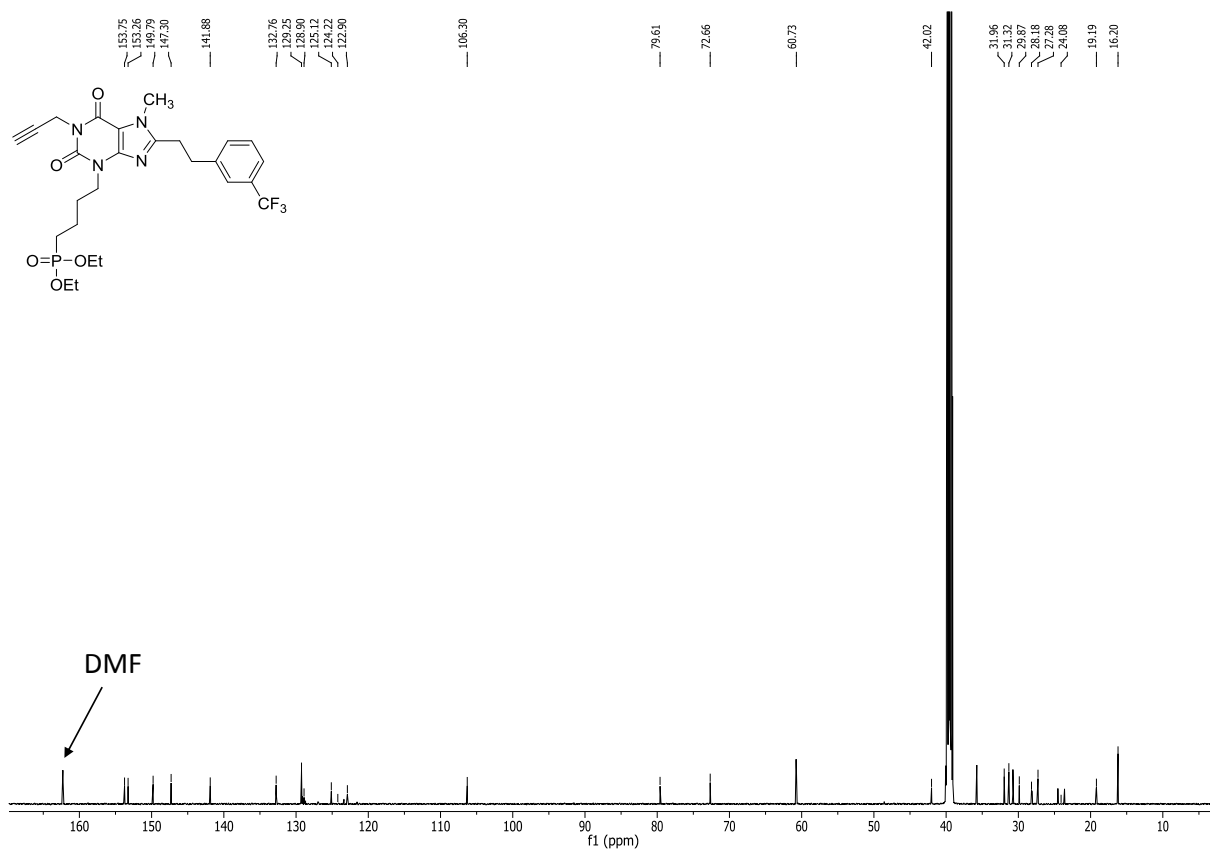


Figure S44: ¹³C-NMR of compound C-5

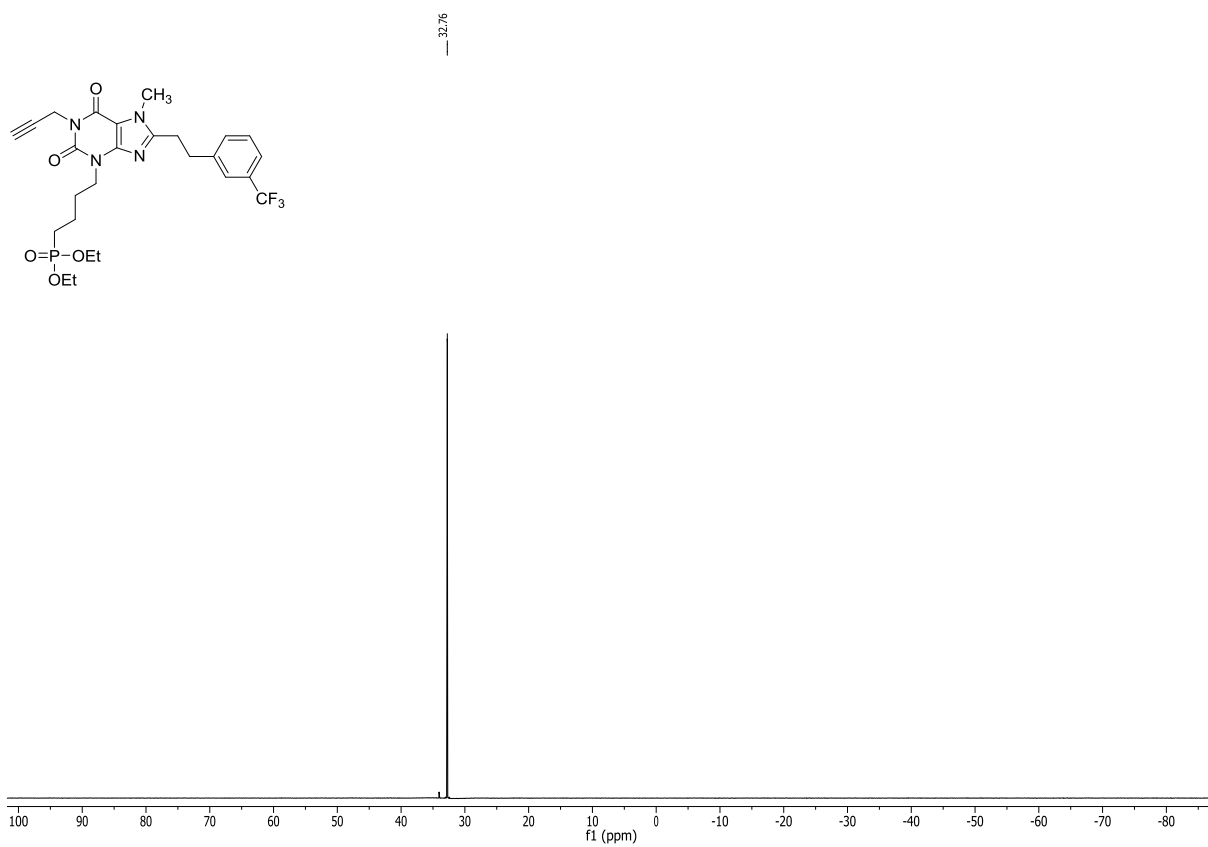


Figure S45: ^{31}P -NMR of compound C-5

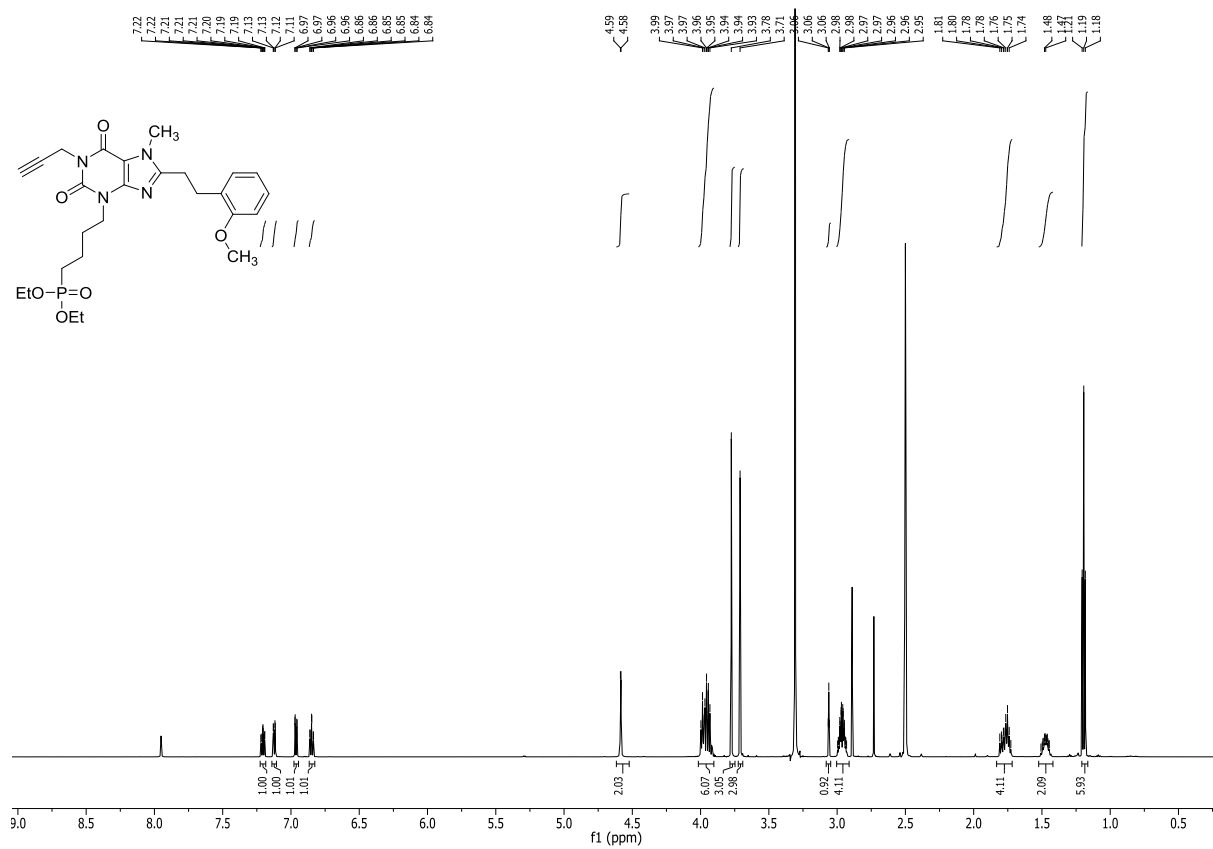


Figure S46: ¹H-NMR of compound C-2

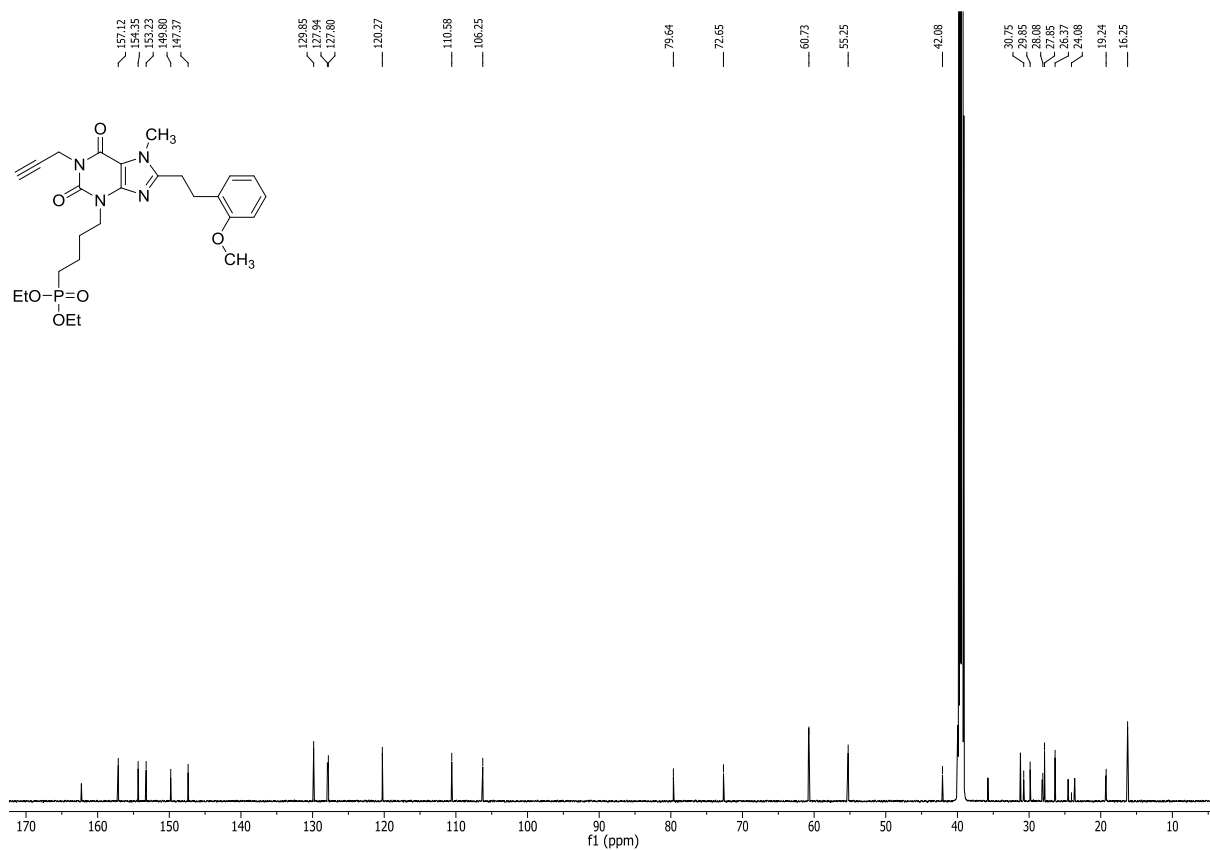


Figure S47: ¹³C-NMR of compound C-2

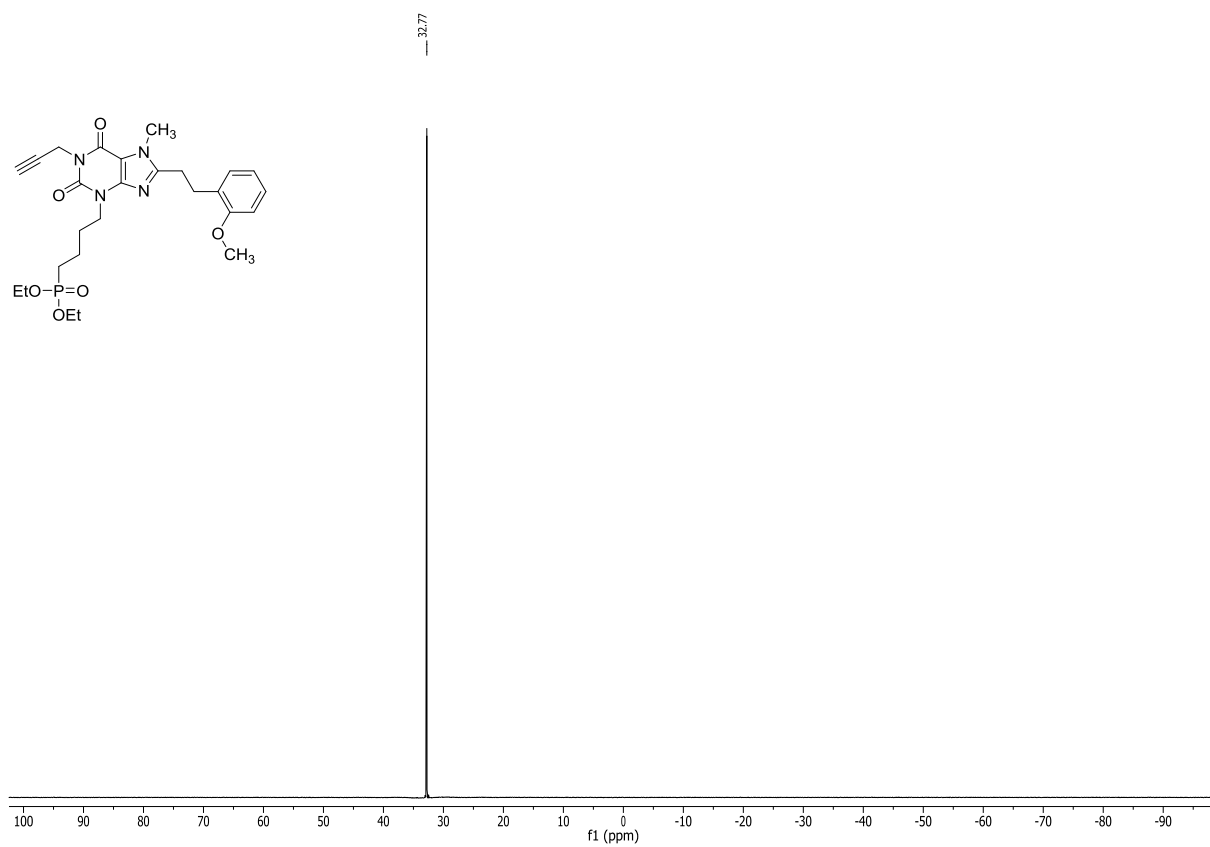


Figure S48: ^{31}P -NMR of compound C-2

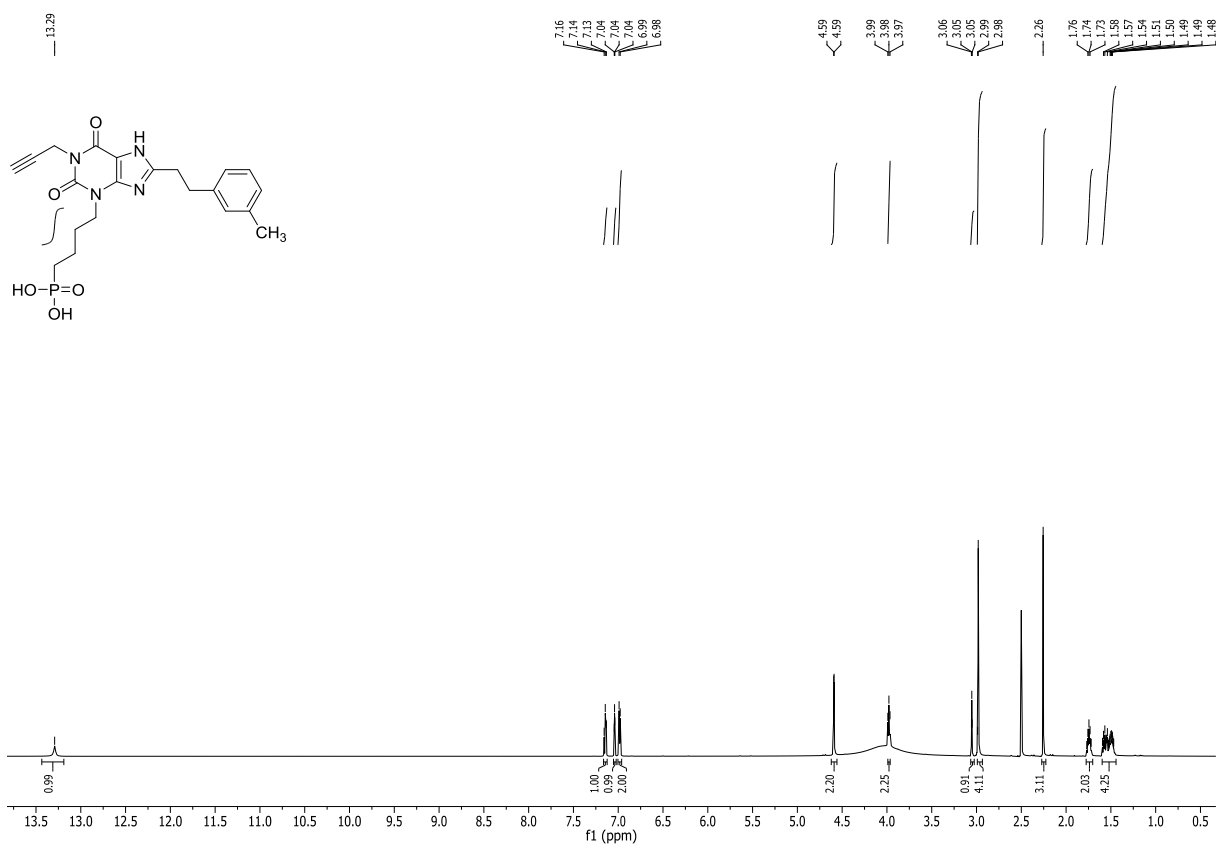


Figure S49: ¹H-NMR of compound B-5

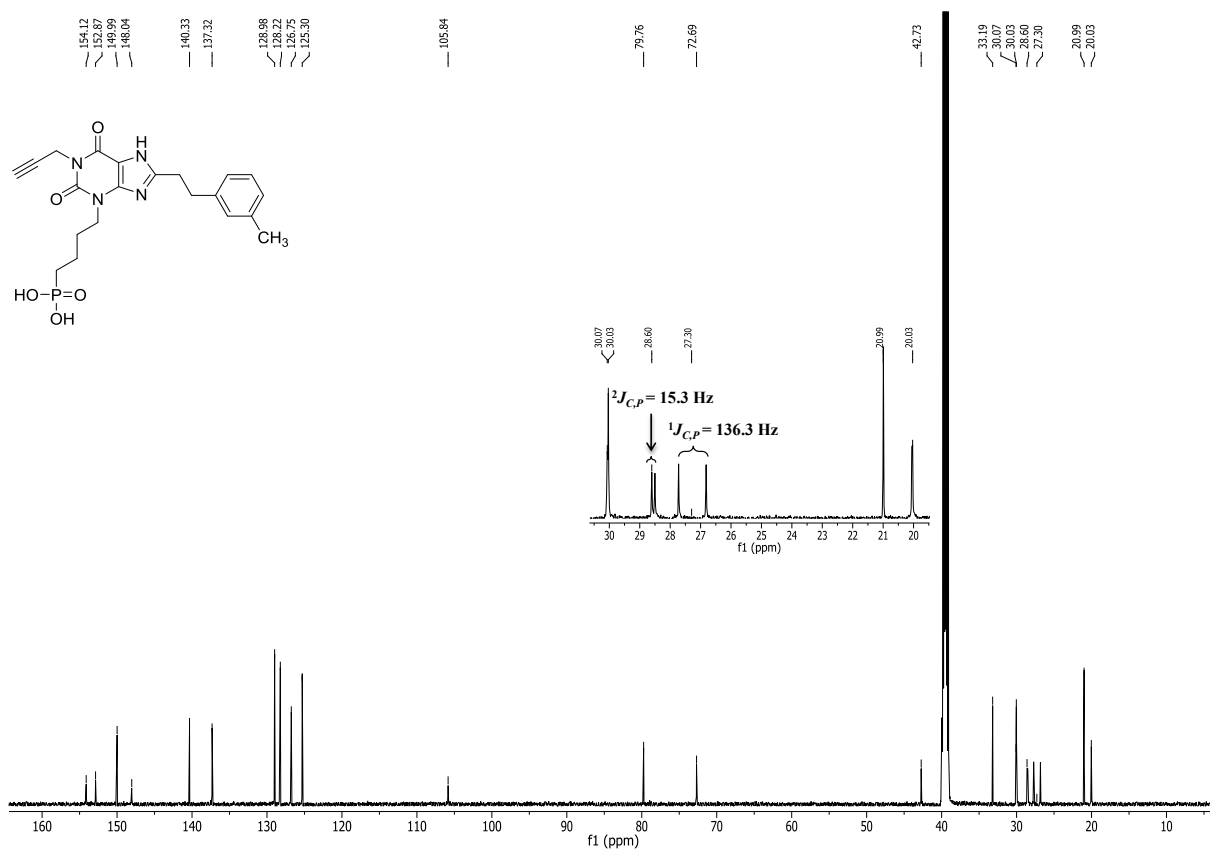


Figure S50: ^{13}C -NMR of compound **B-5**

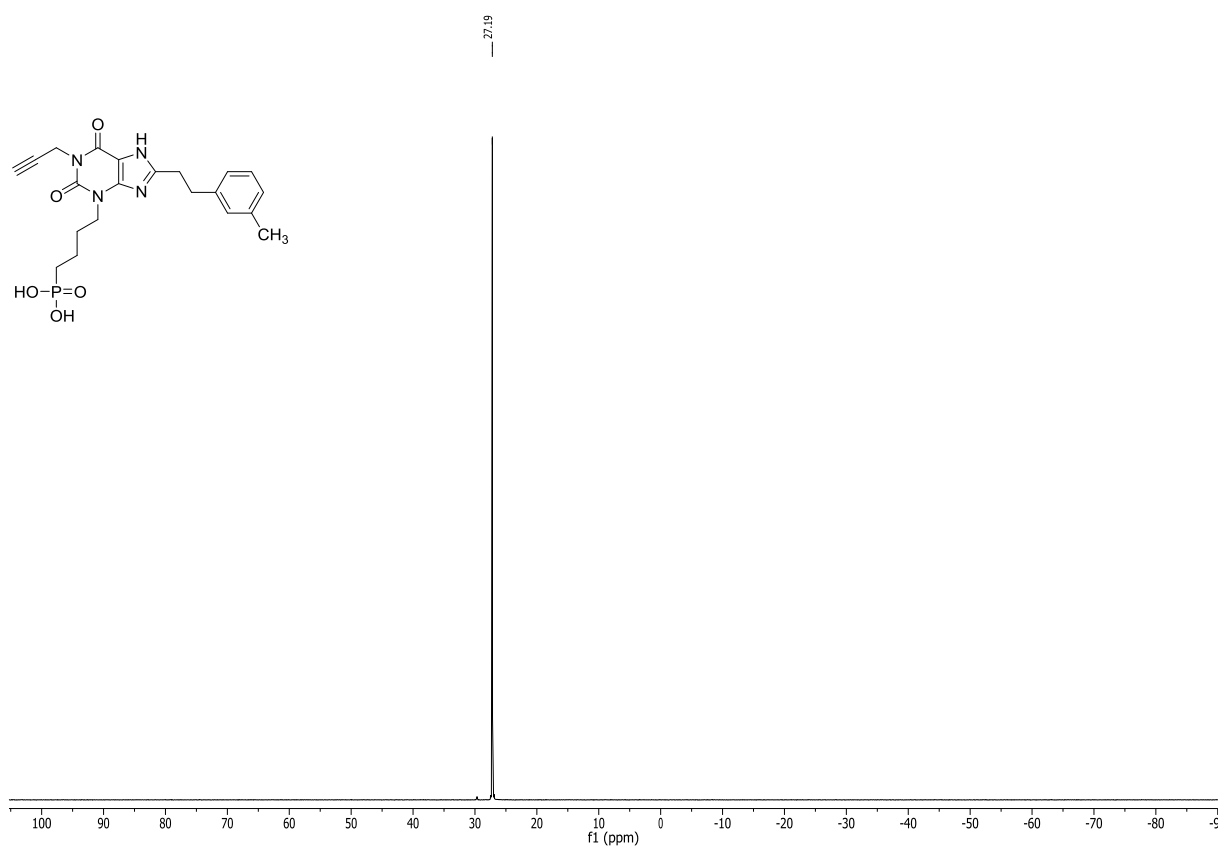


Figure S51: ^{31}P -NMR of compound **B-5**

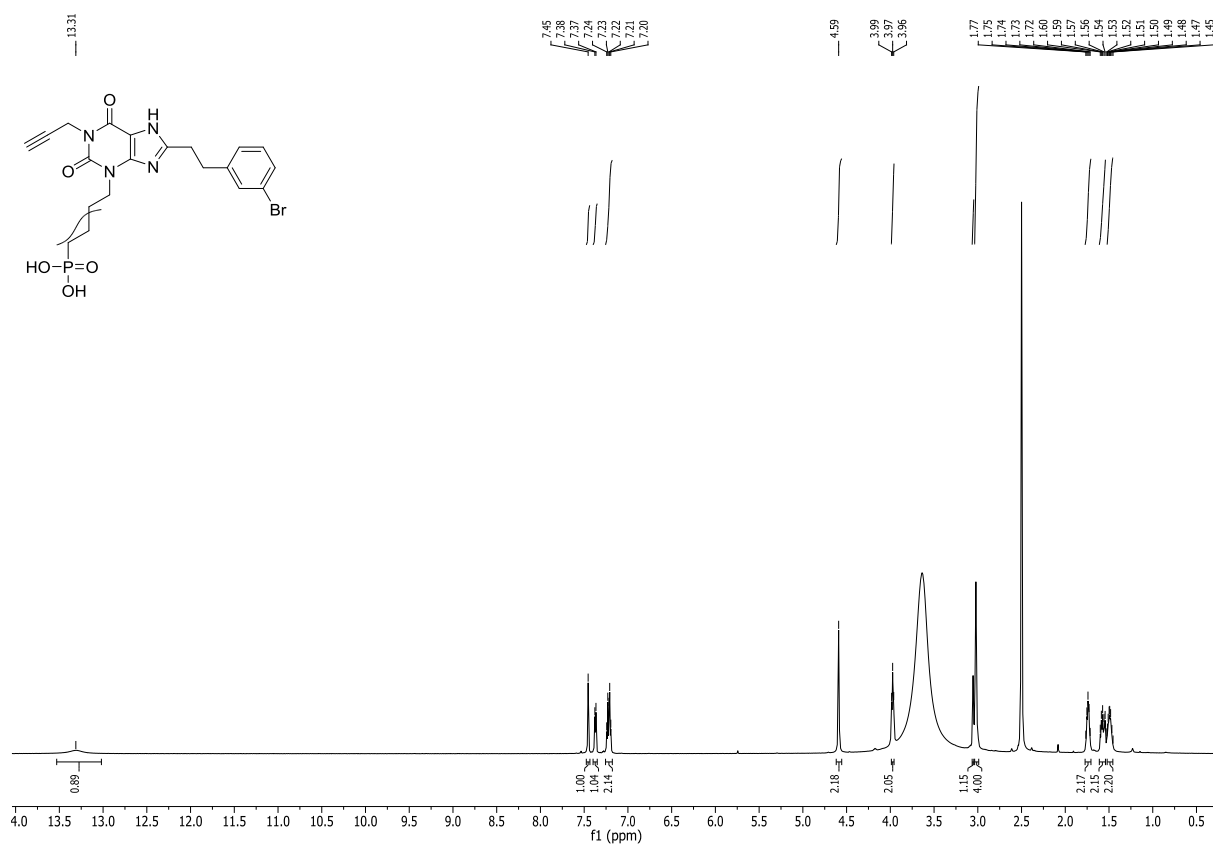


Figure S52: ¹H-NMR of compound **B-4**

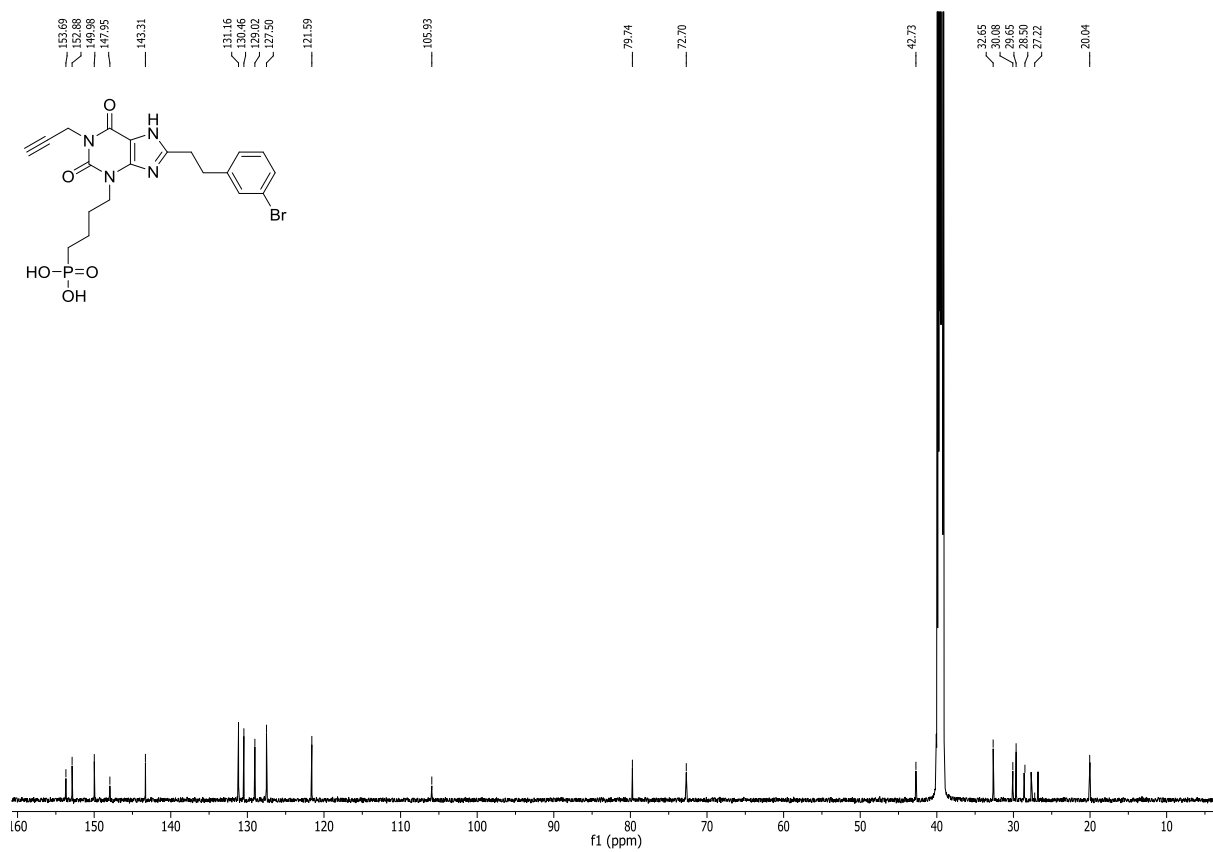


Figure S53: ^{13}C -NMR of compound **B-4**

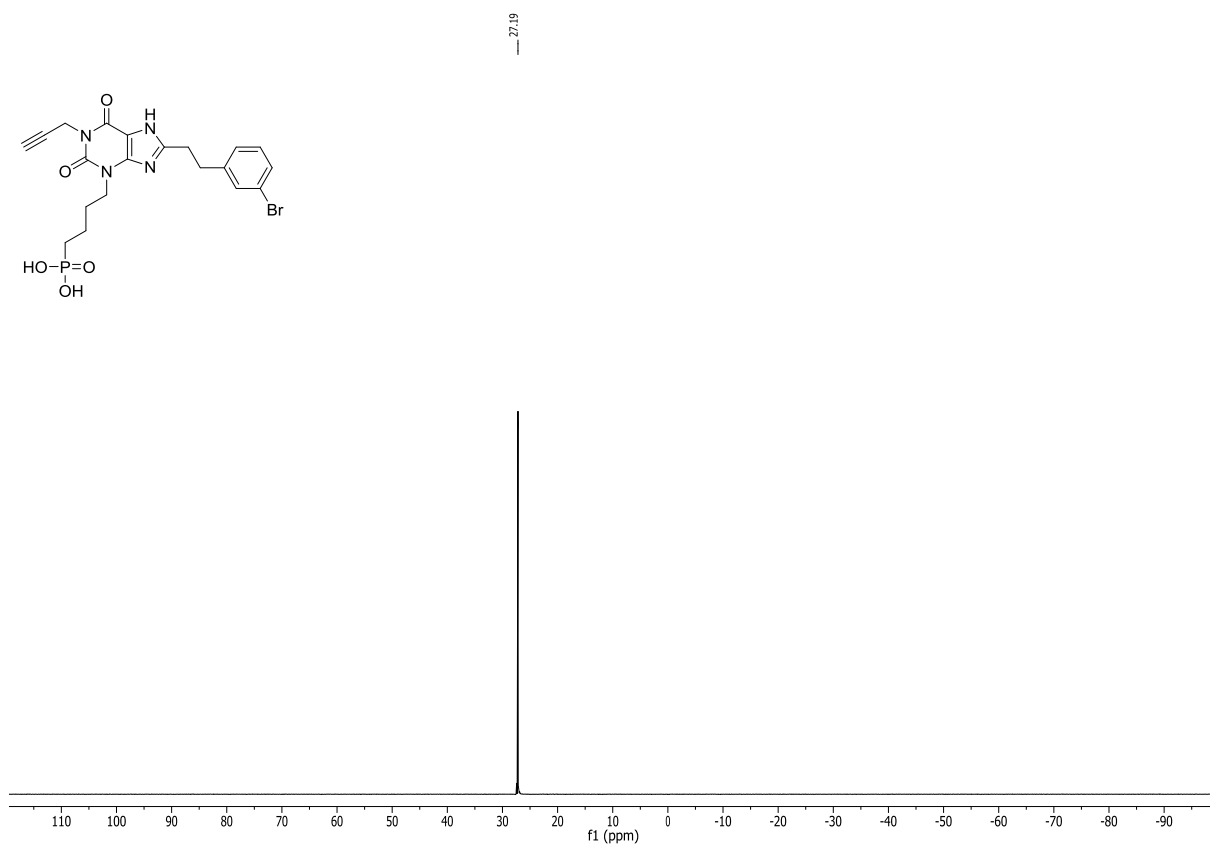


Figure S54: ^{31}P -NMR of compound **B-4**

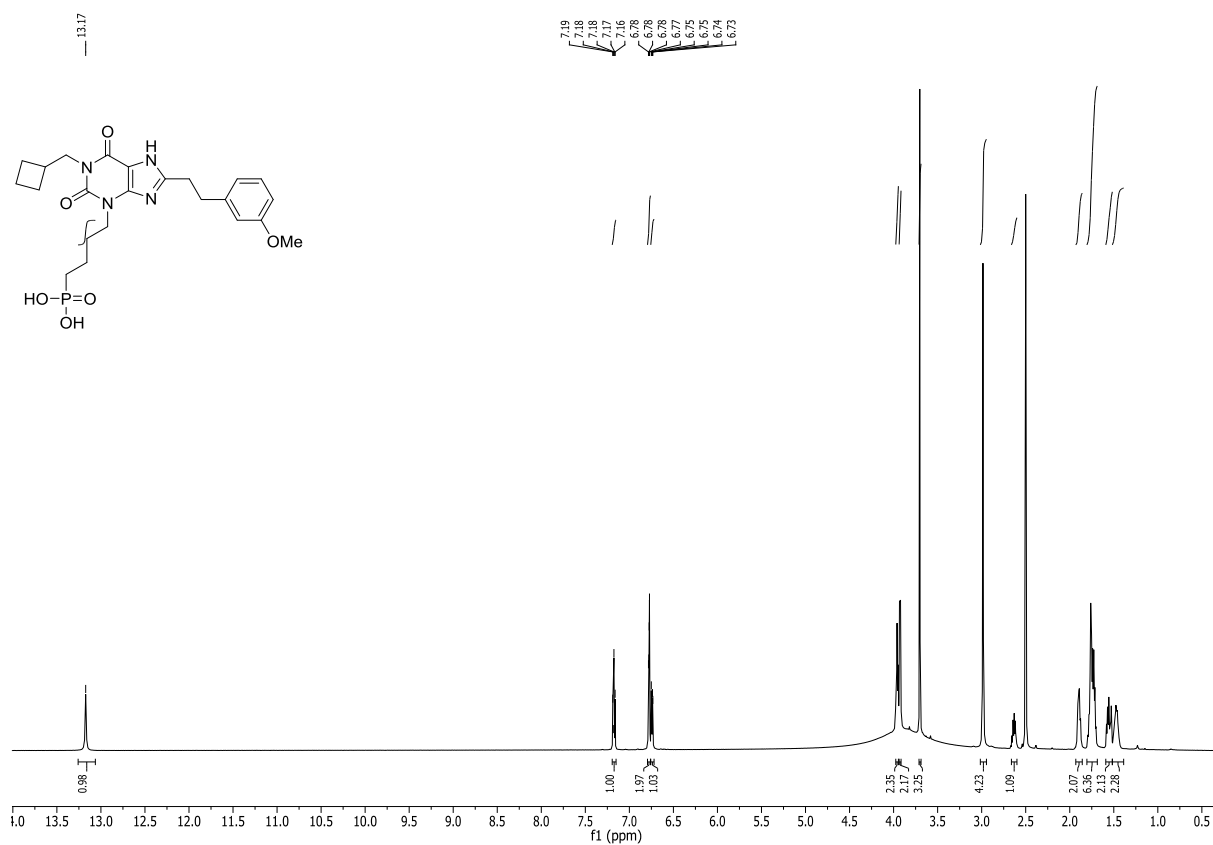


Figure S55: ¹H-NMR of compound **B-13**

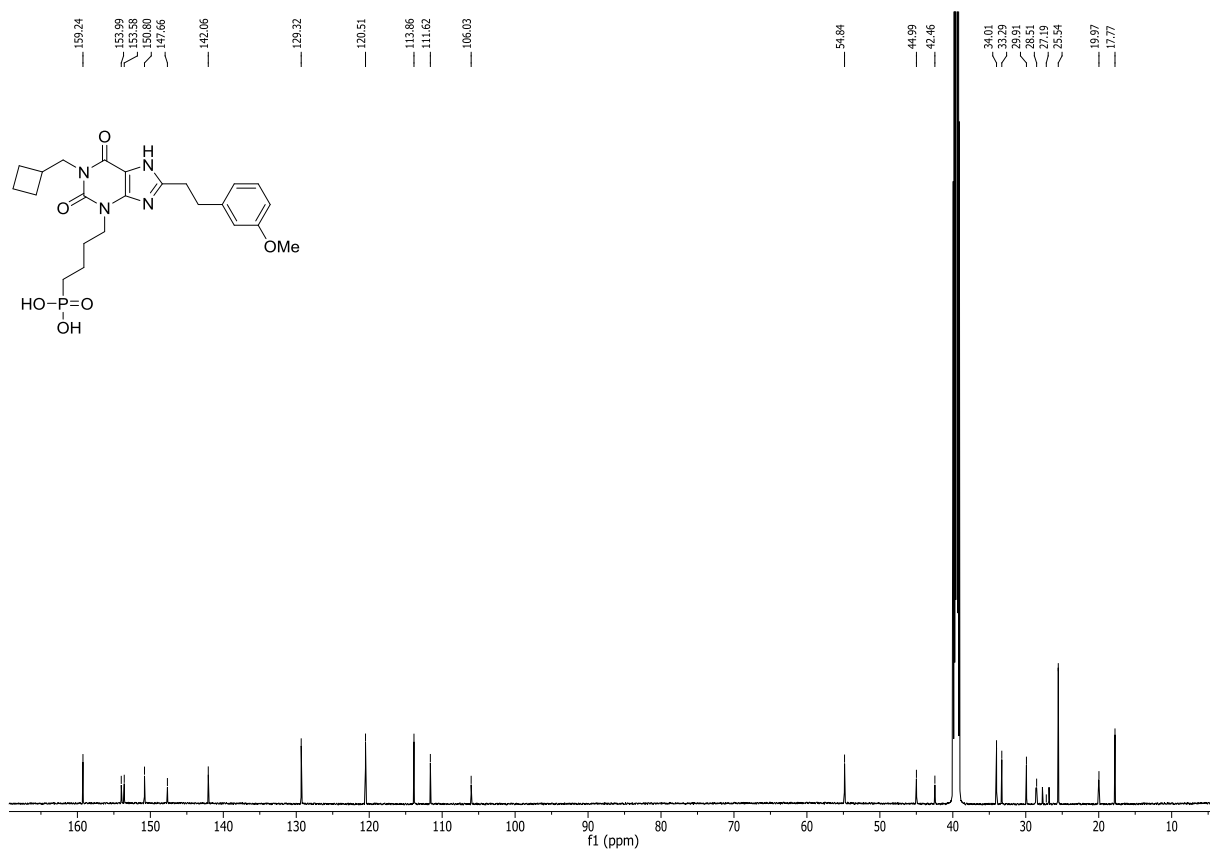


Figure S56: ^{13}C -NMR of compound **B-13**

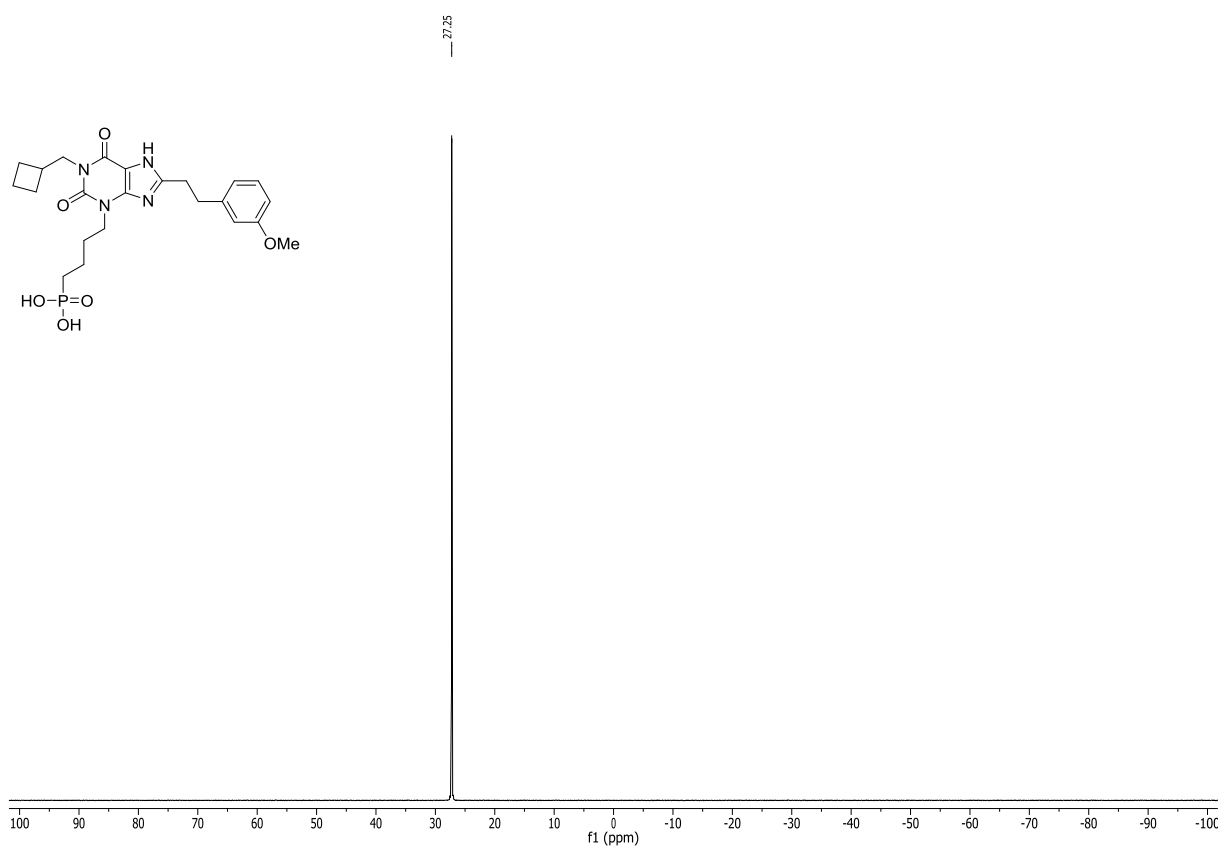


Figure S57: ^{31}P -NMR of compound **B-13**

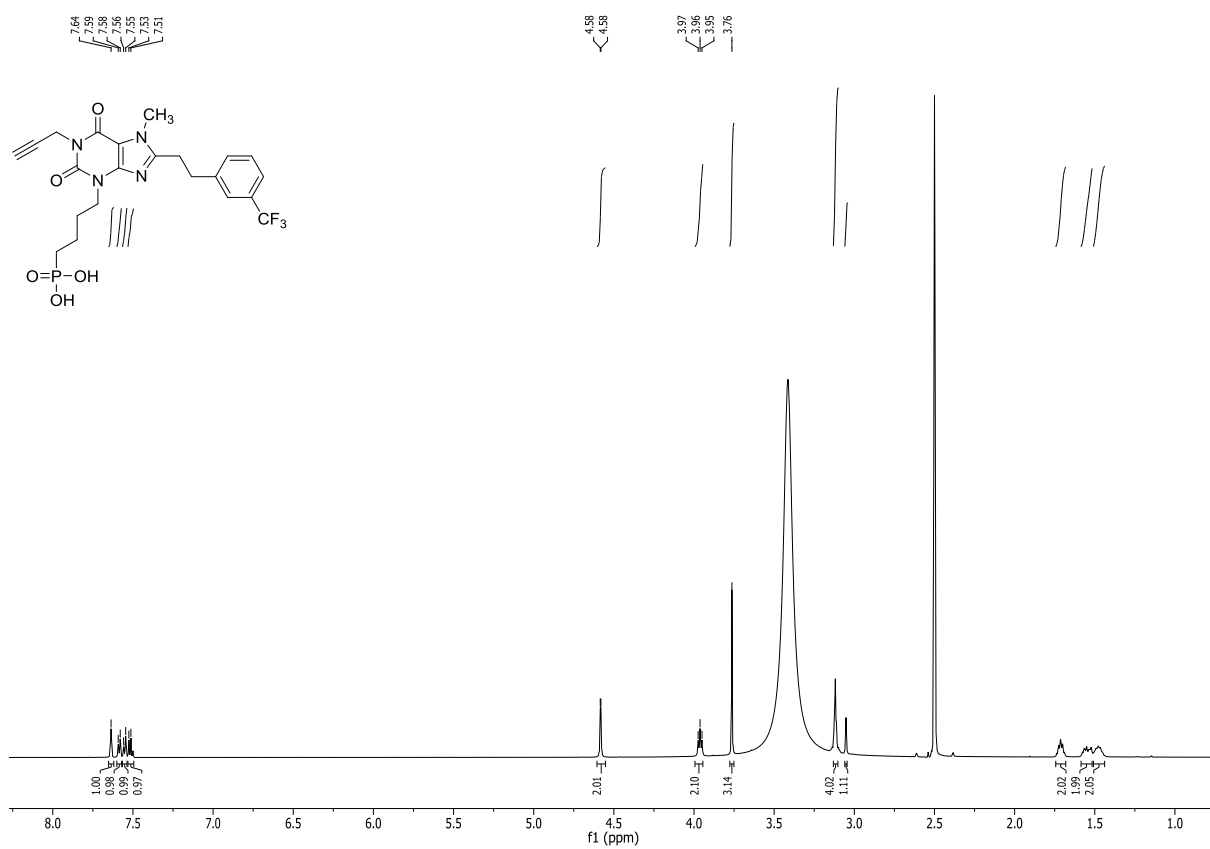


Figure S58: $^1\text{H-NMR}$ of compound **B-16**

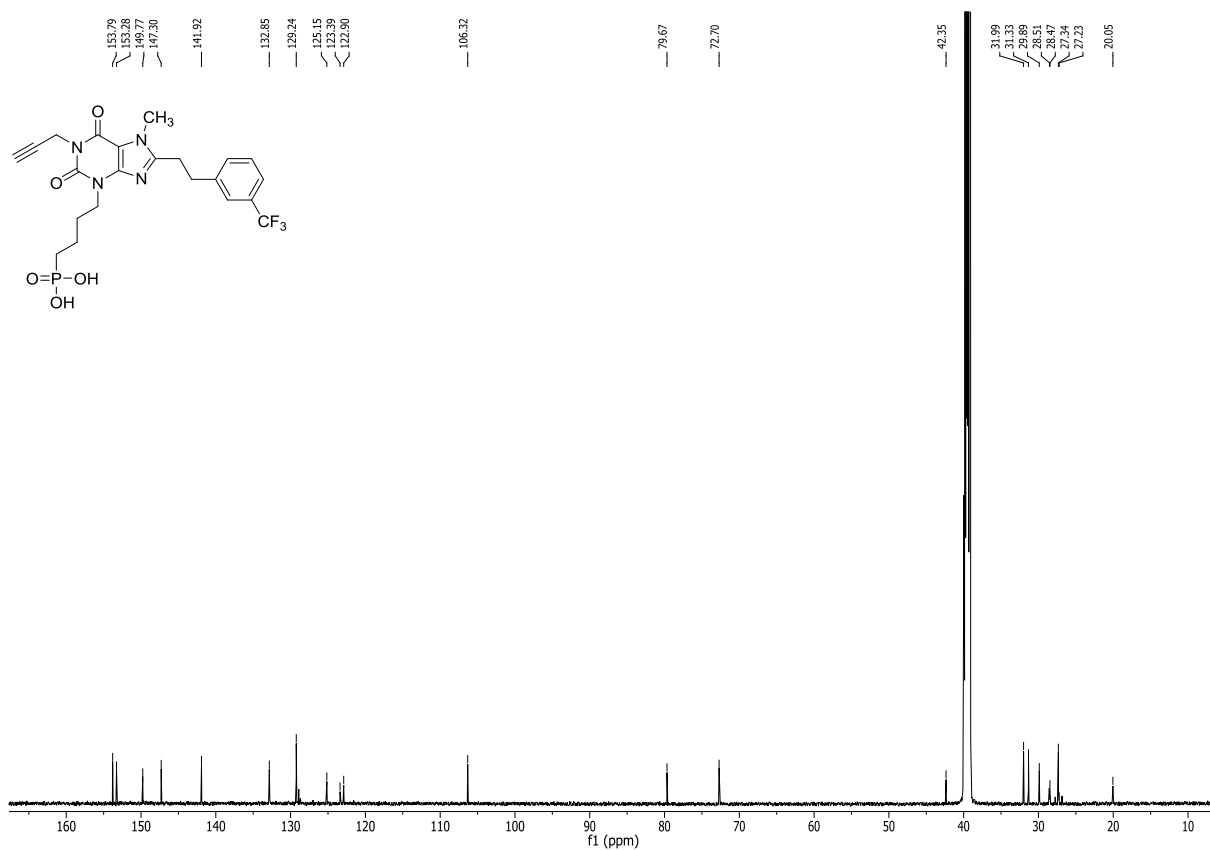


Figure S59: ^{13}C -NMR of compound **B-16**

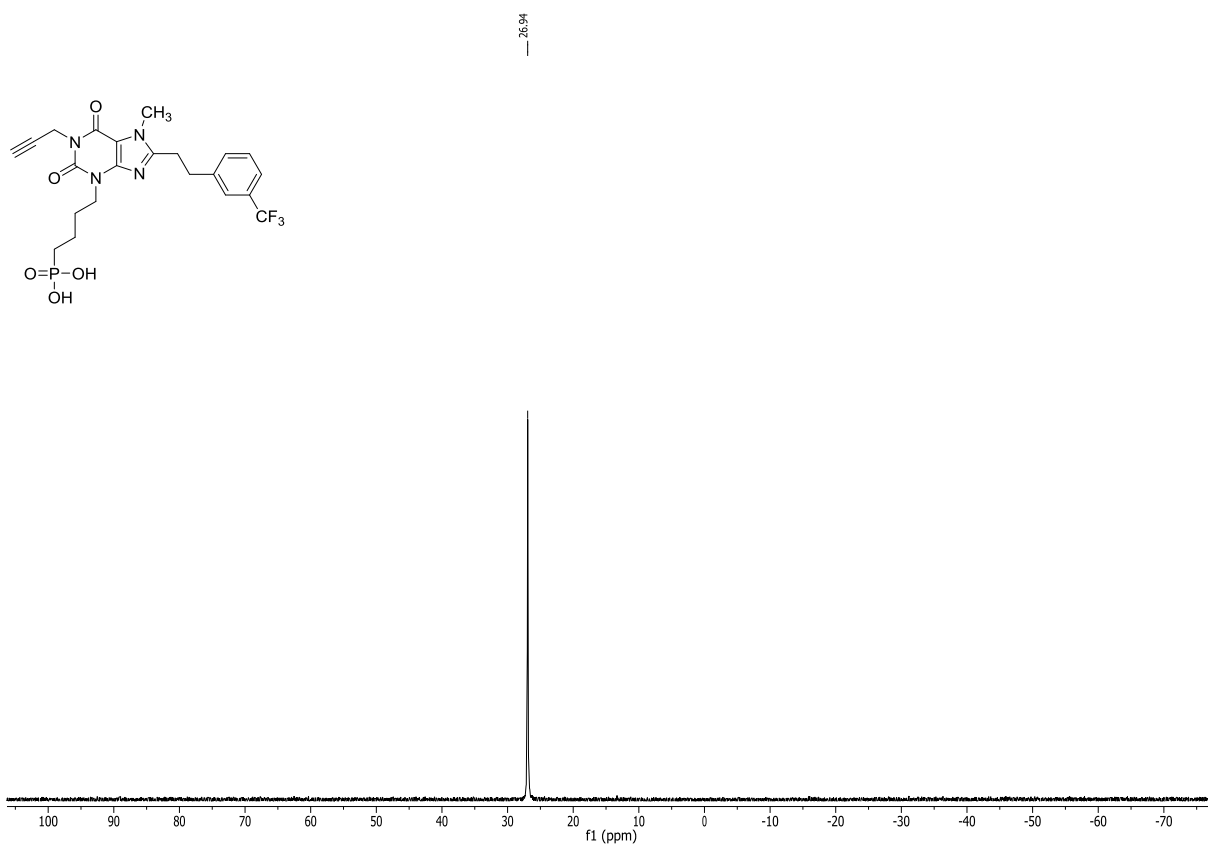


Figure S60: ^{31}P -NMR of compound **B-16**

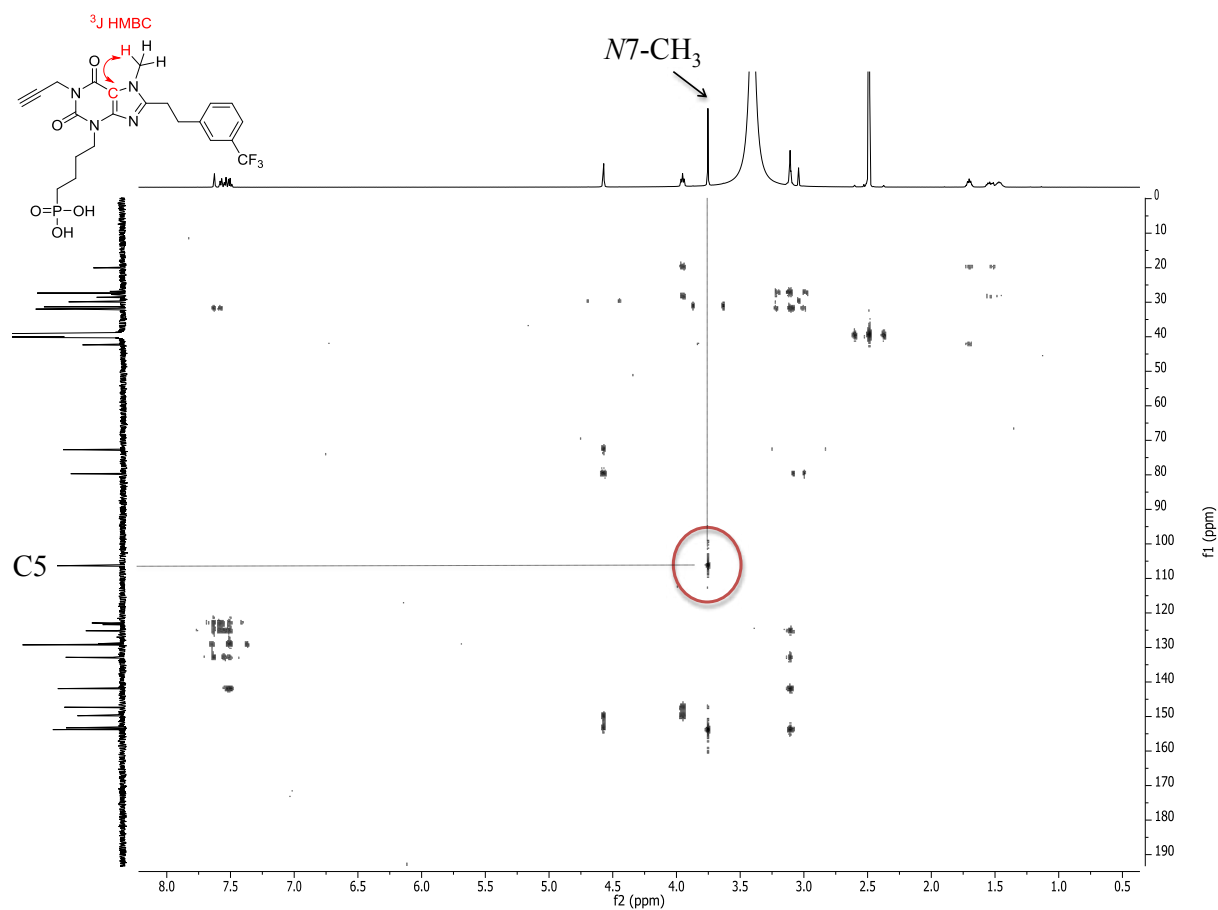


Figure S61: HMBC of compound **B-16**

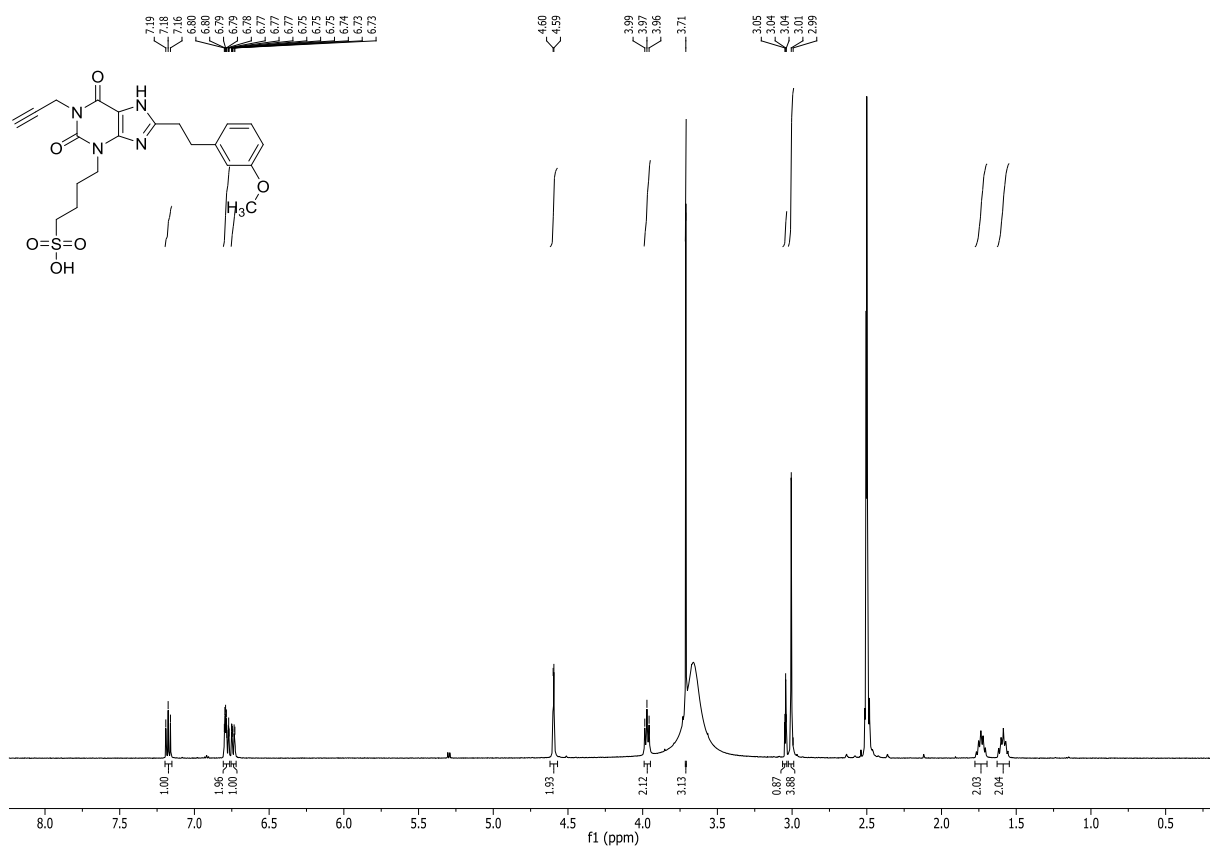


Figure S62: ¹H-NMR of compound E-3

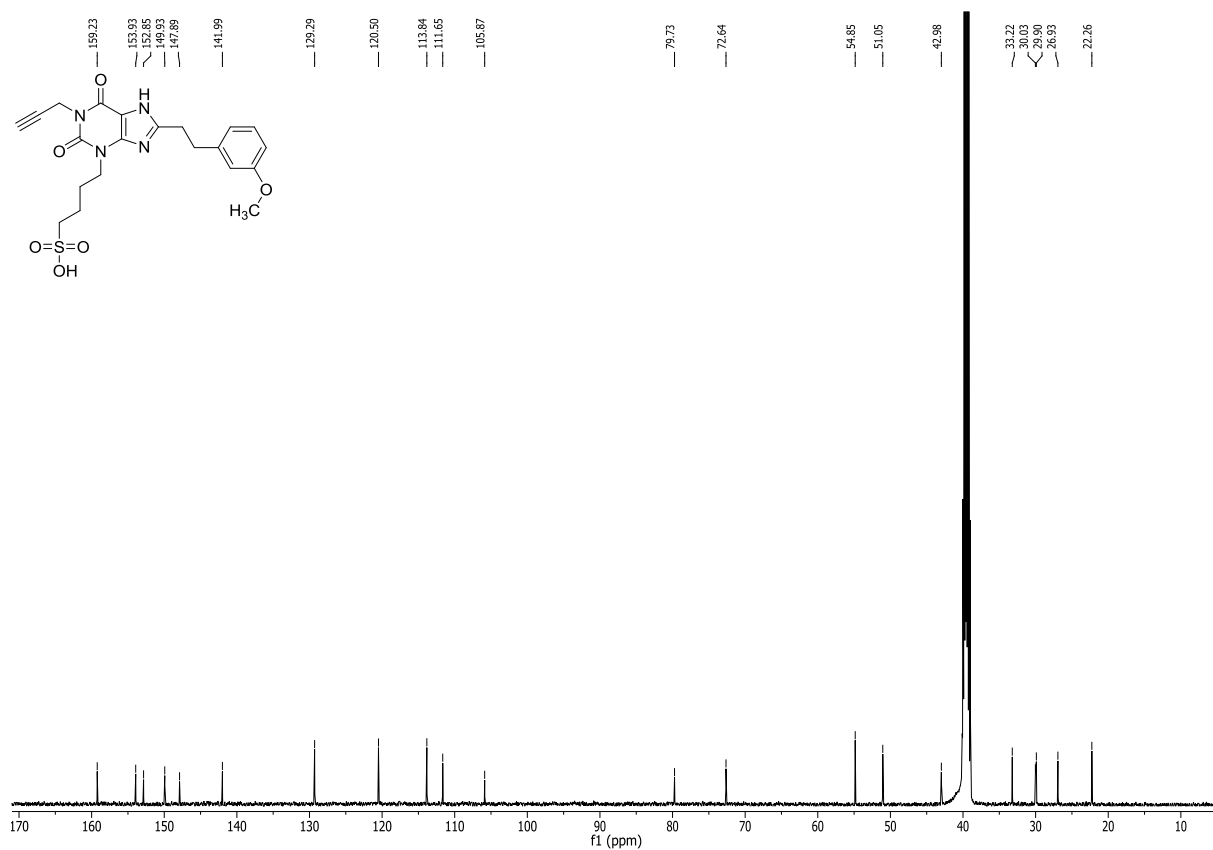


Figure S63: ¹³C-NMR of compound E-3

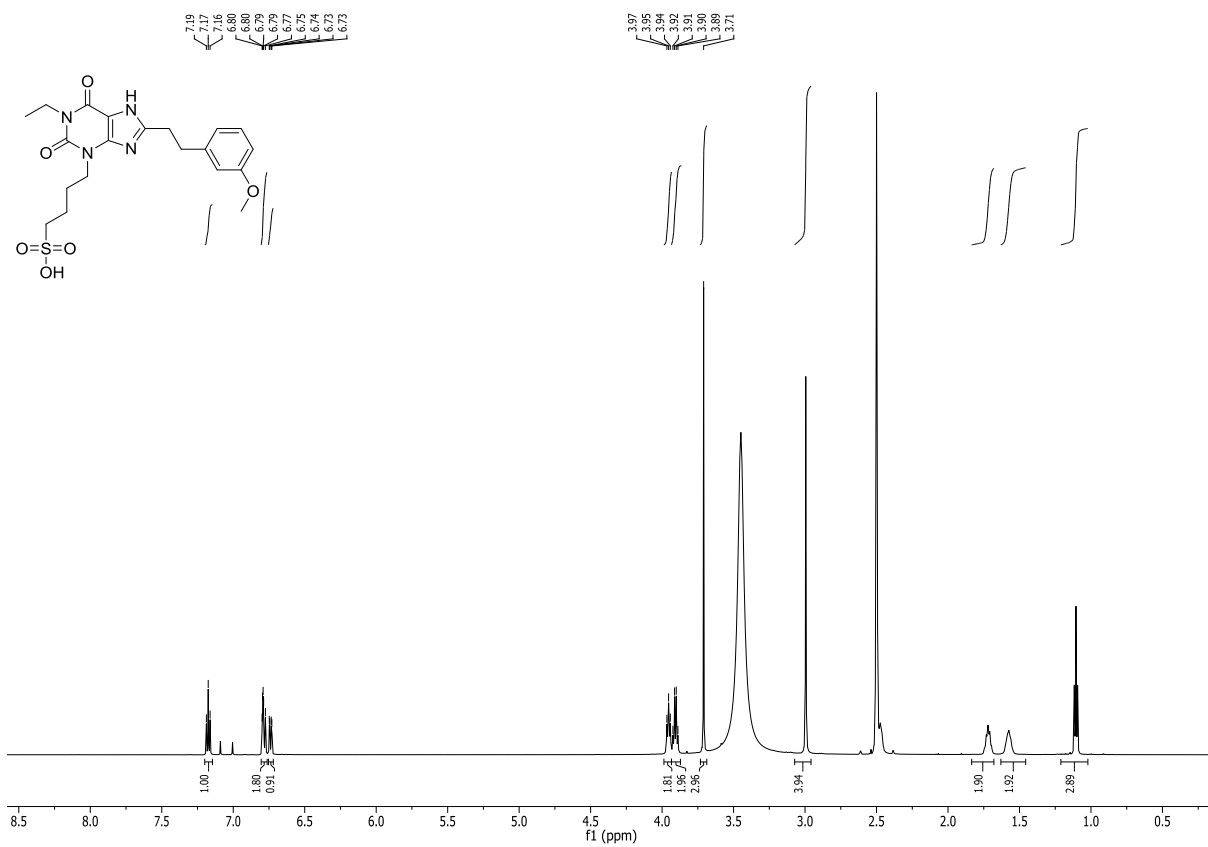


Figure S64: ¹H-NMR of compound E-2

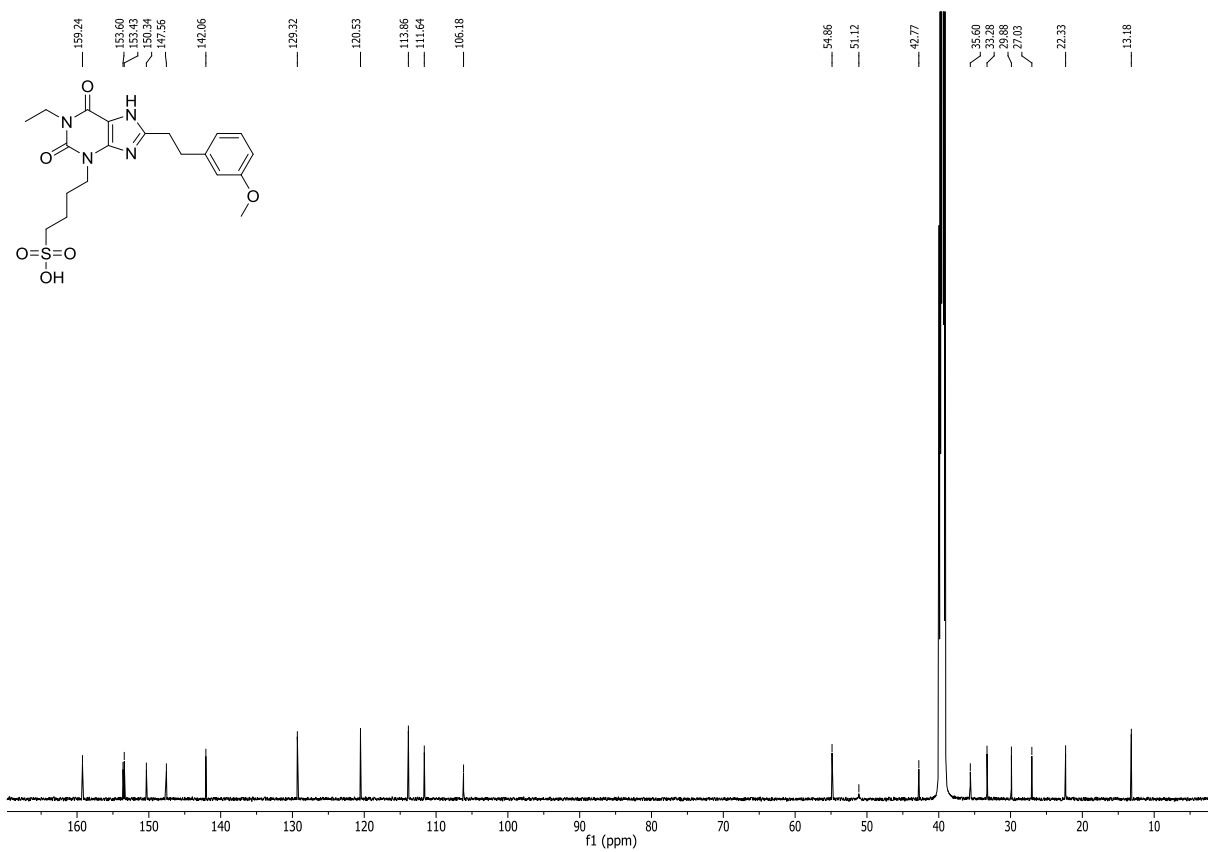


Figure S65: ¹³C-NMR of compound E-2

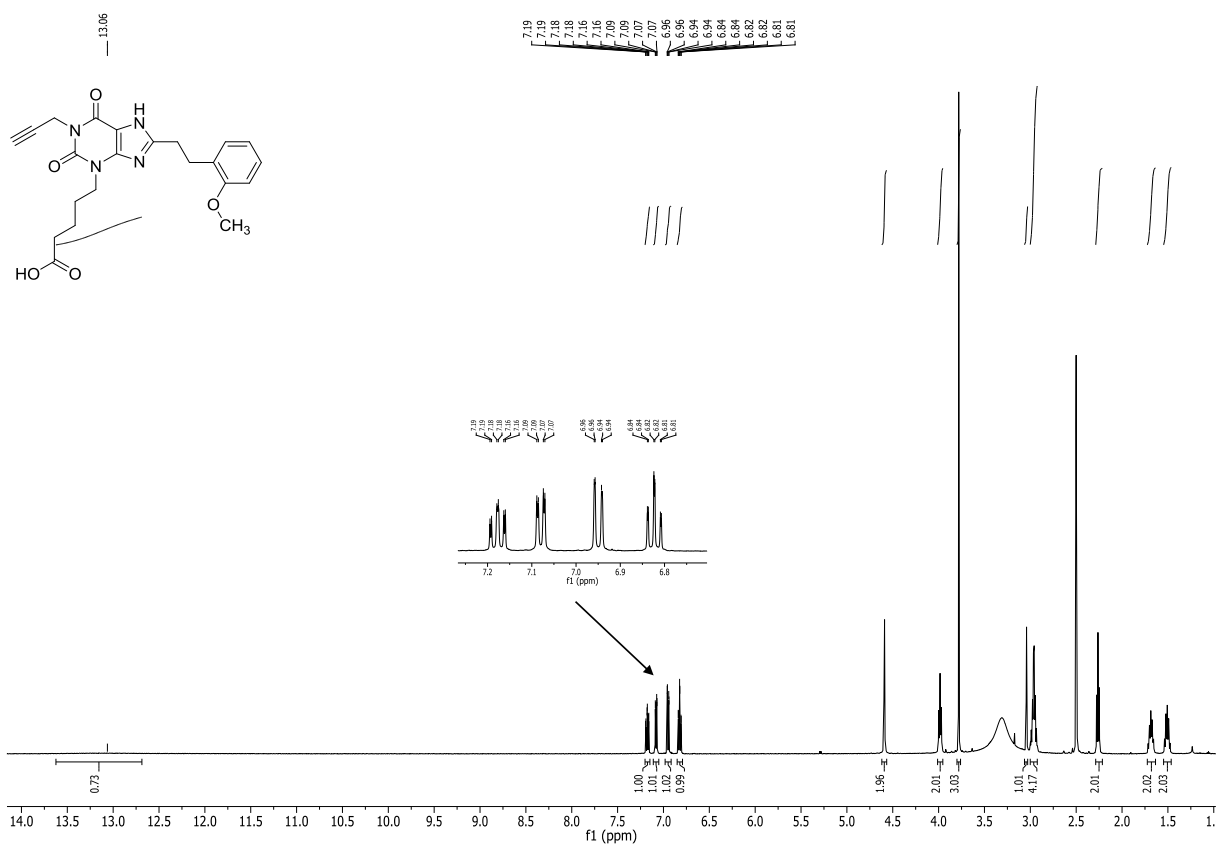


Figure S66: $^1\text{H-NMR}$ of compound **G-1**

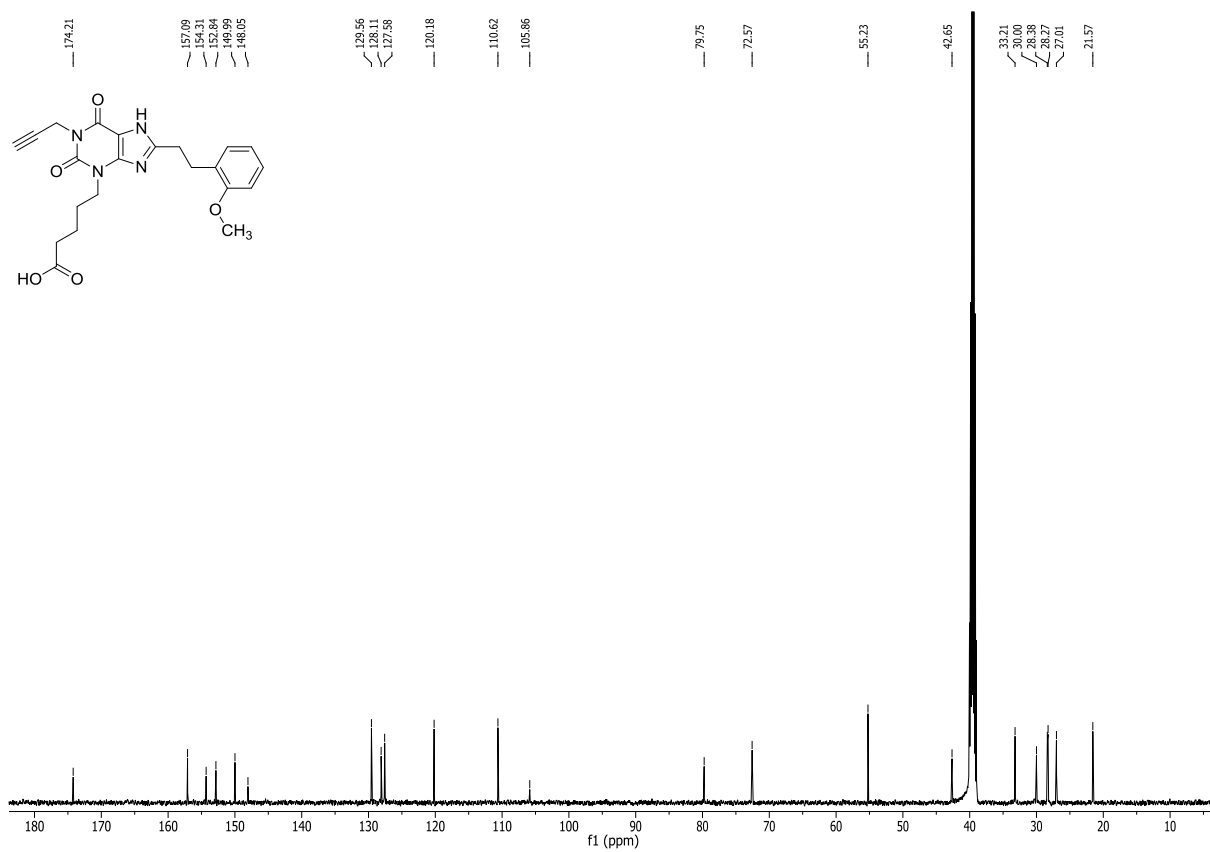


Figure S67: ^{13}C -NMR of compound **G-1**

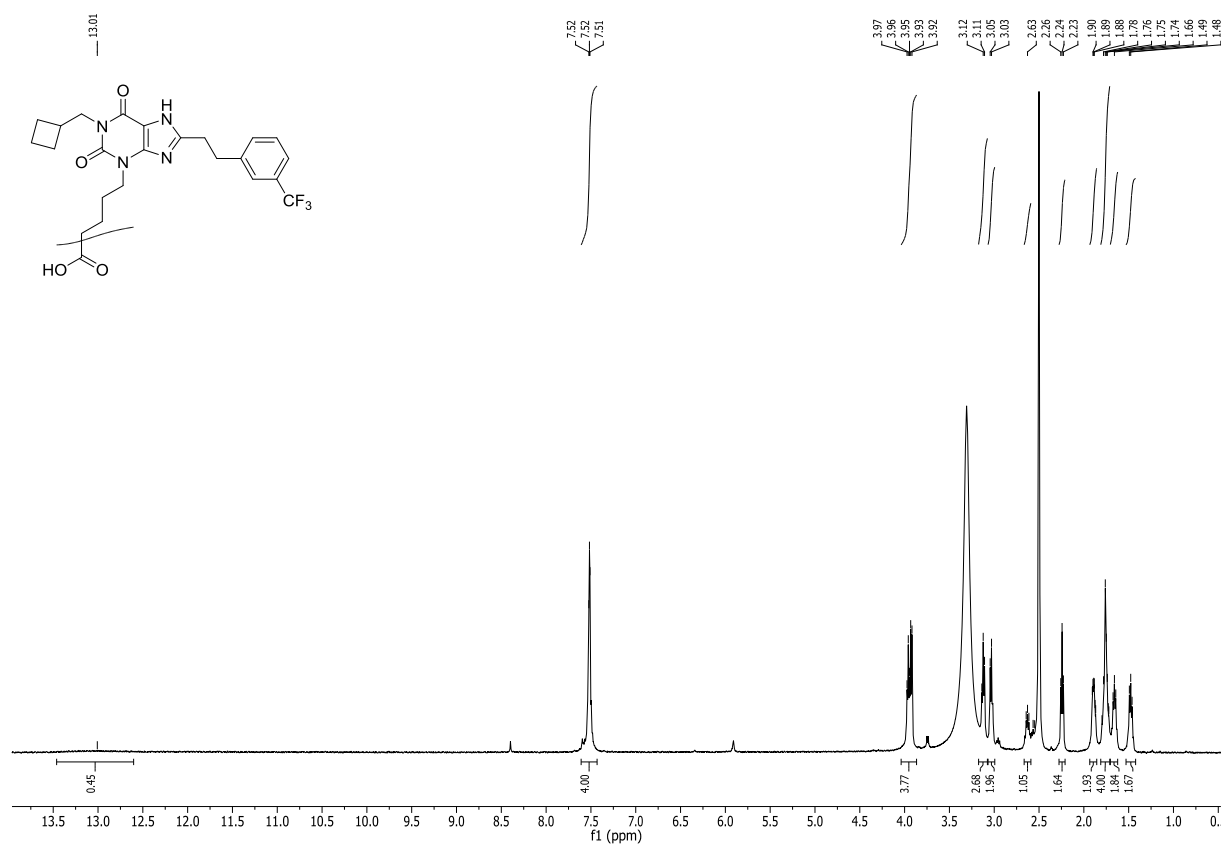


Figure S68: ¹H-NMR of compound G-2

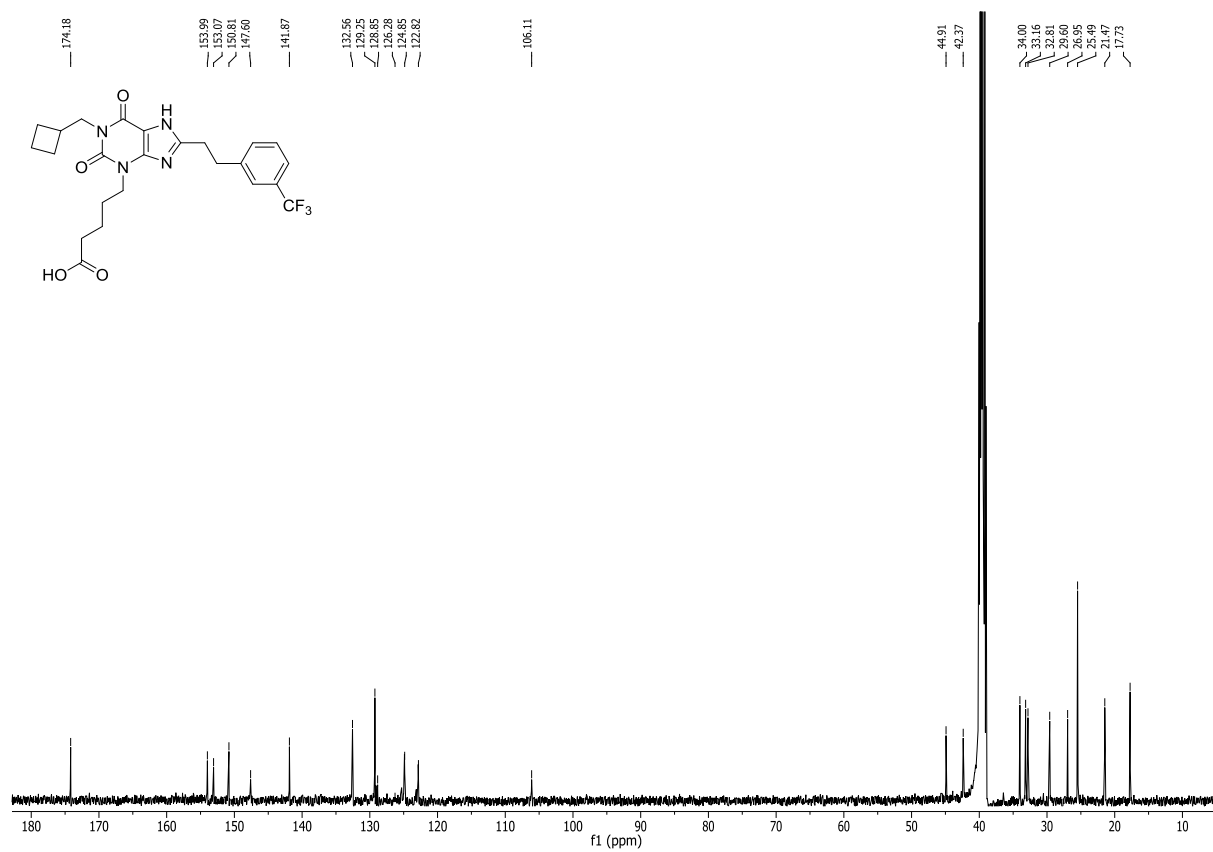


Figure S69: ^{13}C -NMR of compound **G-2**

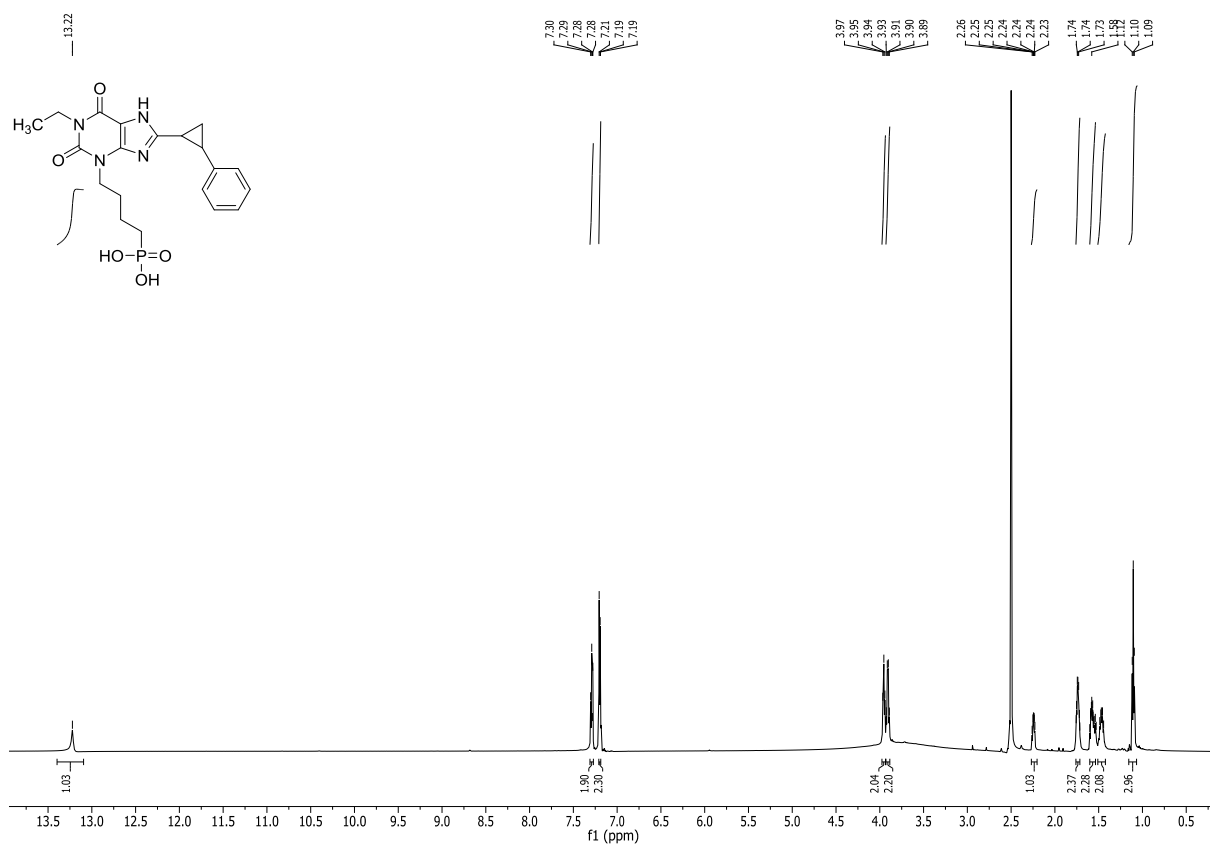


Figure S70: $^1\text{H-NMR}$ of compound **DM195**

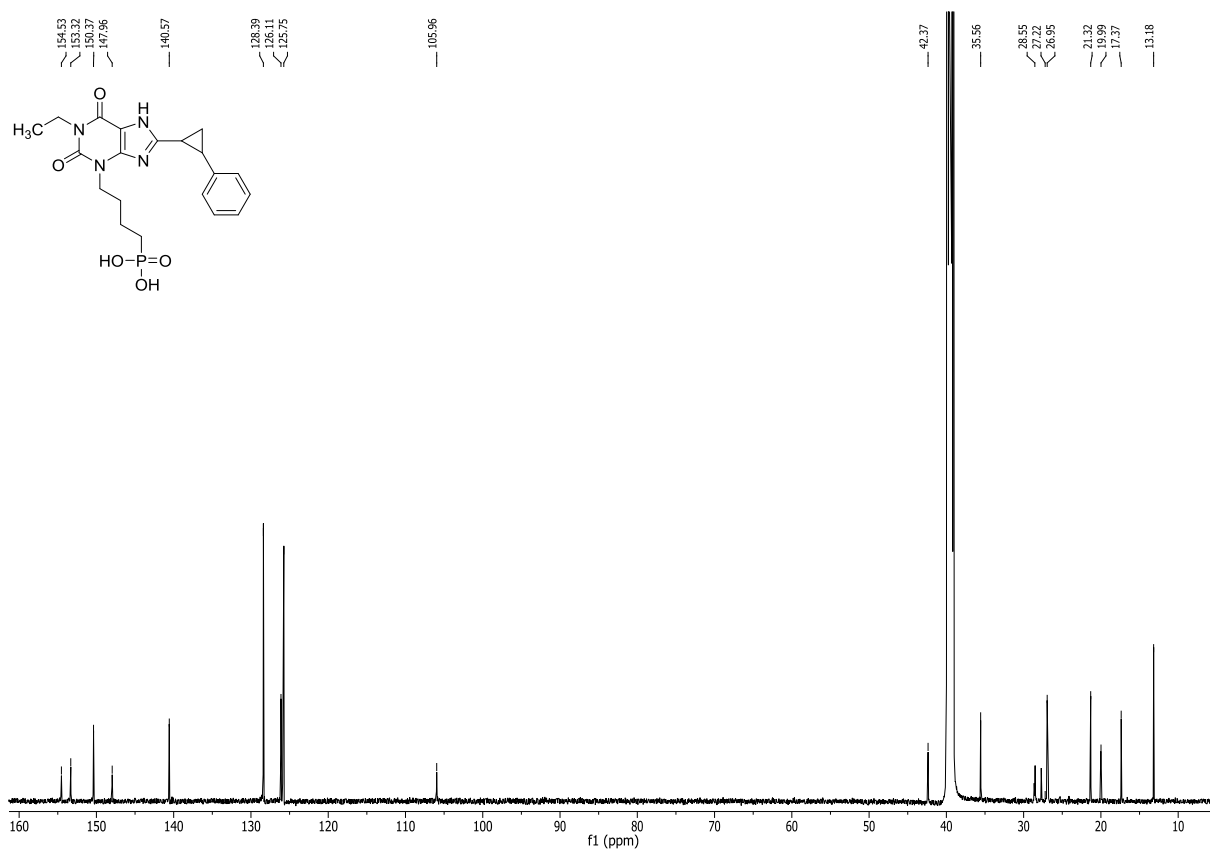


Figure S71: ¹³C-NMR of compound **DM195**

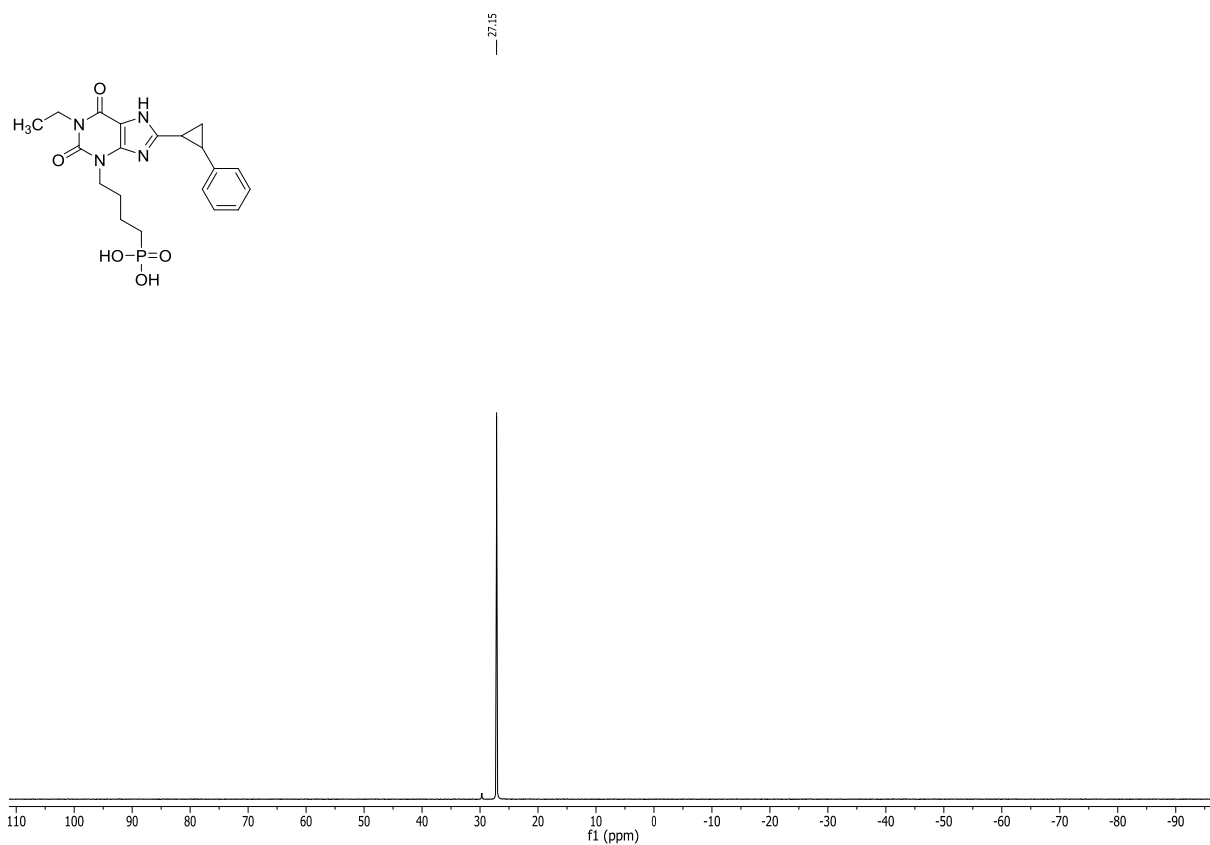


Figure S72: ^{31}P -NMR of compound **DM195**

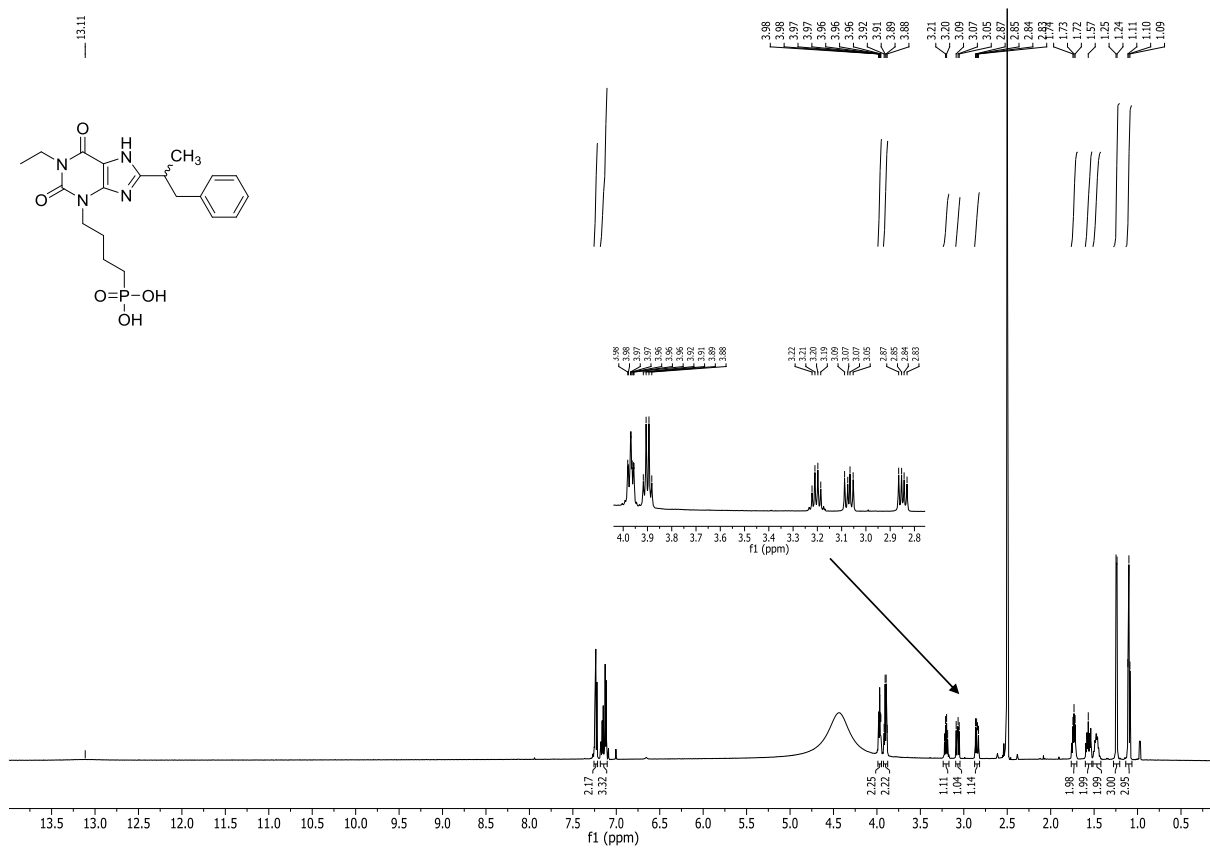


Figure S73: ¹H-NMR of compound DM234

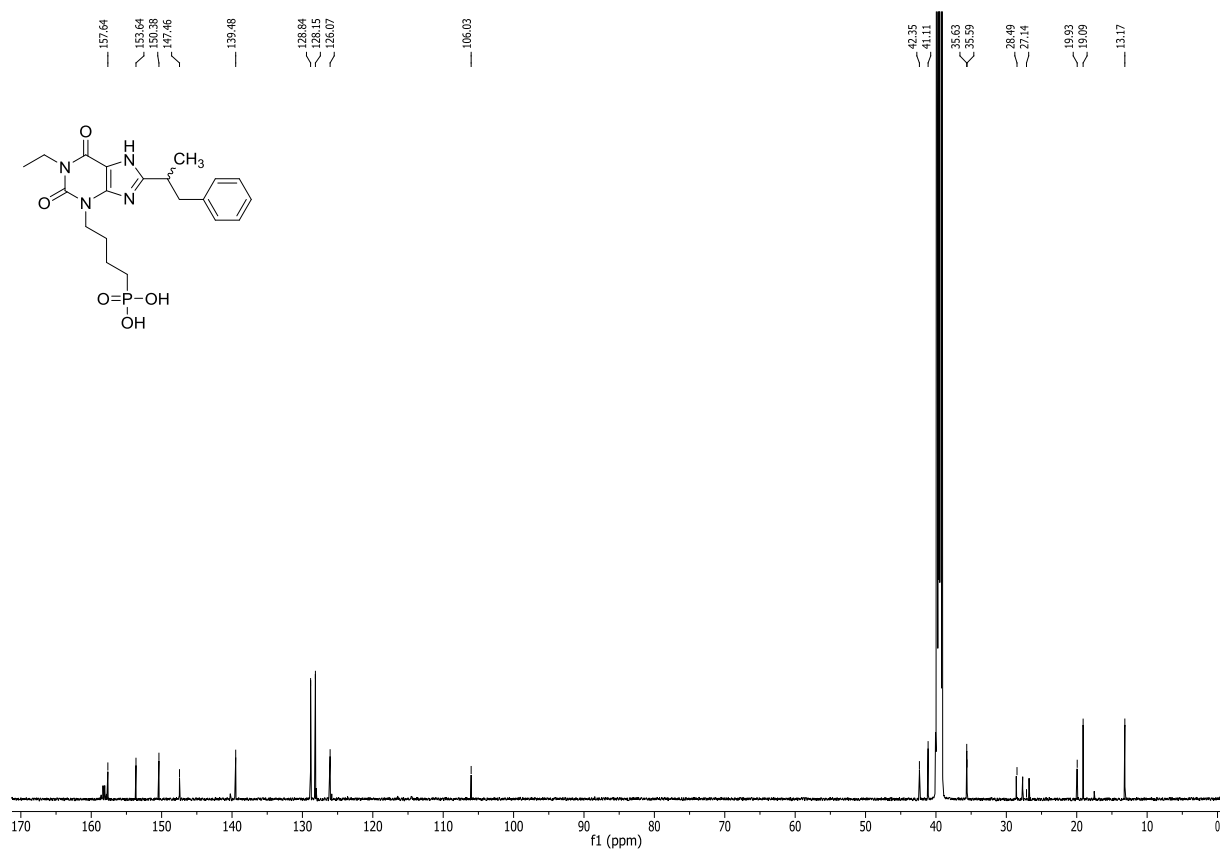


Figure S74: ^{13}C -NMR of compound **DM234**

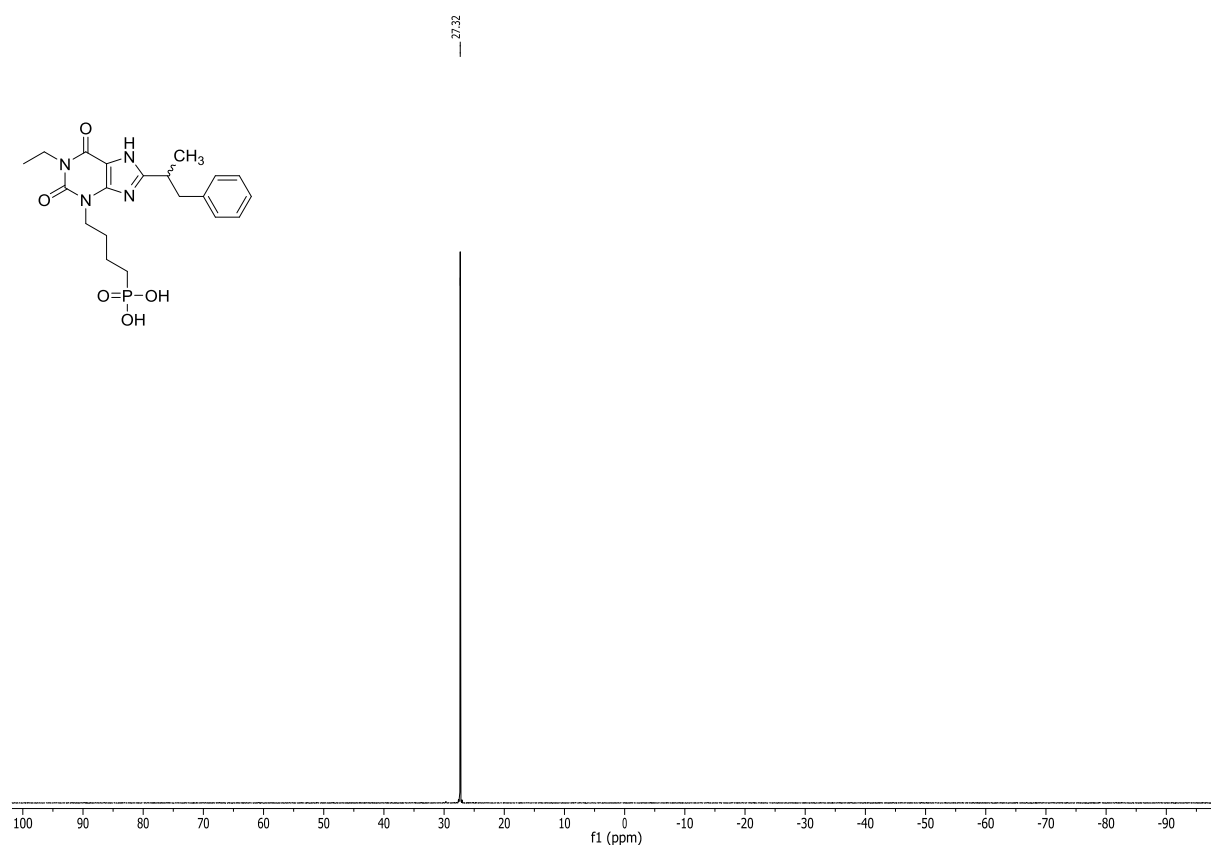


Figure S75: ^{31}P -NMR of compound **DM234**

References:

- (1) Ragulin, V. ω -haloalkylphosphoryl compounds: synthesis and properties. *Russ. J. Gen. Chem.* **2012**, *82*, 12, 1928-1937.
- (2) Hockemeyer, J.; Burbiel, J. C.; Müller, C. E. Multigram-scale syntheses, stability, and photoreactions of $\text{A}_{2\text{A}}$ adenosine receptor antagonists with 8-styrylxanthine structure: potential drugs for Parkinson's Disease. *J. Org. Chem.* **2004**, *69*, 10, 3308-3318.
- (3) Alexander, S. P.; Curtis, A. R.; Kendall, D. A.; Hill, S. J. A_1 adenosine receptor inhibition of cyclic AMP formation and radioligand binding in the guinea-pig cerebral cortex. *British Journal of Pharmacology* **1994**, *113*, 4, 1501-1507.