Chromen-4-ones as novel potent and selective ligands for purinoceptor-related class A δ-branch *orphan* G protein-coupled receptors

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Anne Meyer

aus Trier

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1. Gutachter: Prof. Dr. Christa E. Müller
2. Gutachter: Prof. Dr. Michael Gütschow

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Contents

1 Introduction

About 800 receptors in the human body are G protein-coupled receptors (GPCRs) which represent the largest receptor family.[1] They represent one of the most important classes of targets for drug development, and about 30% of all marketed drugs target GPCRs.[2] Many of the most important drug classes, including beta-adrenergic blockers, antihistamines, opioids and dopamine receptor agonists act via GPCRs. Some GPCRs cannot only be activated by small molecules, but also by peptides, proteins and even by light. In the human body they are involved in various physiological and pathological processes. Neurotransmission, hormone regulation, sensory functions, chemotaxis, cell growth and differentiation, to name a few, are mediated by GPCRs. The award of the Nobel Prize to Brian Kobilka and Robert J. Lefkowitz in 2012 for their research on GPCRs highlights their unique importance.

1.1 G protein-coupled receptors

1.1.1 Receptor pharmacology

Receptors are cellular macromolecules which selectively bind a specific compound which induces a cellular response. This process can be metaphorically described as a "key lock principle" where the so-called ligand like a key perfectly fits into the binding pocket of the receptor, the lock. Receptors can thereby be stabilized in an active or an inactive conformation. Ligands which activate the receptor by stabilizing its active conformation are called agonists. The activation of the receptor results in a further signal transduction cascade in the cell and finally in a physiological or pathological effect. On the other hand, antagonists are ligands that block the receptor and prevent the binding of an agonist but do not induce a signal transduction. Inverse agonists are a specific type of antagonists that stabilize the inactive conformation of the receptor.[3] The part of the receptor where the endogenous ligand

binds is the orthosteric binding pocket. Besides, there may be additional binding sites, which are upon ligand binding also able to influence the binding or effects of orthosteric ligands. Those ligands are called allosteric modulators.[4]

1.1.2 Structure and classification of G protein-coupled receptors

G protein-coupled receptors consist of seven α-helical transmembrane domains that are connected by three intracellular and three extracellular loops. They display an extracellular N-terminal domain and an intracellular C-terminal domain. That is why they are also called 7-transmembrane receptors 7-transmembrane receptors (7TM-receptors) (Figure 1.1).

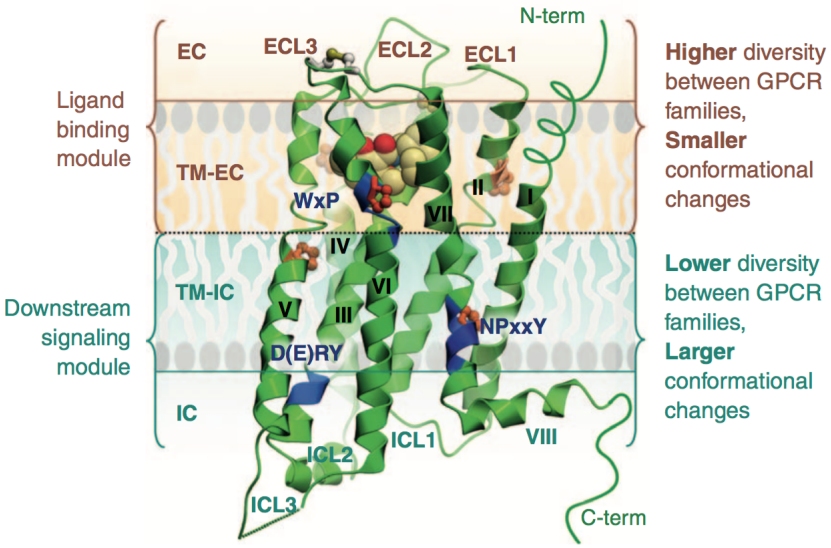


Figure 1.1: General structure of a G protein-coupled receptor shown on the crystal structure of the dopamine D3 receptor. The figure was taken and modified from Katritch et al.[5]

In 1994 Kolakowski et al. grouped all known GPCRs in 6 classes from A to F whereas most of the human GPCRs can be found in classes A-C.[6]

Based on the GRAFS classification system, which was developed in 2003 by Fredriksson et al. using phylogenetic analyses, the GPCRs can be grouped into 5 classes, the rhodopsin (class A), secretin (class B), glutamate (class C), adhesion, and frizzled/taste classes.

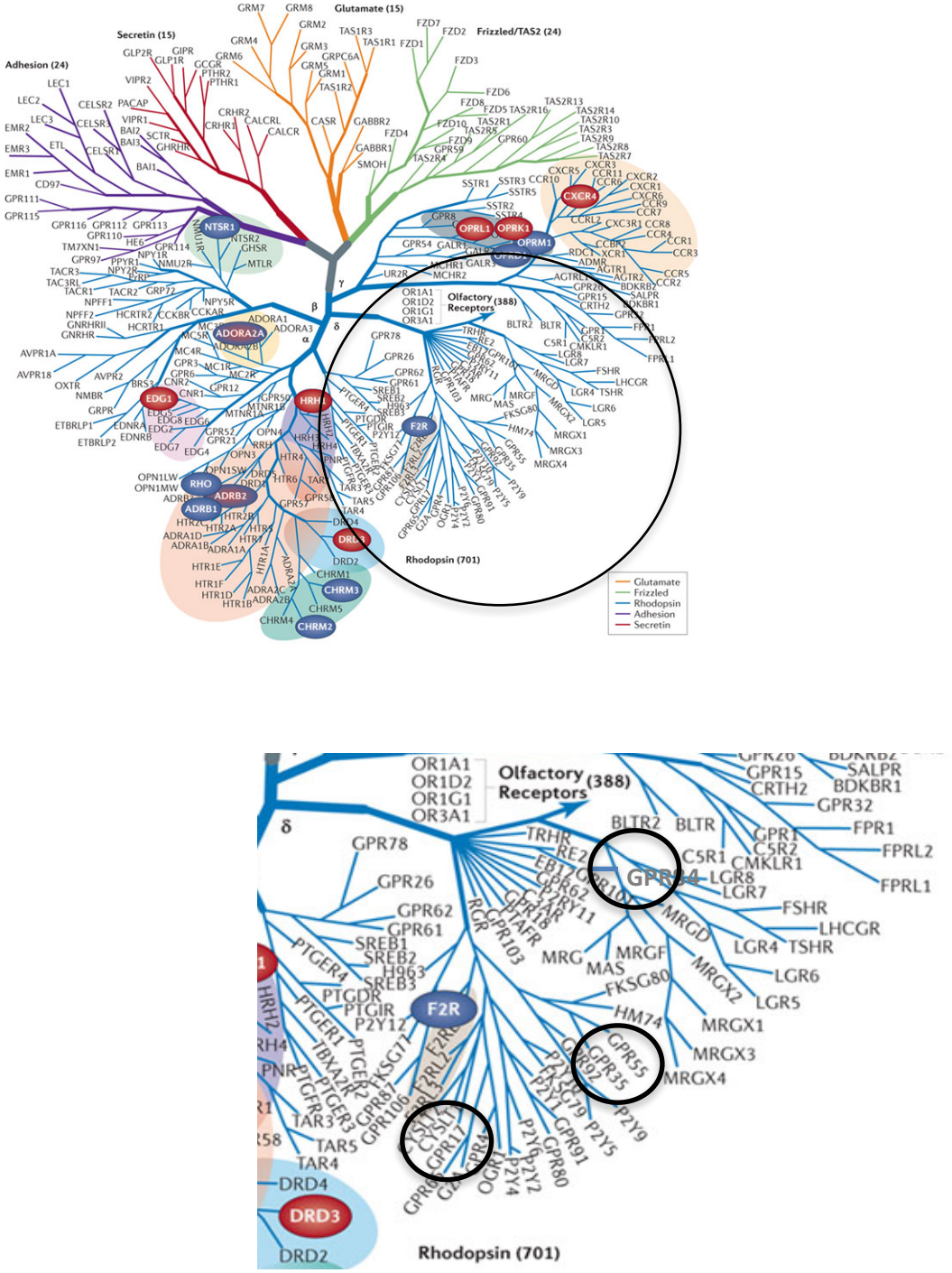


Figure 1.2: Phylogenetic tree of GPCRs with a special view on *orphan* receptors of the δ-branch of class A. The figure was taken and modified from Stevens et al.[7]

The secretin receptor family is named after its first member, the secretin receptor. In this class the N-terminus is important for the binding of the ligands. Well-known and pharmacologically relevant members of this family are among others the calcitonin receptor (CALCR), the corticotropin-releasing hormone receptors (CRHRs), and the glucagon receptor (GCGR).

The adhesion receptor family contains adhesion-like motifs in the N terminus which are likely to participate in cell adhesion.

The glutamate receptor family includes eight metabotropic glutamate receptors (GRMs), two γ-aminobutyric acid (GABA) receptors and a calcium-sensing receptor (CASR).

The frizzled/taste receptor family is a small receptor class with currently only limited relevance for pharmaceutical research.

The rhodopsin (class A) receptors are the largest receptor class. The class is named after the bovine rhodopsin and can be further subdivided into 4 groups, α, β, γ, and δ. The α-branch contains prostaglandin receptors, amine receptors, melatonin receptors, and the melanocortin, endothelial differentiation sphingolipid, cannabinoid, adenosine (MECA) receptor cluster. The β-branch mainly includes peptide receptors. The γ-branch contains the somatostatin, chemokine, galanin (SOG) receptor cluster, the melanin-concentrating hormone (MCH) receptor cluster, and the chemokine receptors.

The δ-branch is subdivided into 4 subbranches: MAS-related receptor cluster, glycoprotein receptor cluster, purine receptor cluster, and the olfactory receptor cluster.[1] It contains also the P2Y-receptor related *orphan* GPCRs GPR17, GPR55 and GPR35, and the *orphan* receptor GPR84 (Figure 1.2). Those receptors will be further investigated in this study.

1.1.3 Signal transduction pathways of G protein-coupled receptors

The name "G protein-coupled receptor" is based on the fact that this receptor class acts via a guanine nucleotide-binding protein, the so called G protein. It was first observed by Martin Rodbell in 1971 in the context of glucagon action.[8] The G protein is a heterotrimeric structure which consists of an α and a heterodimeric $\beta\gamma$ subunit. The binding of an agonist to the receptor results in a conformational change of the

receptor which now is able to interact with the heterotrimeric G protein complex. It functions as a guanosine triphosphate exchange factor (GEF), replacing guanosine diphosphate (GDP) by guanosine triphosphate (GTP). This results in dissociation of the Gα subunit from the G$\beta\gamma$ subunit which both can activate different signaling cascades.[9] The kind of signaling pathway, which is activated, is mainly depending on the type of the Gα subunit (Figure 1.3).

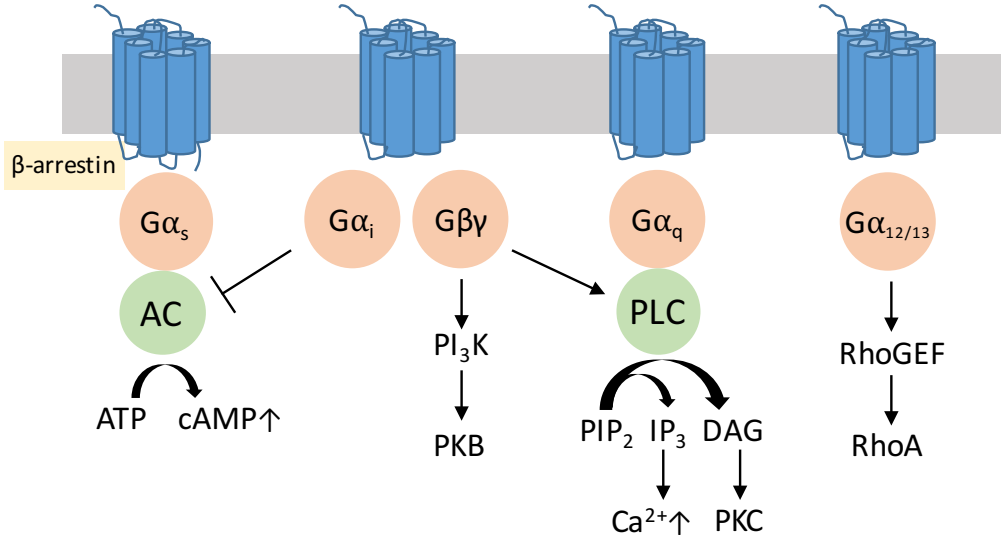


Figure 1.3: Signal transduction cascades of G proteins.

The Gα_s proteins activate adenylate cyclases (ACs) which catalyze the reaction of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). An increase of cAMP results in an activation of protein kinase A (PKA) which is a serine/threonine kinase. It is able to phosphorylate the transcription factor cAMP responsive element binding protein (CREB) in the nucleus. By activation of phosphorylase kinase B (PKB), PKA can also regulate glycolysis. Further effects of PKA activation are lipolysis and relaxation of smooth muscles. Gα_i proteins inhibit AC and the subsequent signaling pathway.

Gα_q proteins activate phospholipase C (PLC) which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mediates the opening of intracellular calcium channels which results in a calcium increase in the cytosol. Calcium activates the Ca^{2+}/calmodulin-dependent protein kinase (CAMK) pathway. DAG activates protein kinase C (PKC). Both pathways are

involved in the regulation of smooth muscle cells and further secretion of mediators.[10]

$G\alpha_{12/13}$ proteins activate Rho guanine nucleotide exchange factors (RhoGEF) which exchange GDP by GTP at RhoA. This results in an activation of RhoA. RhoA regulates the actin cytoskeleton, cell shape, cell polarity, microtubule dynamics, membrane transport pathways, gene transcription, cell adhesion, cell migration, neurite extension/retraction and cell growth.[11]

Not only the $G\alpha$ subunit is able to activate signaling pathways. Also the $G\beta\gamma$ protein is able to activate the PLC pathway. Furthermore it activates PKB via the phosphoinositide-3-kinase pathway resulting in the effects described above.[10]

The termination of the signal cascades can on the one hand be caused by an elimination of the bound agonist. But it was also observed, that on the other hand, the activated receptor itself stops its activity after a certain time. One distinguishes between homologous desensitization, where only the agonist-bound receptor is desensitized, and heterologous desensitization, which affects also other GPCRs.

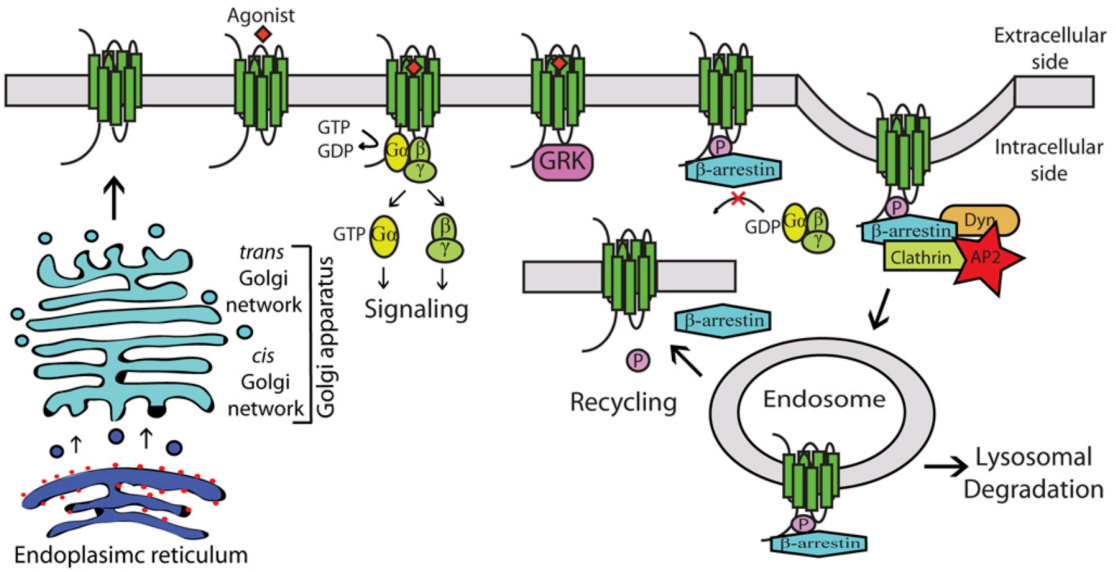


Figure 1.4: The life cycle of a G protein-coupled receptor. The figure was taken and modified from Stadel et al.[12]

Heterologous desensitization is mostly caused by phosphorylation of the GPCRs by second messenger-dependent protein kinases such as PKA and PKC. Homologous desensitization is induced by G protein-coupled receptor-kinases (GRKs) which phosphorylate serine and threonine residues of activated GPCRs. Furthermore the phosphorylated receptor presents new binding sites for the so-called arrestin proteins which completely desensitize the GPCR.[13] This occurs when β2-adaptin (AP-2) and clathrin bind to arrestins. A so-called "clathrin-coated pit" is formed which results in the formation of a vesicle. The desensitized receptors finally can be either recycled by dephosphorylation or can undergo degradation in the lysosomes (Figure 1.4).[12]

In conclusion, GPCR signaling is a complex process in which various signaling cascades are involved. Dependent on the type of G protein, ligands can trigger a broad range of effects in the cell which can influence essential physiological functions in the human body.

1.1.4 *Orphan* G protein-coupled receptors

In the past receptors were mainly identified by the ligands that bind to them. Modern genetic technologies and especially the decoding of the whole human genome as part of the human genome project made it possible to identify new receptors by the comparison of genetic sequences with sequences of known receptors. To some of these new receptors no specific endogenous ligand could be assigned. This is why those receptors are called *orphan* receptors. According to International Union of Basic and Clinical Pharmacology (IUPHAR) about 100 GPCRs exist whose endogenous ligand has not been identified or at least has not been confirmed yet. Most of them belong to the rhodopsin-like, class A GPCR family.[14] The deorphanization of those receptors is of great interest for pharmaceutical research because they represent potential drug targets. Studies with knockout mice and also their expression in tissues related to diseases like cancer indicate a physiological or pathological relevance. However the deorphanization of GPCRs is challenging. In the beginning *orphan* GPCRs were matched with known neurotransmitters. The first receptor which was deorphanized in this way was the 5-hydroxytryptamine receptor ($5HT_{1A}$ receptor) which binds 5-hydroxytryptamine.[15] As the number of *orphan* GPCRs soon exceeded the number of known neurotransmitters it emerged that there

must be also unidentified neurotransmitters that have to be identified. A second idea was that some neurotransmitters do not exclusively bind to only one target. Today GPCR deorphanization is linked also to neurotransmitter identification. As GPCRs bind to chemically diverse compounds such as peptides, small molecules and also lipids, deorphanization is often difficult. The characterization of the binding pocket with surrogate ligands as pharmacological tools has become more and more important. High-throughput screenings combined with medicinal chemistry currently dominates the research field of *orphan* GPCRs.[16]

1.1.5 Purinergic signalling

The history of purinergic signalling began in 1929 when the German researcher Karl Lohmann discovered adenosine ATP.[17] Despite its function as an intracellular energy source, in 1972 Geoffrey Burnstock identified ATP also as a neurotransmitter and cotransmitter.[18] Initially ridiculed, the concept of purinergic signalling today is generally accepted and an interesting and promising research field also with regard to drug development. Because of the fact that receptors and enzymes which are involved in the purinergic signalling system have various physiological and pathological functions in the human body, they represent potential drug targets for the treatment of various diseases.

1.1.6 Purinergic receptors

In 1987 Burnstock proposed the first classification of purinergic receptors by subdividing them into P1 and P2 receptors. P1 receptors can be activated by adenosine whereas P2 receptors are activated by ATP or adenosine 5'-diphosphate (ADP).[19]

P1 receptors are G protein-coupled and can be subdivided into four subtypes, A_1, A_{2A}, A_{2B} and A_3. Adenosine A_1 and A_3 receptors are $G_{i/o}$-coupled and A_{2A} and A_{2B} are coupled to the G_s proteins.[20]

The P2 receptors are subdivided into the G protein-coupled P2Y receptors and the ligand-gated ion channel P2X receptors. P2X receptors are subdivided into seven receptor subtypes $P2X_1$-$P2X_7$. For the P2Y receptors eight receptors have been identified which can be activated by different nucleotides such as uridine triphosphate (UTP), ATP and ADP (Figure 1.5).[21]

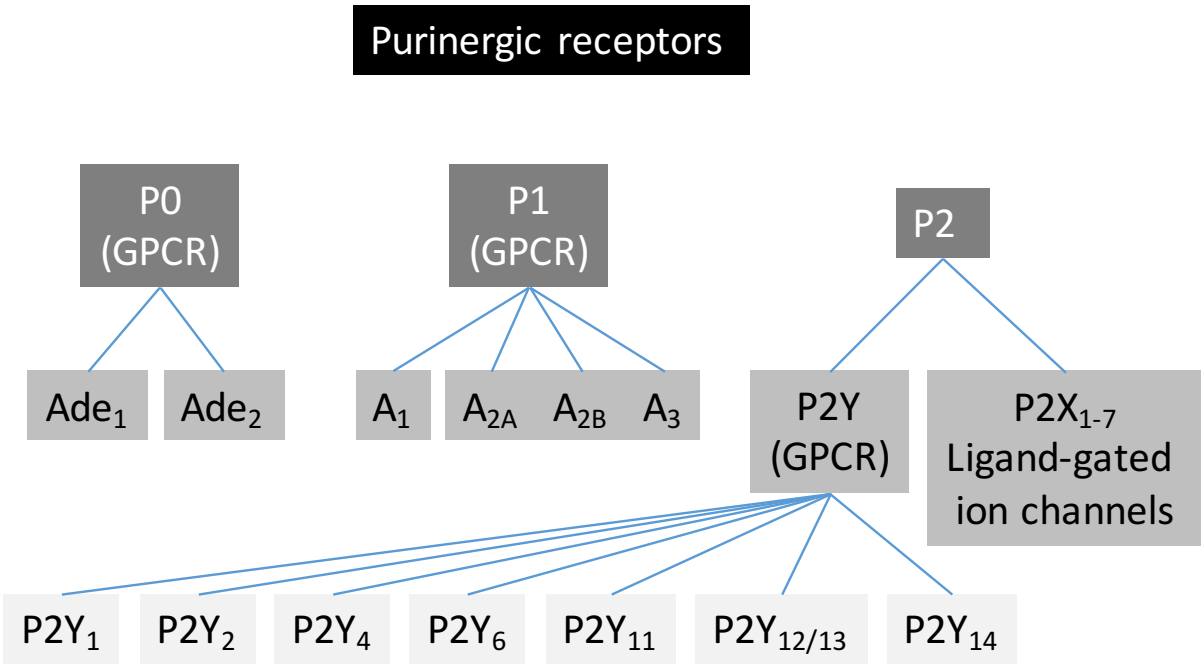


Figure 1.5: Purinergic receptors.

Recently a novel receptor class which is activated by adenine, named P0 receptors, was identified.[22,23]

1.1.7 P2Y purinoceptors

The P2Y purinoceptors are phylogenetically grouped into the δ-branch of the class A (rhodopsin) GPCRs which contains also many *orphan* GPCRs (Figure 1.2). Eight P2Y receptors have been identified so far which differ in their endogenous agonists and their second messenger system. $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ are G_q-coupled whereas $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ are $G_{i/o}$-coupled. $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ are activated by ADP, $P2Y_2$ and $P2Y_{11}$ by ATP, $P2Y_2$ and $P2Y_4$ by UTP, and $P2Y_6$ and $P2Y_{14}$ by uridine diphosphate (UDP). $P2Y_{14}$ is also activated by UDP-glucose and UDP-galactose.[24]

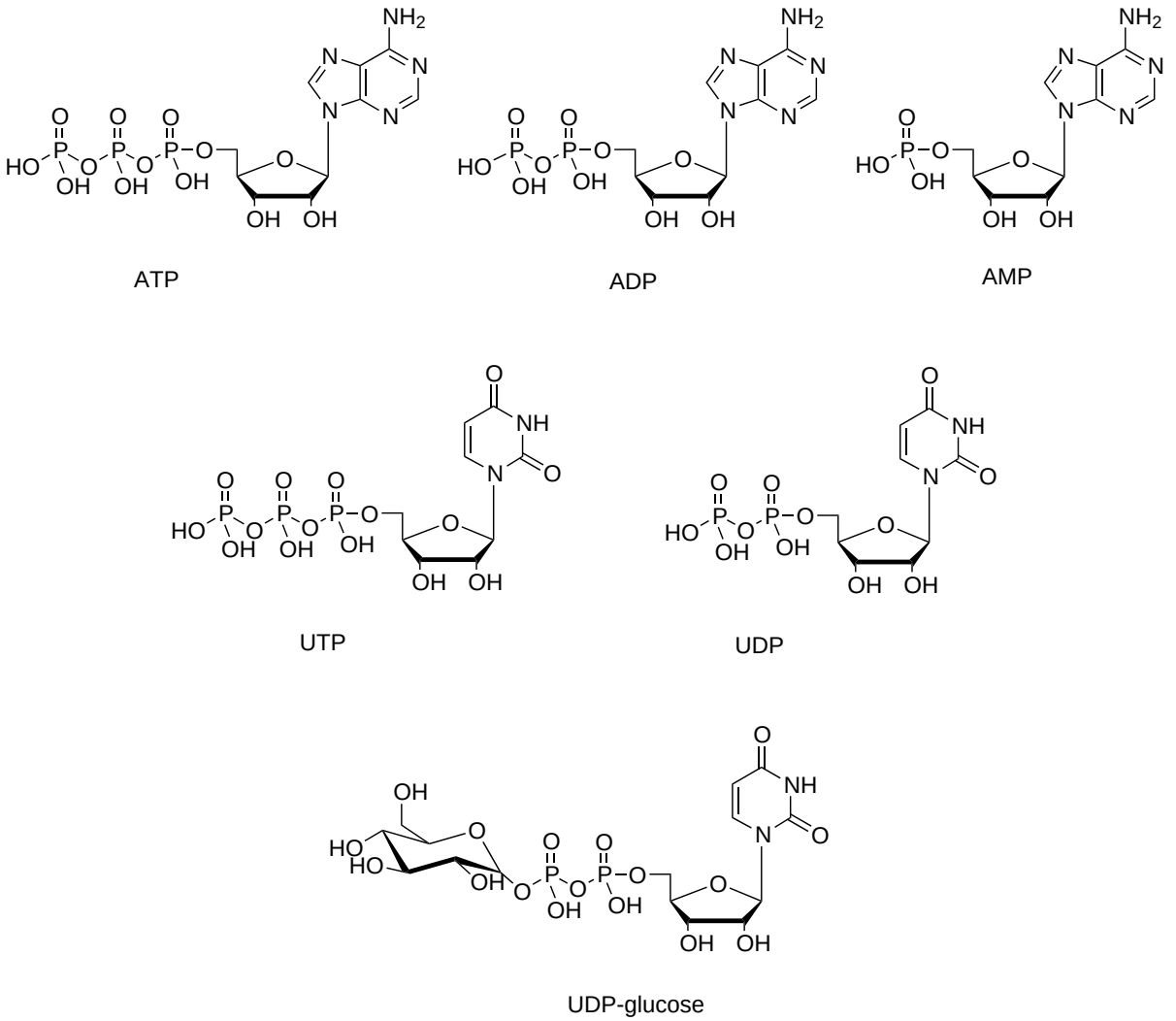


Figure 1.6: Nucleotides that can activate P_2Y receptor subtypes.

P2Y receptors are present in almost every cell of the human body and are involved in many physiological and pathological processes. The widely used drug clopidogrel for example blocks platelet aggregation via the $P2Y_{14}$ receptor. But the receptors also play a role in the nervous system and have been identified as potential targets for alzheimer's disease.[25,26]

1.1.8 The *orphan* receptor GPR17

Demyelinization by immune dysregulation is one of the major pathological hallmarks of multiple sclerosis (MS).[27] Myelin is formed by oligodendrocytes in the central ner-

vous system (CNS) and enables the saltatory conduction of electric signals along nerves. Damage of this layer of insulation of nerves results in symptoms like coordination problems, blindness or muscle weakness. To date, MS therapy is based on immunosuppression (corticosteroids, methotrexate etc.) and immune modulation (natalizumab, glatiramer acetate, β-interferons etc.) to prevent damage of the myelin sheats. However no drug is on the market yet, which can induce remyelination.[28] There is some evidence that purinergic signalling and especially the *orphan* GPCR GPR17 play a role in this remyelination process and therefore represent interesting potential drug targets for neurodegenerative diseases like MS.[29]

GPR17 (initially named R12) was first cloned in 2006 by Rapodo et al. upon searching for new chemokine receptors. They identified homologies of R12 to the P2Y purinoreceptors.[30] Upon screening of a complementary DNA (cDNA) library using degenerate RT-PCR searching for P2Y homologous sequences two years later Bläsius et al. identified two splice variants of GPR17/R12 located in the brain. The longer isoform has a 28 amino acids longer N-terminus than the short isoform.[31]

Phylogenetically GPR17 is located at an intermediate position between the P2Y and the cysteinyl-leukotriene receptors (CysLTs) cysteinyl-leukotriene receptor 1 ($CysLT_1$) and cysteinyl-leukotriene receptor 2 ($CysLT_2$), all of which are class A GPCRs.[32] P2Y receptors consist of 8 receptor subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$. They are stimulated by different nucleotides such as ATP, ADP, UTP, UDP and UDP-glucose and can be found in almost all regions of the body.[33] $CysLT_1$ and $CysLT_2$ bind cysteinyl-leukotrienes which act as mediators of inflammation, and the receptors are involved in allergic reactions like asthma bronchiale.[34]

1.1.8.1 The endogenous ligand of GPR17

The question about the endogenous ligand of GPR17 has been controversially discussed. Ciana et al. described the receptor as a dual receptor activated by both CysLTs (leukotriene D4 (LTD_4) and leukotriene C4 (LTC_4) in the nM range) and uracil derivatives (UDP, UDP-glucose, and UDP-galactose in the μM range) (determined by [^{35}S]GTPγS binding at 1321N1-, COS7-, CHO-, and HEK293-cells). The receptor is coupled to G_i and G_q proteins and through its activation the cAMP level decreases whereas the calcium level increases.[35,36] Meakawa et al. did not

detect significant calcium mobilziation in GPR17 expressing cells (1321N1, CHO, and HEK-293T) with LTC_4, LTD_4, or leukotriene E4 (LTE_4) and also not with UDP-glucose (1321N1 cells). Instead they postulated that GPR17 negatively regulates $CysLT_1$ function. Co-expression of $CysLT_1$ with GPR17 resulted in decreased LTD_4-initiated calcium mobilization in a variety of cell lines, inhibited $CysLT_1$-mediated extracellular-signal regulated kinase (ERK) phosphorylation in response to LTD_4 and supressed [^{3}H]LTD_4 binding. They also observed a co-localization of GPR17 and $CysLT_1$ in human monocytes.[37] Benned-Jensen and Rosenkilde confirmed that UDP activates the short isoform, human GPR17-S, dose-dependently in the low micromolar range, and the long isoform, human GPR17-S, in the high micromolar range. However they did not observe an inhibition of human GPR17-S and human GPR17-L by LTD_4.[38] In 2013 Qi et al. also tried to verify the identity of the endogeous ligands for GPR17. However they did not observe any activation of the receptor, neither by UDP-glucose, UDP-galactose or UDP, nor by CysLTs in human GPR17-transfected C6, 1321N1 astrocytoma, CHO, COS-7, and HEK293 cells (cAMP accumulation, inositol phosphate production, and monitoring of Ca^{2+} mobilization).[39] In the laboratory of E. Kostenis and also in our laboratory no interaction of nucleotides or cysteinyl leukotrienes with GPR17 has been observed so far despite considerable efforts.[40]

1.1.8.2 Physiological and pathophysiological role of GPR17

Ciana et al. showed that GPR17 is located in organs undergoing ischemic damage,[35] but Lecca et al. were the first who connected GPR17 with brain damage and suggested the receptor as a novel target for brain repair. It was previously shown by Maisel et al. that the GPR17 gene is expressed on hippocampus-derived neuroprogenitor cells (NPCs).[41] Lecca et al. showed that GPR17 is present on neurons and on a subset of quiescent oligodendrocyte precursor cells. Especially in neurons expressing the stress marker heat shock protein 70, GPR17 was up-regulated. Knock-down of GPR17 prevented brain infarct evolution and was identified on microglia/macrophages infiltrating the lesioned area at later times of the ischemia. This indicates that the receptor forces neuronal death during the ischemic attack but also plays a role in the remodeling of the damaged brain areas.[42,43] Ceruti et al. investigated GPR17 as a potential drug target for spinal cord injury (SCI). The results of their studies were consistent with the ones by Lecca et al.. SCI in-

duced a dramatic and rapid death of $GPR17^+$ neurons and oligodendrocytes inside the lesioned area. Blocking of GPR17 by injection of a specific GPR17 antisense oligonucleotide into the spinal cord improved improved healing of the tissue damages derived from SCI. Another fact is that GPR17 is expressed by spinal cord cells which supports the hypothesis that GPR17 is a mediator for cell differentiation.[44] Chen et al. found that GPR17 is regulated by the basic helix-loop-helix (bHLH) transcription factor Olig1 which plays an important role in oligodendrocyte myelination and remyelination.[45] They found that GPR17 over-expression results in a blockage of the differentiation of neural progenitor cells into oligodendrocytes and an inhibition of terminal differentiation of primary oligodentrocyte precursor cells (OPCs). Vice versa a knock-out of GPR17 accelerated OPC maturation. In Olig1 null mice GPR17 was dramatically downregulated which indicated an interdependence.[46] The role of GPR17 in cell differentiation is supported by the observation that GPR17 agonists together with neuronal growth factor (NGF) promote neurite outgrowth in PC12 cells and activate the intracellular phosphorylation of both ERK 1/2 and p38 kinases.[47] The same effect was shown by Huber et al. who blocked GPR17 with montelukast and observed increased neural stem and progenitor cell proliferation.[48] Fumagalli et al. described the signalling pathway of GPR17 as follows: The highest level of receptor expression can be found in immature preoligodendrocytes. It is also expressed on early $NG2^+$ polydendrocytes but is completely downregulated on fully mature MAG^+ or MBP^+ oligodendrocytes. GPR17 inhibition by small interfering RNAs (SiRNAs) hindered the differentiation of OPCs whereas activation with UDP led to OPC maturation. They postulated that regarding differentiation of OPCs GPR17 has a positive effect in early stage OPCs but a negative role in late OPCs.[49] This was Coppi et al. found that GPR17 regulates K^+ currents which could activate oligodendrocyte differentiation.[50] The same results were obtained by Hennen et al. who stimulated GPR17 with the agonist MDL29,951.[51]

An interesting study recently showed that mice, treated with the GPR17 antagonist montelukast showed reduced neuroinflammation and an elevated hippocampal neurogenesis. Additionally the mice had an improved ability to learn and an improved memory which makes GPR17 also a potential target for dementia.[52] However, montelukast is only a very weak GPR17 antagonist, and it is much more potent at $CysLT_1$ receptors.

Meakawa et al. showed that GPR17 negatively regulates $CysLT_1$ receptor func-

tions regarding the antigen presentation and the downstream phases of allergic pulmonary inflammation and is therefore proposed as a potential target for allergic asthma.[53]

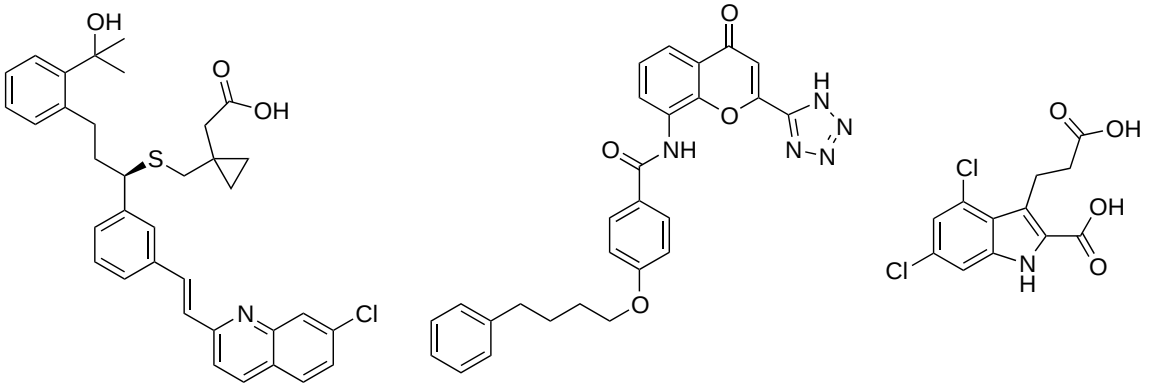
A completely different functional role for GPR17 was described by Ren et al.. They found that Gpr17 knockout mice (Agrp-Gpr17(-/-)) showed reduced food intake. GPR17 seems to be a FoxO1 target which is involved in gluconeogenesis. They also found that GPR17 regulates the sensitivity to insulin and leptin. In consequence GPR17 might be a target for the treatment of obesity.[54,55] In contrast to that Mastaitis et al. did not observe any differences in food intake comparing GPR17 knock-out mice to the wild-type animals.[56]

When considering the results described in this section we have always to keep in mind that some of the studies partly contain experiments that were performed with GPR17 ligands that never could be confirmed. Rather the majority of research groups, including our group, could show that neither nucleqotides nor CysLTs do interact with GPR17. The conclusions of all studies, where the receptor was stimulated with this ligands, therefore have to be seen in a very critical light.

1.1.8.3 Synthetic ligands for GPR17

Several synthetic ligands are known for GPR17. First of all some purinergic and leukotriene antagonists were found to be antagonists at GPR17. Cangrelor[57] (P_2Y_{12}/-P_2Y_{13} antagonist) and MRS2179[58] ($P2Y_1$ antagonist) were reported to be GPR17 antagonists but could never be confirmed. Pranlukast and montelukast[59,60] ($CysLT_1$ antagonists) were identified and confirmed as antagonists at GPR17.[35] MDL29,951[40] and its tritiated form [^{3}H]PSB-12150[61] are agonists for GPR17 (Figure 1.7).

The human and the rat orthologue share an amino acid sequence similarity of 89%.[35] The mouse orthologue shares an amino acid sequence similarity of 90 and 98% with the human and the rat orthologue, respectively.[42] Therefore ligands developed for the human receptor may also be suitable compounds for animal studies. However none of the ligands developed so far is specific for GPR17 since they were originally designed for other targets except for MDL-29.951 which is quite selective for GPR17. We therefore have to continue working on the development of novel potent and selective ligands.



montelukast (IC$_{50}$ = **14.1** μM[40] in calcium assay)

pranlukast (IC$_{50}$ = **2.69** μM[40] in calcium assay)

MDL29.951 (EC$_{50}$ = **1.41** μM[40] in calcium assay)

Figure 1.7: Selected GPR17 antagonists pranlukast and montelukast, and agonist MDL29.951.

1.1.8.4 Conclusion

In summary GPR17 is an interesting target for the treatment of diseases caused by myelin damage because by blocking the receptor it could be possible to induce neurorepair. Additionally it seems also to have an effect in organs undergoing ischemic damage. However only few synthetic and drug-like ligands are known for this poorly studied receptor. It will be necessary to develop potent ligands which pass the blood-brain-barrier because the target is located in the brain. All ligands developed so far contain polar functionalities such as carboxylic acids which may prevent CNS penetration. It is therefore necessary to determine structure-activity relationships (SARs) to further characterize the binding pocket of GPR17 and to design suitable drugs.

1.1.9 The *orphan* receptor GPR35

The *orphan* receptor GPR35 gene was first discovered in 1998 by O'Dowd et al. encoding a receptor of 309 amino acids.[62] In 2004 Okomura et al. identified two different splice variants of the receptor which differ in 31 amino acids at the N-terminal domain. GPR35a is the short isoform and GPR35b the longer one.[63] The human GPR35 is closely related to the *orphan* receptor GPR55 (34% amino acid identity),[64] to lysophosphatidic acid receptors, P2Y receptors[62] and hydroxycar-

boxylic acid receptors.[65] Interestingly the receptor shows a high species diversity. The amino acid sequence identity between the human and the rodent orthologues is only moderate with about 70%.[66] GPR35 belongs to the class A, rhodopsin like GPCRs.

Wang et al. were able to show that the signal transduction of GPR35 is mediated by the pertussis toxin sensitive $G_{i/o}$ protein.[67] This findings could be confirmed by several groups. Guo et al. showed that the treatment of GPR35 with the agonists zaprinast or kynurenic acid inhibited N-type calcium channels. This effect could be blocked by pertussis toxin.[68] Jenkins et al. showed that GPR35 also interacts with the G_{13}-protein.[69] The receptor can furthermore recruit β-arrestins.[69,70]

1.1.9.1 Expression profile and therapeutic potential of GPR35

GPR35 was first found to be expressed in the intestine of the rat[62] by Northern blot analysis. The rat GPR35 is also expressed in the uterus, lung and dorsal root ganglion.[71] Okumura et al. detected GPR35 in human gastric cancer cells. Interestingly the expression of the longer splice form GPR35b in gastric cancer cells and also stomach cells was higher than the expression of GPR35a.[63] Wang et al. detected the human GPR35 also in peripheral leukocytes and spleen. However, also in human it is highly expressed in the small intestine, colon and stomach. A similar situation had been found for the mouse GPR35 which was detected in spleen and the gastrointestinal tract.[67] Low levels of GPR35 have also been found in the liver.[67,71]

The expression profile of GPR35 indicates a potential role in the pathology of the gastrointestinal tract. GPR35 is overexpressed in gastric cancer cells in comparison to healthy tissue. Furthermore the introduction of GPR35-cDNA into a NIH3T3 cell line (embryonal mouse fibroblast cells) resulted in a transformation of the cells.[63] Imielinski et al. identified a single nucleotide polymorphism (SNP) which links GPR35 to ulcerative colitis and inflammatory bowel disease (IBD).[72]

Min et al. connected GPR35 to cardiovascular diseases. GPR35 knock-out mice showed a significantly higher blood pressure than the wild-type mice. The group also compared the GPR35 expression of healthy patients and heart failure patients and detected a higher expression of GPR35 in the diseased patients.[73] Sun et al. postulated a relation of a Ser294Arg SNP in the GPR35 gene to artherosclerotic

changes.[74] GPR35 could also function as a marker for myocardial infarction as it was upregulated in hypoxic tissue.[75]

The expression of GPR35 on various immune cells such as spleen, peripheral leukocytes,[76] monocytes,[77] mast cells and eosinophils[78] and not at least on invariant natural killer T cells (iNKTs)[79] indicates a potential role of the receptor in the pathology of immune-related diseases. Barth et al. postulated a potential role of the receptor in the context of leukocyte recruitment to inflamed tissue. They could show that the GPR35 agonist kynurenic acid induces the adhesion of monocytes to the endothelium, which starts the process of leukocyte recruitment.[77] The fact that a range of mast cell stabilizers like nedocromil or cromolyn function as GPR35 agonists and the expression of GPR35 on mast cells indicate a therapeutic potential of GPR35 for the treatment of asthma.[78]

A connection of GPR35 to diabetes is also discussed in the literature. Horikawa et al. identified SNPs of GPR35 that correlate with diabetes type 2.[80]

Moreover GPR35 is also a potential target for the treatment of pain. The receptor is colocalized in dorsal root ganglionic neurons, which also express the transient receptor potential vanilloid 1 (TRPV1) channel. This compound is known to be involved in nociception, so GPR35 is a potential target for analgesia.[81] Cosi et al. observed an antinociceptive effect when mice were treated with the GPR35 agonists kynurenic acid and zaprinast.[82]

In conclusion, due to its expression profile it is very likely that GPR35 plays a role in gastrointestinal diseases. However various effects in other regions of the body related to GPR35 have been observed, that are poorly studied yet.

1.1.9.2 Ligands for GPR35

Endogenous ligands

The first ligand that was identified for GPR35 was the tryptophan metabolite kynurenic acid. Due to the fact that it is present in tissue which expresses GPR35 it was proposed to be the endogenous ligand for GPR35. However it is much more potent at the rat GPR35 than at the human orthologue. For an activation of the human receptor micromolar concentrations of kynurenic acid are needed.[67]

Oka et al. identified lysophosphatidic acids (LPAs), especially 2-acyl-LPAs as fur-

ther potential endogenous agonists of GPR35. In contrast to kynurenic acid they were able to activate the human receptor in nanomolar concentrations.[83]

Recently Maravillas-Montero et al. reported that GPR35 is activated by the chemokine CXCL17 in nanomolar concentrations. They suggested to rename GPR35 in CXCR8 according to the chemokine nomenclature.[84] However also this result has to be confirmed, and the receptor is still not deorphanized.

Synthetic agonists

The phosphodiesterase (PDE)-inhibitor zaprinast was the first synthetic agonist described for GPR35. It is more potent at the human receptor than kynurenic acid but still much more potent at the rodent orthologues.[71] Besides zaprinast many other small molecules were found to act as GPR35 agonists.

Especially notable is that many mast cell stabilizers are agonists at GPR35. The exact mode of action of these drugs is not clear, so the effect could be GPR35-mediated. Members of this class are the chromones cromoglicic acid and nedocromil, which are more potent at the human receptor than at the rodent receptors[78] and the recently identified bufrolin and lodoxamide, which are the first agonists of GPR35 that can activate both, the human and the rat receptor, in the low nanomolar range.[85]

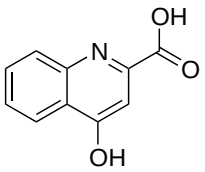
One interesting agonist of GPR35 is pamoic acid, which was assumed to be biologically completely inactive. Because of its properties it is used as an excipient which is present in many marketed drugs. Zhao et al. found that it is a selective agonist for the human GPR35.[70]

Deng et al. identified a broad range of GPR35 agonists, including aspirin metabolites,[86] tyrphostin analogs,[87] benserazide, catechol and pyrogallol,[88] natural phenols,[89] nitrophenols,[90] D-luciferin,[91] 2-(4-methylfuran-2(5*H*)-ylidene)malononitrile and thieno[3,2-b]thiophene-2-carboxylic acid derivatives,[92] gallic acid and wedelolactone.[93]

Yang identified the common loop diuretic drugs bumetanide and furosemide as GPR35 agonists.[94]

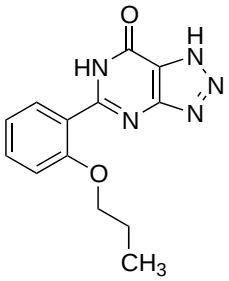
Recently, a very potent and selective class of GPR35 agonists was identified in our group. 8-Benzamidochromen-4-one-2-carboxylic acids are able to activate the receptor in the low nanomolar range.[95] The first radioligand for GPR35, 6-bromo-8-(4-[3H]methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid, is based on this scaffold.[65]

GPR35 agonists



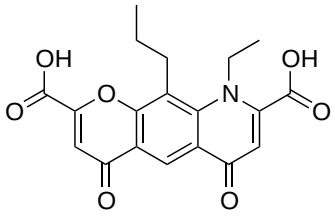
kynurenic acid

(EC_{50} = **217**[a] (h),[70] **10.4**[b] (m),[67] **7.4**[b] (r)[67] μM)



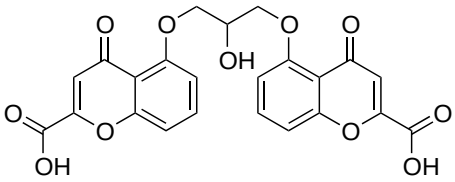
zaprinast

(EC_{50} = **2.75**[a] (h),[69] **0.977**[a] (m),[96] **0.0955**[a] (r)[96] μM)



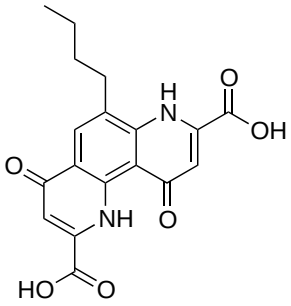
nedocromil

(EC_{50} = **0.126**[a] (h),[78] **7.24**[b] (m),[78] **2.75**[b] (r)[78] μM)



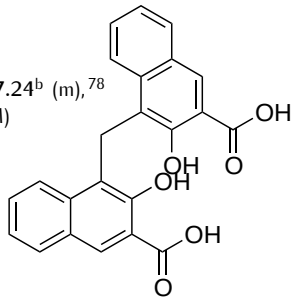
cromoglicic acid

(EC_{50} = **7.57**[a] (h),[69] **57.5**[a] (m),[69] **4.37**[a] (r)[69] μM)



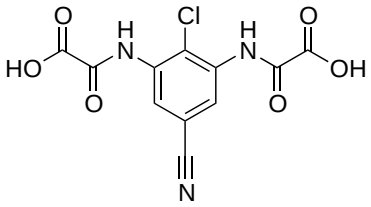
bufrolin

(EC_{50} = **0.0126**[a] (h),[85] **0.010**[a] (r)[85] μM)



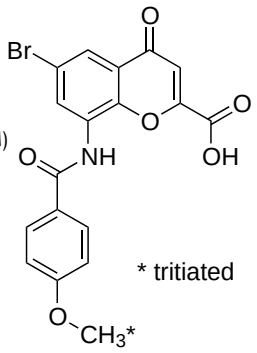
pamoic acid

(EC_{50} = **0.079**[a] (h),[70] inactive[a] (m),[96] inactive[a] (r)[69] μM)



lodoxamide

(EC_{50} = **0.00363**[a] (h),[85] **0.0126**[a] (r)[85] μM)

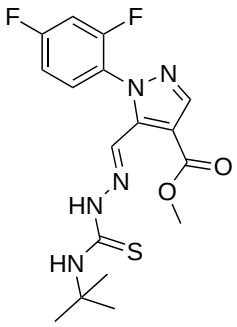


* tritiated

[^{3}H]PSB-13253

(EC_{50} = **0.0111**[a] (h),[95] **4.17**[a] (m),[95] inactive[a] (r)[95] μM)

GPR35 antagonist



CID274587

(IC_{50} = **0.0128**[a] (h),[70] inactive[a] (m),[96] inactive[a] (r)[96] μM)

[a] β-arrestin recruitment assay
[b] calcium ion mobilization assay assay

Figure 1.8: Selected GPR35 agonists and antagonists and IC_{50}/EC_{50} values at the human (h), mouse (m) and rat (r) GPR35.

Synthetic Antagonists

Only few antagonists for GPR35 are known so far. The most common and widely used one is CID2745687, which was discovered in 2010 by Zhao et al.[70]

1.1.9.3 Conclusion

GPR35 is an interesting target for drug research but poorly investigated so far. Its physiological function as well as its endogenous agonist still remain unclear. Although several physiological agonists of GPR35 have been identified most of them activate the receptor only at high concentrations. It is questionable if these compounds are present in adequate concentrations in the body to activate the receptor. LPAs and the chemokine CXCL17 are more potent but test results have not been confirmed yet. These proposed agonists are not suitable as tool compounds or as drug candidates because they are too expensive and, what is more important, because of their inappropriate physiochemical properties. Therefore it is necessary to put further effort into the development of synthetic GPR35 ligands with drug-like properties, high selectivity and increased stability.

1.1.10 The *orphan* receptor GPR55

The endocannabinoid system is a part of the nervous system originally based on the two GPCRs CB_1 and CB_2 which bind constituents of the plant Cannabis sativa, the so called cannabinoids. Besides the cannabinoids derived from plants, there are also endogenous ligands for the two receptors including *N*-arachidonylethanolamide also known as anandamide (AEA) and 2-arachidonylglycerol (2-AG). The endocannabinoid system is pharmacologically related to movement, memory, appetite, nausea, pain, cancer and also immune diseases.[97] The endocannabinoids AEA and 2-AG have also been described to bind to the *orphan* GPCR GPR55. It is therefore discussed wether GPR55 could represent a third cannabinoid (CB) receptor, however, sequence homology is low.[98]

The GPR55 gene was first identified in 1999 by Sawzdargo et al. from an expressed sequence tag (EST) database encoding a receptor of 319 amino acids. Its closest relatives are $P2Y_5$ (29%), GPR23 (30%), GPR35 (27%) and CCR4 (23%).[64] It shares only an amino acid sequence identity of about 14% with the two CB-receptors CB_1

and CB_2.[99]

1.1.10.1 Pharmacology of GPR55

GPR55 belongs to the class A (rhodopsin-like) GPCRs.[1] In 2007 Ryberg et al. reported that GPR55 is coupled to $G\alpha_{13}$. They observed an activation of RhoA during an activation with AEA and this effect was blocked by cannabidiol.[98] Lauckner et al. observed an increase of intracellular calcium in HEK293 cells and postulated that GPR55 could couple to $G\alpha_q$ or $G\alpha_{12}$.[100] The $G\alpha_{12/13}$ coupling could be confirmed by Henstridge et al. in 2009.[101]

1.1.10.2 Expression profile and therapeutic potential of GPR55

When GPR55 was identified in 1999, Sawzdargo et al. already defined an expression profile of the receptor performed by Northern blot analysis. It turned out that it is abundantly expressed in the brain, especially in caudate nucleus and putamen.[64] Later on Ryberg et al. analysed the GPR55 expression in mouse using quantitative polymerase chain reaction (PCR) and detected GPR55 messenger RNA (mRNA) in the gastrointestinal tract and the brain.[98] There is also evidence for GPR55 expression in the liver and adipose tissue.[102] Furthermore GPR55 can be found in osteoblasts and osteoclasts.[103]

The expression of GPR55 in metabolically relevant tissues and the relation to CB-receptors, which are known to be involved in the regulation of food intake, indicate a therapeutic potential of the receptor for the treatment of metabolic disorders like diabetes or obesity.[104] Moreno-Navarrete et al. observed a correlation of GPR55 and lysophosphatidylinositol (LPI), a GPR55 agonist, with several metabolic parameters like weight, body mass index (BMI), and percent fat mass.[102] Meadows et al. investigated GPR55 knock-out mice regarding body weight and insulin resistance. They found that the body weight of GPR55 deficient mice was not increased significantly whereas the percentage of fat and the insulin resistance was. The movement of the knock-out mice was decreased.[105] Very recently Liu et al. postulated that GPR55 regulates the insulin secretion of the islets of Langerhans.[106]

GPR55 is an interesting target for the treatment of cancer. In 2000 the GPR55 agonist LPI was first identified as a marker for ovarian cancer.[107] Sutphen et al.

confirmed this results.[108] In 2011 it was shown that GPR55 plays a role in the regulation of proliferation and anchorage-independent growth of ovarian cancer and also of prostate cancer cells.[109] Andradas et al. showed that GPR55 promotes cancer cell proliferation via ERK and the receptor was found to be associated to tumor growth in glioblastoma[110] and to enhance skin cancer growth.[111] A further interesting fact is that CB_2 and GPR55 form heteromers in cancer cells which display modified signalling.[112] Recently it was reported by the same group that GPR55 expression in human tumors is associated with the aggressive basal/triple-negative breast cancer population. The authors found that an activation of GPR55 promotes metastasis in this cancer type.[113] Also in colon cancer GPR55 promotes adhesion and migration.[114]

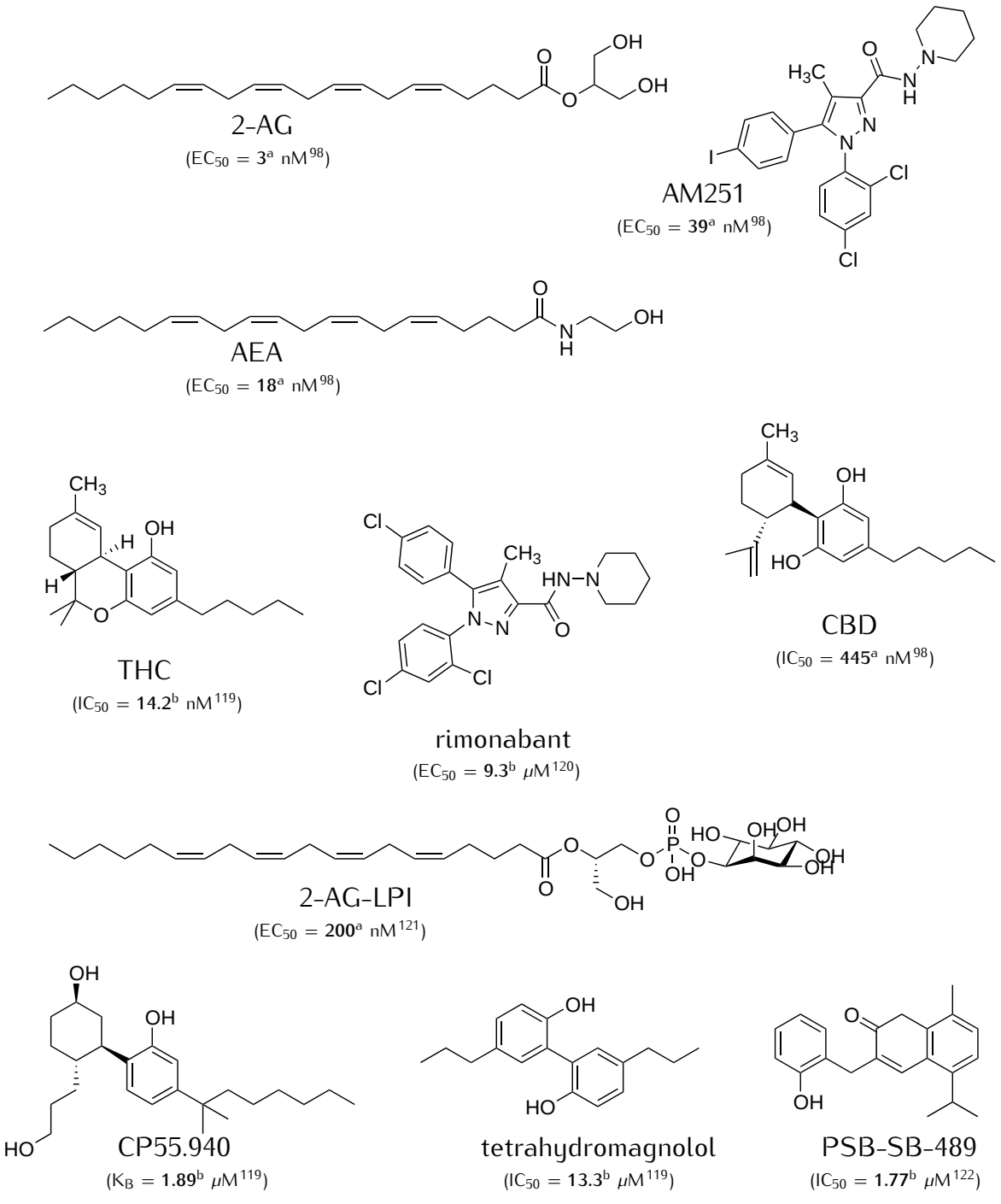
As previously described GPR55 is also expressed in bone cells. It was shown that LPI is able to inhibit osteoclast formation On the other hand it increased their activity. A modulation of GPR55 therefore could be starting point for the treatment of osteoporosis.[103]

A proinflammatory role of GPR55 especially in the gastrointestinal tract is also discussed. Stancic et al. induced a colitis in mice by adding 2.5% dextran sulfate sodium (DSS) supplemented to the drinking water, or by application of trinitrobenzene sulfonic acid (TNBS). Then the mice where treated with the GPR55 antagonist CID16020046[115] which reduced the inflammation.[116]

The physiological role of GPR55 is diverse. There is strong evidence that it is a potential target for the treatment of cancer. Furthermore its role in the context of metabolic diseases, osteoporosis and inflammation has to be further investigated.

1.1.10.3 Ligands for GPR55

The idea that GPR55 is a new CB receptor was mainly based on the fact that initially known endocannabinoids were found to bind to GPR55.[98] But GPR55 does not have the classical CB binding pocket.[117] Ryberg et al. identified AEA, 2-AG and CBD as GPR55 agonists[98] and Johns et al. found that O-1602 stimulates GPR55.[118]



[a] [35S]GTPγS binding assay
[b] β-arrestin recruitment assay

Figure 1.9: Selected GPR55 agonists and antagonists.

Lauckner et al. identified further agonist for GPR55: tetrahydrocannabinol (THC), the aminoalkylindole JWH015, the eicosanoids AEA and methanandamide (MEA),

and the endogenous lipid LPI increased the calcium level in HEK293 cells and large dorsal root ganglion (DRG) neurons, but they did not observe an effect with CP55,940, WIN55,2122, and 2-AG.[100] However, Whyte et al. found that CBD functions as an GPR55 antagonist[103] which was confirmed by Rempel et al. who found that THC and CP55.940 act as antagonists in the β-arrestin recruitment assay.[119]

In 2007 Oka et al. identified 2-AG-LPI as a possible endogenous ligand for GPR55 ([^{35}S]GTPγS binding assay in HEK293 cells; EC_{50} = 200 nM).[123] In 2009 the same group found that the activity of 2-arachidonoyl-LPI is about 3 times higher than LPI.[121] Ryberg et al. identified AM251, which is known a CB_1-antagonist, to be an agonist at GPR55.[98] This finding could be reproduced by Yin et al. who performed a β-arrestin PathHunter assay at a large number of *orphan* GPCRs including GPR55. Besides AM251 they also identified the cannabinoid antagonist rimonabant (SR141716A) and LPI as strong agonists but did not observe any effect with endogenous cannabinoids.[120] The same results were repoduced by Kapur et al. who identified CP55949, which is also a CB agonist, as a partial agonist at GPR55, and by Henstridge et al. who observed an agonistic effect of LPI but also an agonistic effect for AM251.[124,101]

Lauckner et al. described rimonabant as a GPR55 antagonist. Heynen-Genel et al. from the NIH did a high throughput screening to identify small molecule agonists as well as antagonists for GPR55. They identified 3 different chemical scaffolds: morpholinosulfonylphenylamide, ML186 (CID15945391) (305 nM), tricyclic triazoloquinoline, ML185 (CID1374043) (658 nM) and piperazine, ML184 (CID2440433) (263 nM).[125] They also identified antagonists: quinoline aryl sulfonamide ML193 (CID1261822) (221 nM), thienopyrimidine ML192 (CID1434953) (1080 nM) and piperadinyloxadiazolone ML191 (23612552) (160 nM).[115] Rempel et al. identified tetrahydromagnolol and later on also coumarins (PSB-SB-489) as lead structures for new GPR55 antagonists.[119,122] In the last year several groups started to work on the optimization of ligands for GPR55. Hess et al. identified constituents of spice as weak GPR55 antagonists,[126] Yrjölä et al. synthesized *N*-(4-sulfamoylphenyl)thiourea derivatives as GPR55 agonists[127] and Meza-Aviña et al. designed piperidine-substituted 1,3,4-oxadiazol-2-ones as GPR55 antagonists.[128]

In conclusion the cognate agonist of GPR55 still lacks confirmation. LPI turned out to be a GPR55 agonist and it is therefore used as a standard agonist. Although a broad range of synthetic agonists and antagonists have been identified in the last

years there is still a need for more drug-like and also more potent compounds.

1.1.11 The *orphan* receptor GPR84

GPR84 was discovered in 2001 by Wittenberger et al. by an EST data mining strategy.[129] In the same year Yousefi et al. identified EX33 (GPR84) as a novel gene using degenerate primer reverse transcription polymerase chain reaction (RT-PCR). Performing Northern blot analysis they could show that EX33 (GPR84) is abundantly expressed in bone marrow, lung, and peripheral blood leukocytes, especially in neutrophils and eosinophils.[130 67]

Venkataraman et al. were the first to generate GPR84-deficient mice. The proliferation of T and B cells in those mice was normal, but T-cells stimulated with anti-CD3 produced an increased level of interleukin-12 (IL-12). The IL-12 level in Th2 effector cells isolated from these knock-out mice was also higher compared to the ones generated from wild-type mice.[131] The first ligands for GPR84 were discovered by Wang et al.. medium-chain free fatty acid (MCFFA) with chain lengths from C9-C14 showed agonistic activity at GPR84. short-chain free fatty acids (SCFFAs) which are known to activate GPR41 and GPR43[132] and long-chain free fatty acids (LCFFAs) which activate GPR40[133] were not able to activate GPR84. Capric acid (C10), undecanoic acid (C11) and lauric acid (C12) were the most potent MCFFAs in the cAMP assay (EC_{50}-values of 4, 8, and 9 μM, respectively). The same group also postulated that GPR84 activation by MCFFAs is coupled to a pertussis toxin-sensitive Gi/o pathway. Furthermore they found that an activation of GPR84 by the surrogate ligand diindolylmethane (DIM) in the presence of lipopolysaccharides (LPSs) leads to an up-regulation of interleukin (IL)-12 p40 mRNA. Thus GPR84 amplifies the LPS-stimulated production of IL-12 p40.[67] In mice suffering from endotoxemia GPR84 is also expressed in microglia and in peripheral macrophages[134] and monocytes induced by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-1. The same group also showed GPR84 expression during experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, which is induced by the injection of various proteins.[135] Perry et al. demonstrated the existence of a GPR84 homologue in Xenopus laevis where it seems to be involved in eye/lens formation.[136] Interestingly GPR84 is also expressed in 3T3-L1 adipocytes and is up-regulated in the adipose tissue from high-fat chow (HFC)-induced obese mice.

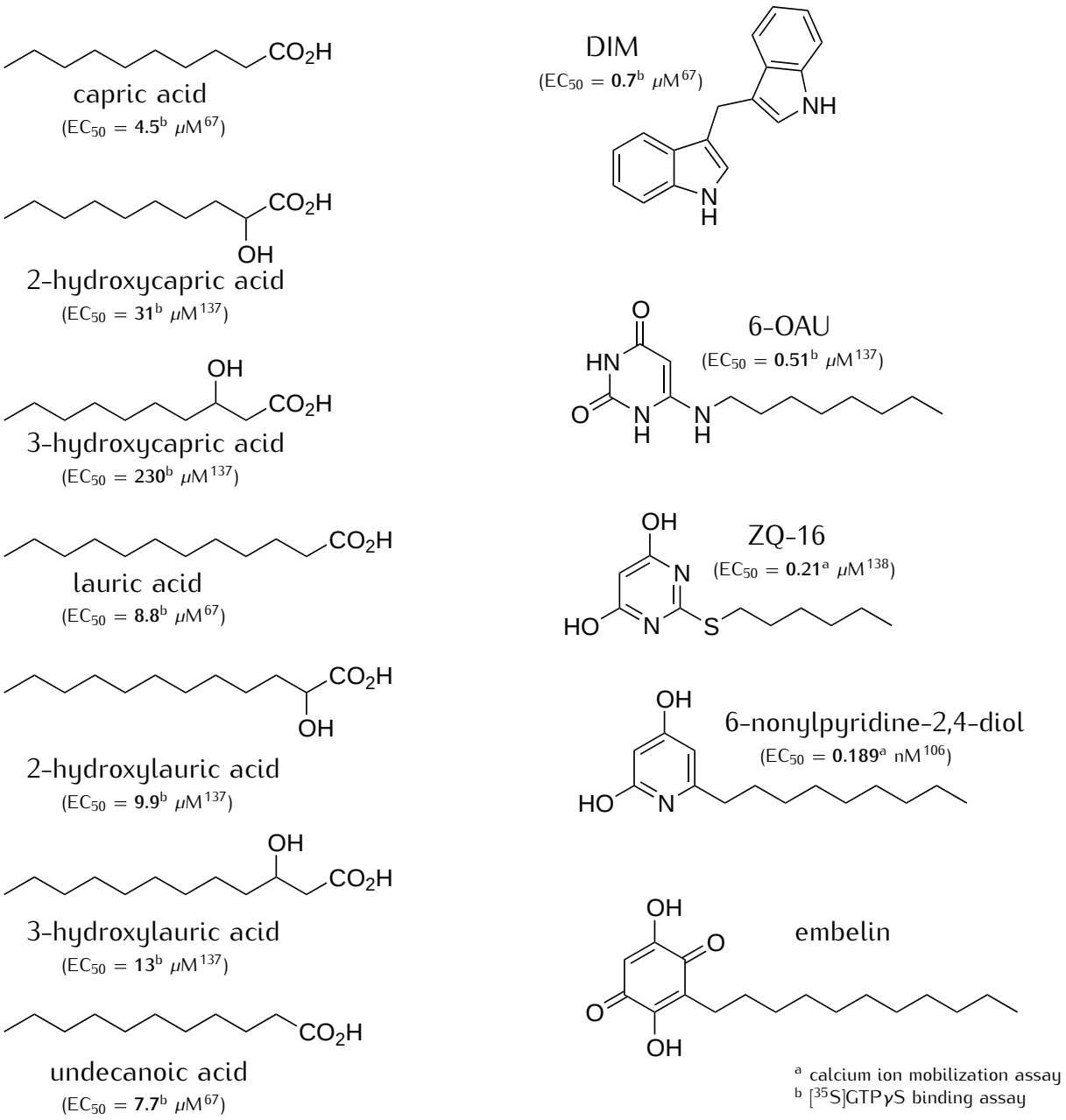


Figure 1.10: Selected GPR84 agonists.

They also showed that RAW264, TNF-α and LPS stimulated the GPR84 expression in 3T3-L1 adipocytes and human adipose-derived stem cell (ADSC) adipocytes. It may be concluded that GPR84 plays a role in the pathogenesis of diabetes and obesity.[139]

A further agonist at GPR84 is the natural product embelin (2,5-dihydroxy-3-undecyl-

[1,4]benzoquinone) which was studied as a modulator for the treatment of atherosclerosis and mcp-1- (monocyte chemoattractant protein-1) related diseases.[140] Suzuki et. al. identified also the hydroxy-MCFFAs 2-hydroxycapric acid (C10), 3-hydroxycapric acid (C10), 2-hydroxylauric acid (C12), and 3-hydroxylauric acid (C12) and the surrogate ligand 6-*n*-octylaminouracil (6-OAU)[141] as agonists for GPR84.

They also confirmed what previous studies had indicated: GPR84 seems to be a pro-inflammatory receptor because it amplifies LPS-stimulated IL-8 production in polymorphonuclear neutrophil (PMN) and TNF-α production in macrophages.[137] Research on the homologue in the zebrafish (zGPR84) where the receptor is mainly expressed in tissues of intestine, heart and liver supported these findings.[142]

As reported by Dietrich et al. GPR84 is a regulator of β-catenin, a signalling molecule for the differentiation of leukemic stem cells (LSCs). It induces the G1-phase cell-cycle arrest in pre-LSCs and reduces LSC frequency. GPR84 is furthermore up-regulated in acute myeloid leukemia (AML) LSCs and in mixed lineage leukemia (MLL) AML, which is a very aggressive and almost untreatable type of AML.[143] Madeddu et al. found that GPR84 is upregulated in the CNS following virus infection.[144]

Nikaido et al. performed the first mutagenesis study and investigated the two GPR84 agonists capric acid and DIM. As a result they postulated that DIM is a positive allosteric modulator (PAM) at GPR84.[145]

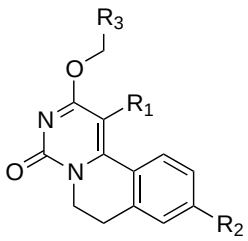
A study published in 2015 in "Brain, Behavior, and Immunity" associated GPR84 with Alzheimer's disease (AD). It was observed that GPR84 is up-regulated in microglia of APP/PS1 transgenic mice, a model of AD. Knock-down of GPR84 in these mice has a negative effect on cognition and the number of microglia. The authors postulated that GPR84 could be a signaling molecule for a yet unknown ligand that promotes microglia recruitment and may have a positive effect in amyloid pathologies.[146]

Recently Nicol et al. investigated the role of GPR84 in association with pain. They found that GPR84 does not affect acute pain but it seems to have an effect on mechanical and thermal behavioral hypersensitivity in mice after partial sciatic nerve ligation (PNL) which was reduced in GPR84 knock-out mice. GPR84 mRNA was increased in the sciatic nerve and spinal cord after PNL. Thus GPR84 might play a role in chronic pain including neuropathic pain.[147]

GPR84 also has a potential role in the pathology of gastroesophageal reflux disease

(GERD) being involved in the regulation of the immune answer in the inflammatory process at esophageal cells.[148] Zhang et al. recently identified and further optimized 2-alkylpyrimidine-4,6-diols and 6-alkylpyridine-2,4-diols as potent GPR84 agonists (Figure 1.10).[106,138]

So far only one class of antagonists for GPR84 is described in a patent and investigated in a first-in-human single dose and multiple dose clinical study for the treatment of ulcerative colitis. However the dihydropyrimidinoisoquinolinones did not meet the endpoints for efficacy (Figure 1.11).[149]



dihydropyrimidinoisoquinolinones core structure

IC_{50} = 0.1 - 100 nM ([^{35}S]GTPγS)

Figure 1.11: Core structure of dihydropyrimidinoisoquinolinone-based antagonists.[149]

Summing up the research that has been done so far on the field of GPR84 there are strong indications that the receptor plays a role in the immune system and therefore is of considerable interest as a target for the development of new drugs. However the receptor has been poorly investigated so far and its physiological/pathophysiological role is not completely understood. There is strong evidence that it is involved in inflammation and immune responses, in metabolic diseases, CNS diseases and even in reflux disease. It is therefore necessary to develop potent ligands to further investigate the receptor pharmacology in more detail.

1.2 Nucleotide-metabolizing ecto-enzymes

Extracellular nucleotides that activate purinergic receptors undergo a sequence of hydrolyses producing adenosine which is subsequently deaminated to inosine and further hydrolyzed to hypoxanthine. A broad range of specific extracelluar enzymes is involved in this process.

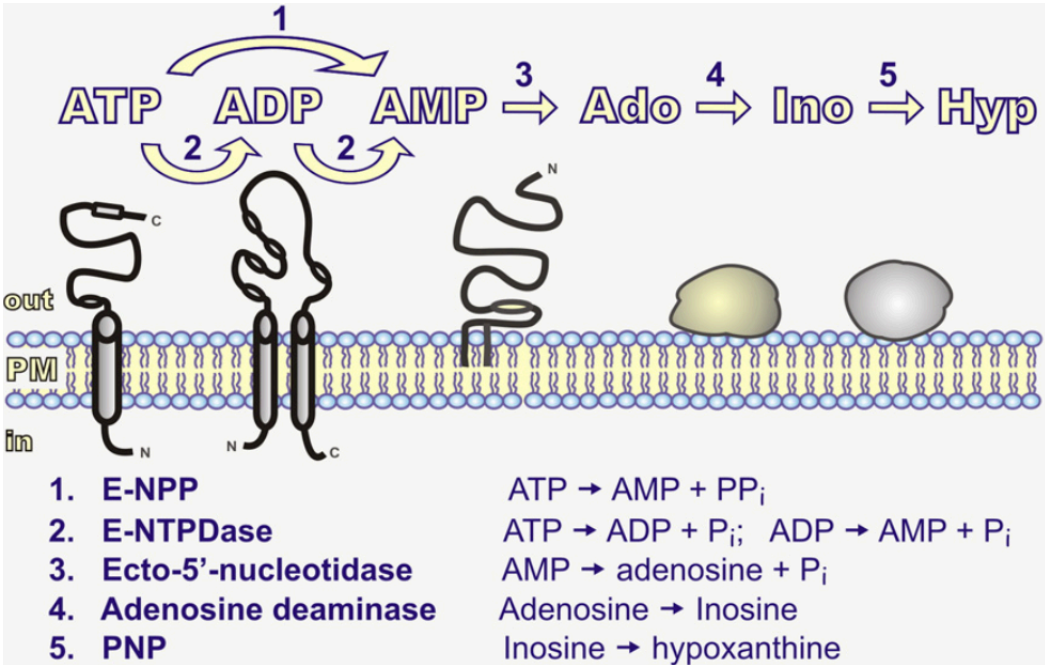


Figure 1.12: The metabolic hydrolysis cascade of extracellular nucleotides. The figure was taken from G. Yegutkin[150]

ecto-nucleotide pyrophosphatases/phosphodiesterases (*e*NPPs), ecto-nucleoside triphosphate diphosphohydrolases (*e*NTPDases), alkaline phosphatase (AP) and ecto-5'-nucleotidase (*e*N) are able to hydrolyse nucleotides yielding adenosine which can be further hydrolysed by ecto-adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) (Figure 1.12).

1.2.1 Ecto-nucleotide pyrophosphatases/phosphodiesterases (*e*NPPs)

The *e*NPP family consists of seven members numbered from NPP1-NNP7. Three members of this enzyme family are able to hydrolyze nucleotides: NPP1 (PC-1), NPP2 (autotaxin), and NPP3 (gp130RB13-6, B10, phosphodiesterase 1β). They are able to degradate nucleoside triphosphates (NTPs) directly to nucleoside monophosphates (NMPs).[151]

1.2.2 Ecto-nucleoside triphosphate diphosphohydrolases (*e*NTPDase)

The *e*NTPDases hydrolyse nucleoside tri- and diphosphates to the corresponding NMPs in the presence of Ca^{2+} or Mg^{2+} at physiological pH.[151] Only four out of the eight subtypes (NTPDase1 (CD39, ATPDase, ectoapyrase), NTPDase2 (CD39L1, ecto-ATPase), NTPDase3 (CD39L3, HB6), and NTPDase8 (hATPDase)) are located extracellularly and therefore function as ectonucleotidases. They differ in their substrate specificity: NTPDase2 is selective for NTPs, NTPDase3 and NTPDase8 have a preference for NTPs and NTPDase1 hydrolyses both NTPs and nucleoside diphosphates (NMPs) without any preference.[152]

1.2.3 Alkaline phosphatases

Alkaline phosphatases are able to hydrolyze ATP via ADP and adenosine 5'-monophosphate (AMP) to adenosine.[151]

1.2.4 Ecto-5'-nucleotidase (CD73)

The ecto-5'-nucleotidase (*e*N, CD73) hydrolyzes NMPs. The preferred enzymatic reaction is the degradation of 5'-AMP to adenosine.[153] *e*N is a Zn^{2+}-binding glycosylphosphatidylinositol (GPI)-anchored homodimeric protein. Adenosine, the product of the CD73 enzyme reaction, can activate P1-receptors (A_1, A_{2A}, A_{2B}, and A_3).[151]

*e*N was first isolated in 1987 by Grondal et al. from the electric organ of the electric ray Torpedo marmorata. They found that is has a Km value of 38 μM for AMP. The enzyme consists of an N-terminal domain, which contains the metal ion binding site, and a C-terminal domain, which contains the substrate binding site and to which the GPI anchor is linked (Figure 1.13. Besides the anchored form a soluble form of the enzyme exists.

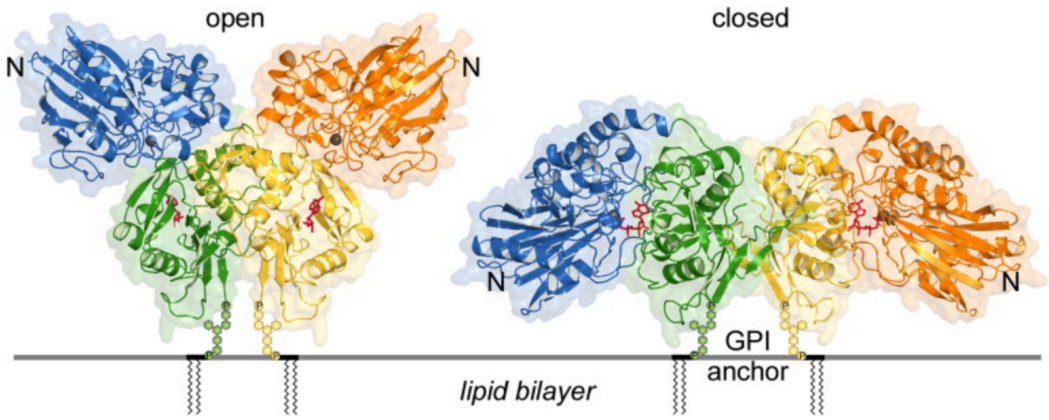


Figure 1.13: Crystal structures of ecto-5'-nucleotidase in the open form (with adenosine) and the closed form (with the inhibitor α,β-methylene-ADP). The picture was taken from Knapp et al.[154]

Human *e*N exists as a noncovalent homodimer which is connected via the C-terminal domains. Rotation of the N-terminal domain results in a change of the enzyme from the open to the closed form and vice versa. This domain motion determines the substrate selectivity of *e*N towards NMPs, and the inhibitory properties of NMPs and NTPs.[154]

1.2.4.1 Therapeutical potential of ecto-5'-nucleotidase

The physiological and pathological functions of *e*N expression in the human body are mostly related to adenosine formation which is regulated by the enzyme. Adenosine is able to activate P1-receptors, and an important mediator of the purinergic system. *e*N is expressed in various tissues and organs such as colon, liver,[155] kidney,[156] brain, heart, cardiovascular system, immune cells and lung.[151,157]

It is known to play a role in the context of immune responses. By hydrolysis of pro-inflammatory ATP to the anti-inflammatory adenosine milieu CD73 modulates the immune response. It is therefore a potential target for immune related diseases.[158]

The expression of *e*N by tumor cells and the immunosuppressive effects identified in last years caused great interest in *e*N as a target for anticancer therapy.[159] Mikhailov et al. found that *e*N is involved in the multiresistance of different cancer types. The apoptosis-resistant T cell leukemia clone (A4) showed a high CD73

surface expression and significant *e*N activity.[160] Stagg et al. also found that CD73 knock-out mice have a reduced growth of tumors and an increased antitumor immunity. Also metastasis was reduced in the CD73-deficient mice. This effects could also be observed when mice where treated with CD73 antibodies.[161,162] Yegutkin et al. treated mice with the known CD73 inhibitor α,β-methylene-ADP (AOPCP) which resulted in retarded tumor progression. They also showed that CD73-deficient mice have a higher immunity against tumors.[163] These effects could be explained because it is known that adenosine in contrast to ATP is a negative regulator of immune cells in protecting normal tissues from inflammatory damage. An activation of adenosine receptors in the tumor has the same effect, protecting the tumor from immune attack.[164] Taken together there is strong evidence that *e*N is a potential target for the immunotherapy of cancer.

1.2.4.2 Inhibitors of ecto-5'-nucleotidase

The first inhibitors that were identified for *e*N were the nucleotides ADP and ATP. They block the enzyme in the micromolar range.[151,152]

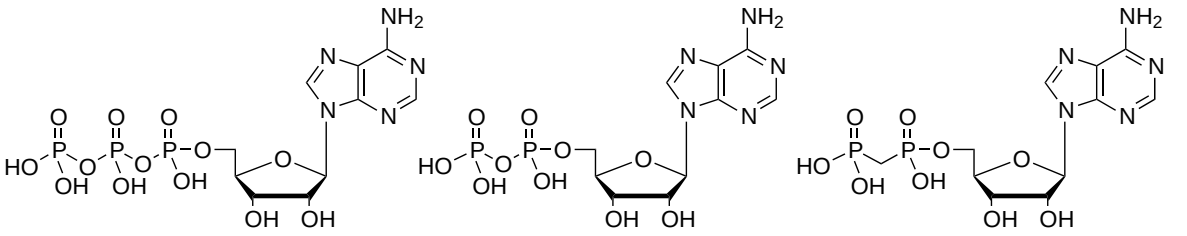


Figure 1.14: Selected inhibitors of ecto-5'-nucleotidase.

The ADP-analogue AOPCP was identified as a more potent *e*N inhibitor, with improved properties regarding stability compared to the endogenous inhibitors.[166] Besides this compound anthraquinones,[167] sulfonamides,[168] various polyphenols,[169] and some polyoxometalates (POMs)[170] were found to inhibit CD73. Recently the widely used inhibitor AOPCP was structurally optimized and a series of analogues with inhibitory potencies in the low nanomolar range have been developed in our group.[171]

2 Aims of the study

2.1 Development of potent and selective ligands for *orphan* G protein-coupled receptors

About 100 of the roughly 800 members of the human G protein-coupled receptor (GPCR) family are so-called *orphan* GPCRs whose endogenous ligand is not known or not confirmed yet. As the *orphan* receptors might qualify as drug targets there is an enormous potential in this poorly studied group of receptors. Our aim is to develop and optimize synthetic ligands for several promising *orphan* receptors of the δ-branch of class A GPCRs. Such ligands will be useful as pharmacological tools to study receptor functions and explore their potential application for the treatment of diseases. Our second aim is to optimize the compounds with regard to their physiochemical properties.

Funke et al. identified 8-benzamidochromen-4-one-2-carboxylic acids as potent and selective agonists for the human orthologue of the *orphan* receptor GPR35.[95] In this study we aim to further optimize the structure of the 8-benzamidochromen-4-one-2-carboxylic acids regarding their activity as GPR35 agonists. A limitation of the GPR35 agonists developed so far is their species selectivity for the human receptor. A second objective is to develop potent and selective agonists for the rodent orthologues of GPR35. Those compounds would be important tools to perform animal studies which can give insights into the functions of the receptor in vivo. Furthermore it will be interesting to determine structure-activity relationships (SARs) for the different orthologues to find out which structural elements are important for orthologue selectivity. This is a point that has been poorly investigated for GPR35 so far.

Some of the compounds, which were originally based on the structure of the cysteinylleukotriene receptor-1 antagonist pranlukast also showed weak activity at the

orphan receptors GPR84 and GPR17.[172] However the compounds are not very potent and are thereby not suitable as pharmacological tools to further study those *orphan* receptors. We plan to modify the structure of the moderately potent compounds to determine SARs and to develop more potent GPR17 and GPR84 antagonists.

Besides the optimization of the compounds regarding their potency at the different targets the selectivity of the compounds should be preserved or enhanced. Structure-activity relationships (SARs) have to be determined for each receptor, which will provide information about the shape of the binding pocket. We may also be able to gain information about structural elements of the endogenous agonist based on the structure-activity relationship (SAR) analysis.

Moreover, it will be of interest to test the chromen-4-ones for their activity at additional targets, like the GPR35-related GPR55 which is still lacking potent and selective ligands.

2.2 Biological evaluation of potent and selective ecto-5'-nucleotidase inhibitors

The objective of a second project is the biological evaluation of selected ecto-5'-nucleotidase (*e*N) inhibitors which were developed in our group.[171] They show inhibitory potencies in the nanomolar range at the rat enzyme but have not been tested at the human enzyme so far. We want to perform a comprehensive study with the most potent compounds using a broad range of techniques in several biological systems including the recombinant human enzyme, human blood serum, and different cell lines and tissues to further characterize the compounds. This will provide information on how the compounds will behave in vivo, for example in animal studies. We can further determine whether species differences exist, between the murine and the human enzyme.

3 Results and discussion

3.1 Synthesis of chromen-4-one-2-carboxylic acid derivatives

3.1.1 Introduction

Chromones represent privileged scaffolds for the synthesis of new drugs.[173] The cysteiny leukotriene receptor antagonist pranlukast, which contains the chromen-4-one core structure, was identified as a moderately potent GRP17 antagonist.[35] The compound was further modified by Mario Funke to increase its inhibitory potency at GPR17. This led to the discovery of agonists for the *orphan* receptor GPR35 based on the chromen-4-one structure.[95,172] Moreover 8-benzamidochromen-4-one-2-carboxylic acids with long lipophilic residues were found to be weak antagonists at the *orphan* receptor GPR84.[172] We therefore decided to develop new ligands based on this promising core structure for several class A *orphan*G protein-coupled receptors (GPCRs). A large number of modifications were realized at the chromen-4-one heterocycle and at various side-chains (Figure 3.1).

We mainly targeted 4 structural elements of the scaffold. Firstly the 5-, 6-, and 7-position of the chromen-4-one were modified. Here we mainly focused on the 6-position which was substituted with small groups including halogen atoms, but also with larger phenyl residues or longer alkyl chains. The second modification at the chromen-4-one core was the replacement of the oxygen atom at the 4-position by sulfur. At the 8-position we introduced many different side chains, from small carboxylic acids up to large lipophilic residues containing one or more phenyl rings, and alkyl chains of different length. Finally, the linker which connects the side chain to C8 of the chromen-4-one core was modified in several ways (see below).

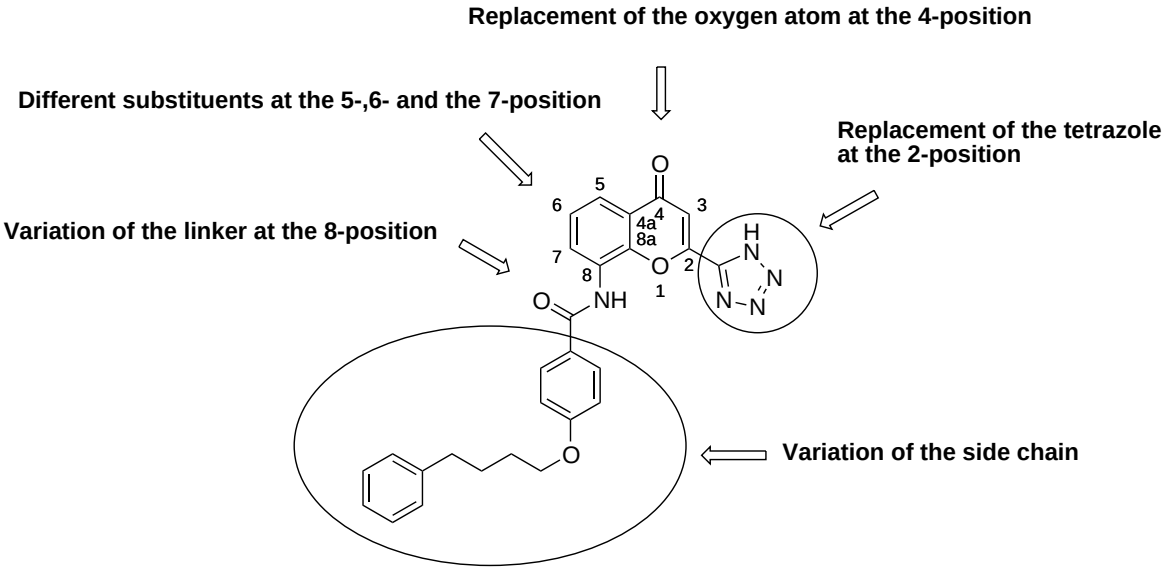
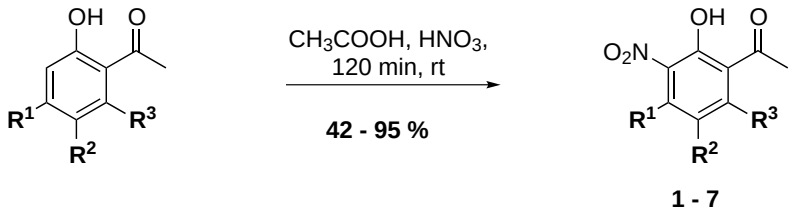


Figure 3.1: Structural modifications of pranlukast

3.1.2 Syntheses

3.1.2.1 Synthesis of ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylates

The preparation of ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylates began with the nitration of commercially available 2-hydroxyacetophenones with various substituents (R^1 - R^3) as shown in Scheme 3.1 based on a method from Burdeska.[174]

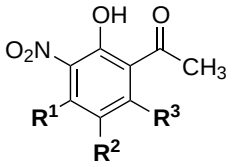


Scheme 3.1: Synthesis of 1-(2-hydroxy-3-nitrophenyl)ethanones. For **R^1 - R^3** see Table 3.1.

The starting material was dissolved in glacial acetic acid. Then a mixture of fuming nitric acid and glacial acetic acid was slowly added to the 2-hydroxyacetophenone and the reaction mixture was stirred for 120 min at room temperature (rt). After pouring it onto ice the product precipitated as yellow solid and could be isolated in good yields ranging from 42 - 95% as shown in Table 3.1.[174] Only mononitration at

the desired position 3 was observed.

Table 3.1: Yields of 1-(2-hydroxy-3-nitrophenyl)ethanones



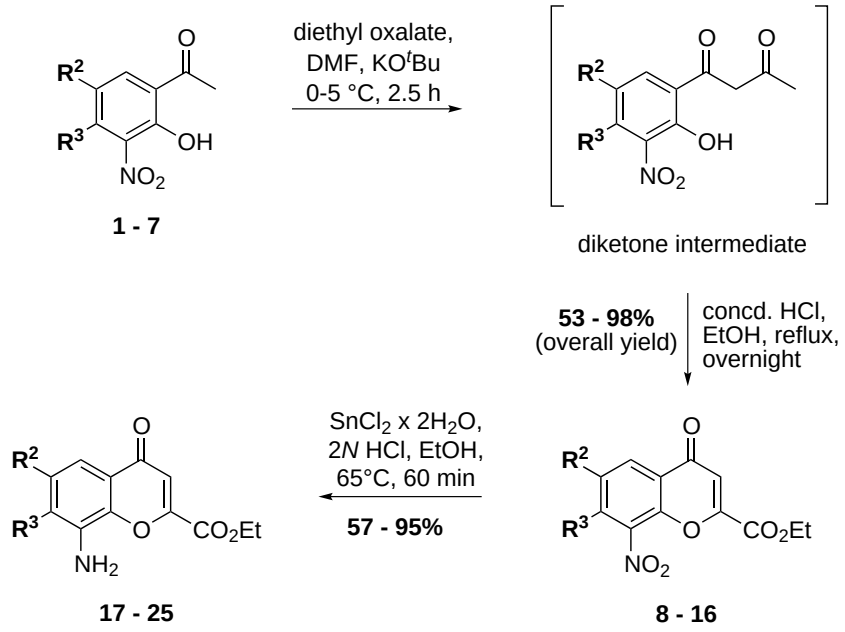
compound	R^1	R^2	R^3	yield [%]	mp (lit. mp) [°C]	purity[a] [%]
1[175]	H	OCH_3	H	62	(112-114)	100.0
2	H	OCF_3	H	42	87-88	100.0
3[176]	H	ethyl	H	92	(124-126)	92.7
4[176]	H	F	H	50	(93-94)	99.4
5	H	Br	methyl	95	163-164	80.0
6[177]	methyl	methyl	H	84	(143-144)	87.0
7	H	O-TBDMS	H	62	87-89	74.0

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

The ring closure was performed by a modified Claisen condensation which was recently developed in our group.[95] Therefore compounds **1** - **7** were reacted with diethyl oxalate and potassium *tert*-butoxide in dry dichlormethane (DMF) under an argon atmosphere to get the diketone, as shown in Scheme 3.2. Since the diketone is not stable it was immediately reacted with concd. HCl in EtOH without further purification to perform the ring closure with an overall yield from 53-98%.

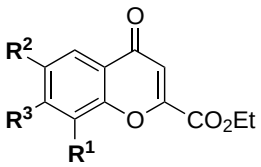
All in all we synthesized 9 chromen-4-ones. The ring closure did not work for compound **7** which has a *tert*-butyldimethylsilyl (TBDMS) protected hydroxy group at the 5-position, probably because of steric hinderance.

The next step was the reduction of the nitro group to the corresponding amine. Since the reduction with H_2 and Pd/C resulted in a debromination at position 6 of the chromen-4-one it was decided to use $SnCl_2$ x 2 H_2O and 2*N* HCl in EtOH for reduction.[178] The amine could be isolated in good yields ranging from 57 - 95 % and with high purities (Table 3.2).



Scheme 3.2: Synthesis of ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylates. For **R^2 and R^3** see Table 3.2.

Table 3.2: Yields of ethyl 4-oxo-4*H*-chromene-2-carboxylates



substitution pattern		$R^1 = NO_2$				$R^1 = NH_2$			
R^2	**R^3**	**compd.**	**yield** [%]	**mp** (lit. mp) [°C]	**purity**[a] [%]	**compd.**	**yield** [%]	**mp** (lit. mp) [°C]	**purity**[a] [%]
Br	H	**8**[179]	93	(141-142)	95.3	**17**[180]	95	(161-162)	99.4
OCF_3	H	**9**	53	120-121	91.3	**18**	57	100-102	90.0
H	H	**10**[32]	89	(122-123)	99.0	**19**[181]	91	(153-154)	100.0
ethyl	H	**11**	65	116-117	90.1	**20**	76	132-133	98.2
F	H	**12**[95]	68	(112-113)	90.2	**21**[95]	60	(164-165)	98.8
Cl	H	**13**[182]	98	(126-127)	85.6	**22**[95]	62	(150-151)	99.2
methyl	H	**14**[183]	69	(148-151)	95.4	**23**[183]	91	(127-132)	98.0
OCH_3	H	**15**[65]	78	(175-176)	98.4	**24**[184]	95	(144-145)	98.5
Br	methyl	**16**	63	204-205	91.5	**25**	92	164-166	97.5

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

3.1.2.2 Modifications of the ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylates

The amine **17** (see Scheme 3.2 and Table 3.2) was the key intermediate for further modifications at the 6-position of the chromen-4-one. Especially for the receptors GPR35 and GPR84 it seemed that there was a lot of space in the binding pocket at this position since a large halogen atom like bromine was beneficial for the activity of the compounds. Thus we decided to introduce a phenyl substituent at that position.

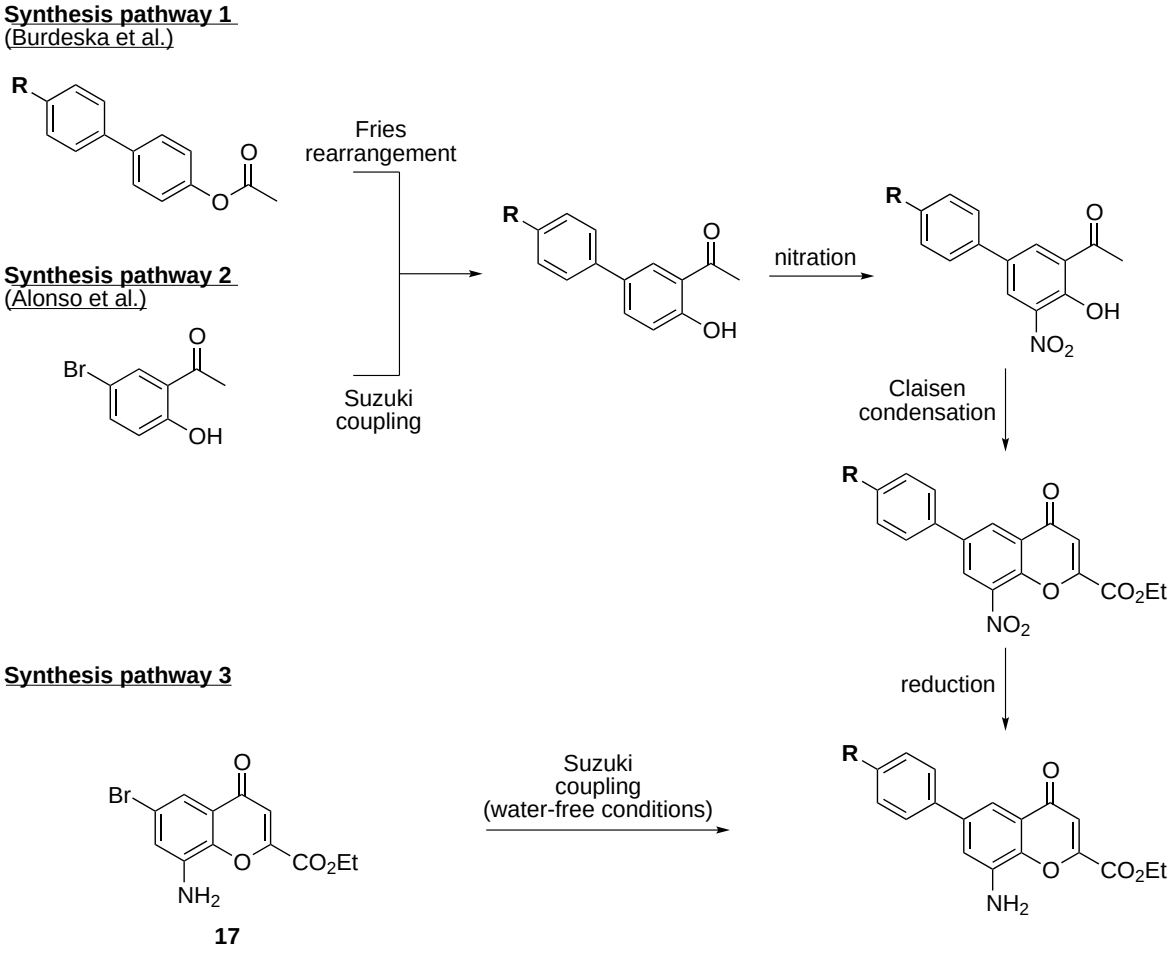
In the literature some chromen-4-ones with a phenyl substituent at the 6-position were described by Witiak et. al.[185] The synthesis starts with the biphenylacetophenone which is reacted in a Claisen condensation with diethyl oxalate to the corresponding ethyl 4-oxo-6-phenyl-4*H*-chromene-2-carboxylate (Scheme 3.3).

Scheme 3.3: Synthesis of ethyl 4-oxo-6-phenyl-4*H*-chromene-2-carboxylates by Witiak et. al.[185]

Ethyl 4-oxo-6-phenyl-4*H*-chromene-2-carboxylates with a nitro group at position 8 are not described yet in the literature. A nitration of the 8-position of the chromen-4-one after the Claisen condensation carries the risk of many undesired by-products and as a consequence tedious purification steps and low yields. It was therefore necessary to introduce the nitro group before the ring closure.

In the literature two pathways are described for the synthesis of ethyl 8-nitro-4-oxo-6-phenyl-4*H*-chromene-2-carboxylates (Scheme 3.4). Burdeska et al. started with a Fries rearrangement of the [1,1'-biphenyl]-4-yl acetate to get the 1-(4-hydroxy-[1,1'-biphenyl]-3-yl)ethanone (Scheme 3.4 - pathway 1).[174] However the Fries rearrangement reaction entails many disadvantages: the reaction is catalyzed by strong Lewis acids such as $AlCl_3$, which are used in excess of the stoichiometric amount. Those catalysts are hazardous, corrosive and react violently with water which makes them

inconvenient. Secondly the Fries rearrangement does not work with many substituted starting compounds which makes it unsuitable for the synthesis of more complex structures. Alonso et al. started from 1-(5-bromo-2-hydroxyphenyl)ethanone reacting it via Suzuki coupling to the 1-(4-hydroxy-[1,1'-biphenyl]-3-yl)ethanone (Scheme 3.4 - pathway 2).[186]

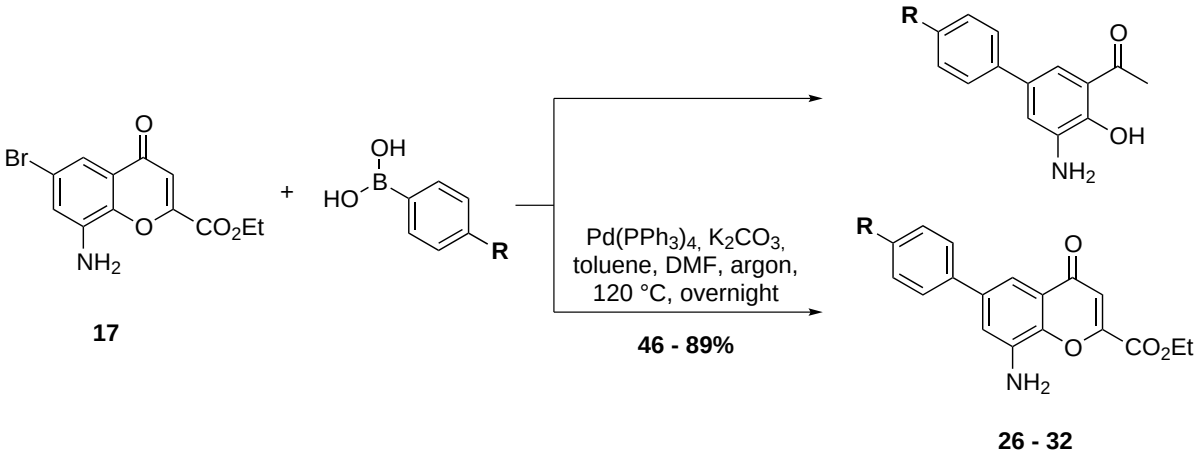


Scheme 3.4: Strategies for the introduction of phenyl substituents at the 6-position of the chromen-4-one scaffold.

This method is much better suitable since it avoids the use of hazardous chemicals and has no limitations regarding highly substituted starting compounds. This could represent a suitable method for our purpose if we would start from 1-(5-bromo-2-hydroxy-3-nitrophenyl)ethanone. However what both methods have in common is

the large number of steps in case of introducing a larger number of phenyl substituents with various substitution patterns. Due to the fact that the phenyl substituent has to be coupled at such an early stage we would always have to run the whole linear synthesis route for each product which is not efficient.

We therefore decided to develop a method which allows to perform the Suzuki coupling reaction of compound **17** which could easily be synthesized in a large scale of up to 15 mmol per reaction (Scheme 3.4 - pathway 3). Another advantage is the good solubility of compound **17** due to the amino group compared to derivatives with a nitro group. We started with a standard procedure for Suzuki coupling using palladium acetate/triphenylphosphine as a catalyst in EtOH containing 2 M aquaous (aq.) Na_2CO_3 solution.[187]



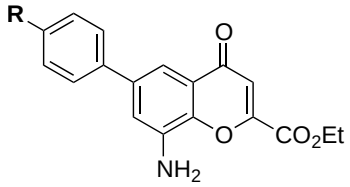
Scheme 3.5: Suzuki coupling with ethyl 8-amino-6-bromo-4-oxo-4*H*-chromene-2-carboxylate. For **R** see Table 3.3

The mixture was then refluxed under an argon atmosphere for 1 hour. The problem that occurred was a hydrolysis of the chromen-4-one ring due to the basic aqueous conditions. We therefore changed the solvent to toluene containing a small amount of DMF, which increases the solubility of **17** significantly. The best catalyst in the new solvent turned out to be tetrakis(triphenylphosphine)palladium(0) in combination with K_2CO_3 as a base. To increase the yield we degassed the reaction mixture for 5 min in an ultrasonic bath to remove all oxygen which would react with the catalyst. The new method worked fine in the microwave oven (150 °C, 45 min, 120 Watt, 6 bar) but also in an ACE® pressure tube (120 °C, overnight) in larger batches.

The product could not be separated from the starting material on a column of silica gel because of similar polarities of starting material and product. Therefore it was absolutely necessary to run the reaction until 100% of the starting material was converted.

We successfully developed an efficient method to introduce different phenyl substituents into the hydrogenolytically unstable chromen-4-ones. It allowed us to synthesize a set of compounds with differently substituted phenyl residues at the 6-position which represent important intermediates.

Table 3.3: Yields of ethyl 4-oxo-6-phenyl-4*H*-chromene-2-carboxylates



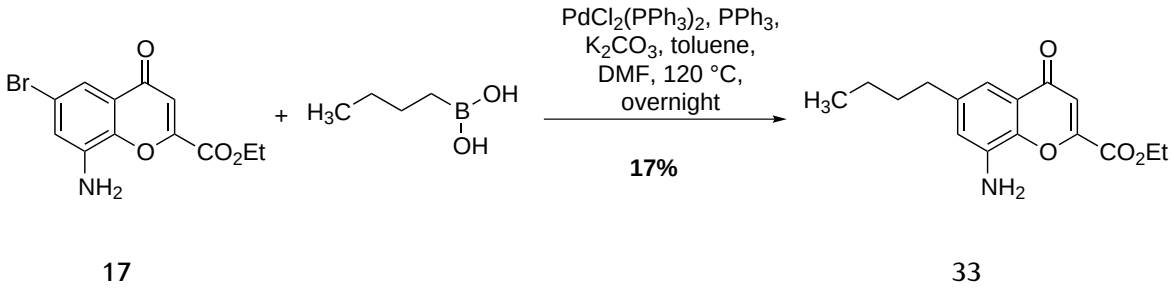
compound	R	yield [%]	mp [°C]	purity[a] [%]
26	H	89	138-139	99.1
27	Cl	46	183-184	94.8
28	methyl	59	127-128	97.1
29	OCH_3	75	162-163	95.3
30	F	74	176-177	98.3
31	O-TBDMS[b]	64	155-157	97.7
32	N-Boc	60	222-223	99.5

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

[b] O-TBDMS = *tert*-butyldimethylsilyl

We were also interested in the introduction of alkyl substituents at the 6-position of the chromen-4-one. Methyl- or ethyl-substituted acetophenones were commercially available, but for longer alkyl substituents like butyl, which could be interesting for obtaining GPR35 agonists, we had to find a synthetic method. In the literature alkylated acetophenones are described to be synthesized by acetylation followed by the Fries rearrangement reaction. The disadvantages of the Fries rearrangement reaction have already been discussed, and it was pointed out that this common way was not an option for our purposes. In the literature there are also procedures described for Suzuki coupling reactions with alkylboronic acids, so we decided to

optimize our method with regard to the coupling of butylboronic acid. We found that the reaction worked with a mixture of $PdCl_2(PPh_3)_2$ and triphenylphosphine as a catalyst. However the yield was very low. Nevertheless we could isolate sufficient amounts of product **33** for the next reaction steps.

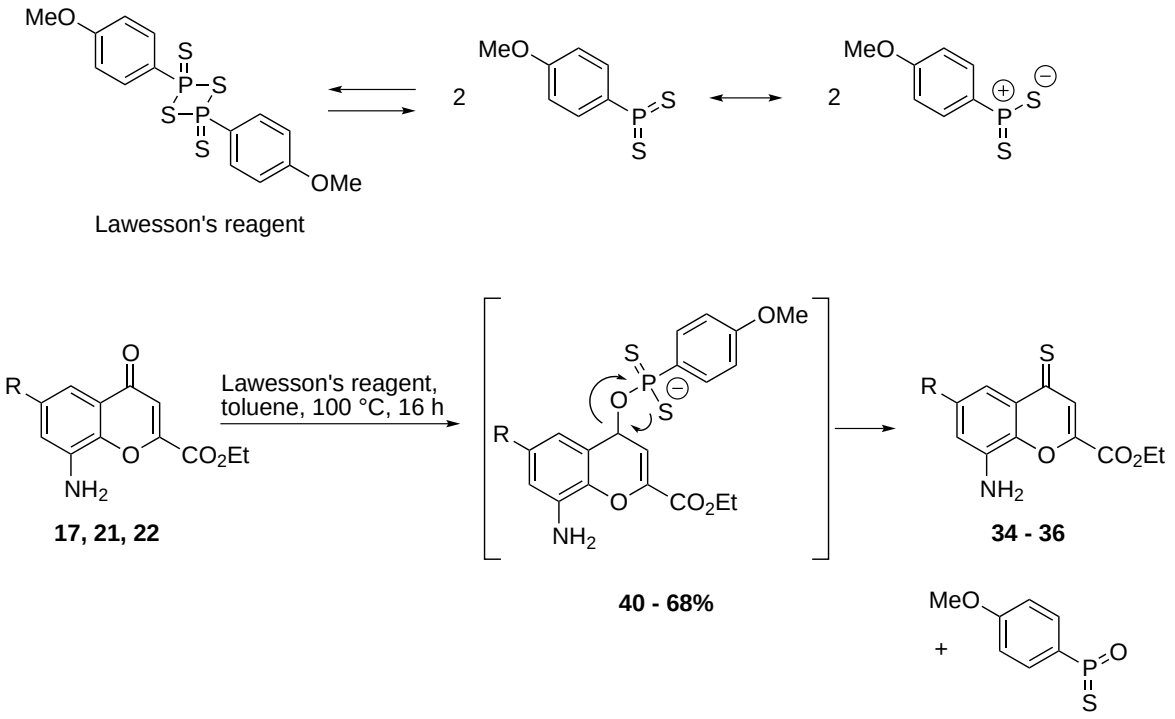


17 **33**

Scheme 3.6: Suzuki coupling with butylboronic acid.

Using the new Suzuki coupling method with butylboronic acid we successfully synthesized ethyl 8-amino-6-butyl-4-oxo-4*H*-chromene-2-carboxylate **33** which is an important intermediate for the development of GPR35 agonists.

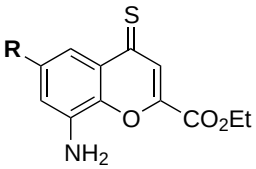
For the determination of further structure-activity relationships (SARs) we replaced the ketone function in the 4-position of chromen-4-one by a thioketone. Lawesson's reagent[188] was used, which is a commercially available, mild and effective reagent for the thioation of ketones. For the reaction, the amines **17**, **21** and **22** were dissolved in toluene, and Lawesson's reagent was added to the mixture which was heated at 100 °C for 16 hours. The mechanism is similar to a Wittig reaction (Scheme 3.7).



Scheme 3.7: Synthesis of ethyl 4-thioxo-4*H*-chromene-2-carboxylates using Lawesson's reagent. For **R** see Table 3.4.

We successfully synthesized three different ethyl 4-thioxo-4*H*-chromene-2-carboxylates in satisfactory yields of 40-68% (Table 3.4).

Table 3.4: Yields of ethyl 4-thioxo-4H-chromene-2-carboxylates

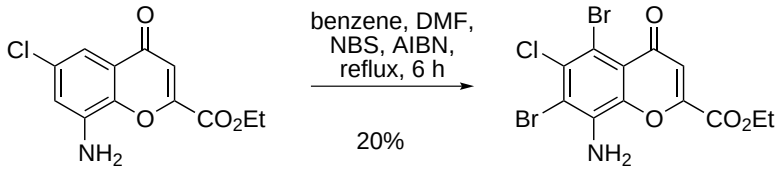


compound	R	yield [%]	mp [°C]	purity[a] [%]
34	Br	63	176-177	84.9
35	Cl	40	190-192	98.1
36	F	68	175-177	84.0

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

The commercial availability of acetophenones with substitutents at the 4- and 6-position is limited because their synthesis can be complex due to directing effects of

other substituents. We therefore introduced bromine atoms at the 5- and 7-position of the chromen-4-ones which could be further modified. Compound **37** was dissolved in benzene and DMF and after the addition of *N*-bromosuccinimide (NBS) and a catalytic amount of azobisisobutyronitrile (AIBN), it was heated to reflux for 6 h (Scheme 3.8).

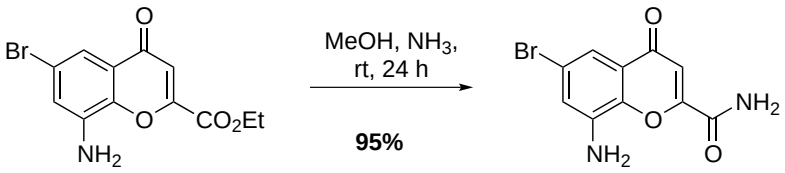


37 **38**

Scheme 3.8: Bromination of **37** with NBS/AIBN

Compound **38** was isolated only in a low yield and was found to display an extremely low solubility which made further modifications impossible.

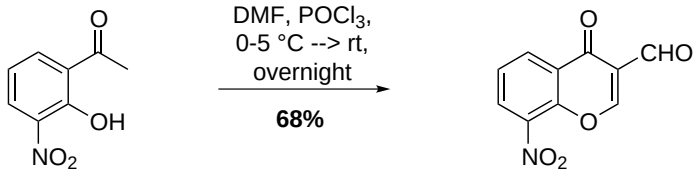
The same problem of low solubility occurred when the ethyl ester at the 2-position of **17** was reacted to a carboxyamide by dissolving it in MeOH and adding ammonia solution.[189] The product **39** could be isolated in an excellent yield of 95% but was practically insoluble in all common solvents (Scheme 3.9).



17 **39**

Scheme 3.9: Conversion of the ethyl ester at position 2 to a carboxamide.

We also synthesized a chromen-4-one with an aldehyde at position 3. Therefore we reacted 1-(2-hydroxy-3-nitrophenyl)ethanone with $POCl_3$ in DMF in a Vilsmeier-Haack reaction.[190] We isolated the product **40** in a good yield of 68% (Scheme 3.10). However it was not possible to reduce the nitro group because the aldehyde was insoluble in all common solvents.



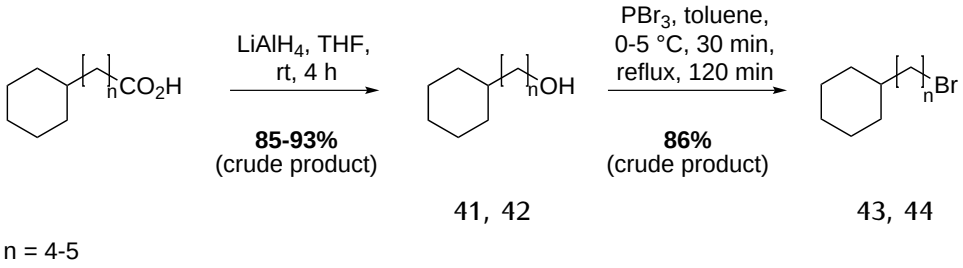
40

Scheme 3.10: Synthesis of 8-nitro-4-oxo-4*H*-chromene-3-carbaldehyde.

Compound **17** turned out to be a perfect intermediate for structural modifications of the chromen-4-one core structure. It shows a good solubility in contrast to the nitro analogue, which is an essential property for most reactions. Furthermore it can be synthesized on large scale which avoids multiple reaction steps. However it is hydrogenolytically unstable under basic conditions, so it is absolutely required to work under dry conditions. By developing new methods it was possible to introduce phenyl and alkyl residues at position 6 of the chromen-4-one, and the ketone at position 4 was replaced by a thioketone. Those compounds represent important intermediates for the development of ligands for the targeted *orphan* GPCRs. However, the introduction of bromine at position 5 and 7 as well as the replacement of the ester at position 2 by an amide and the introduction of an aldehyde at position 3 resulted in compounds with extremely low solubility which could not be further reacted.

3.1.2.3 Synthesis of benzoic acid derivatives

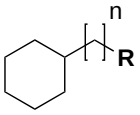
For the synthesis of precursors for side-chains to be introduced in position 8 we started from the commercially available *N*-alkylcarboxylic acids. In the first step they were reduced with $LiAlH_4$ in tetrahydrofurane (THF) to the corresponding alcohols.[191] The alcohol was subsequently brominated without further purification to the alkyl bromide using PBr_3 (Scheme 3.11).[192]



Scheme 3.11: Synthesis of alkyl bromides.

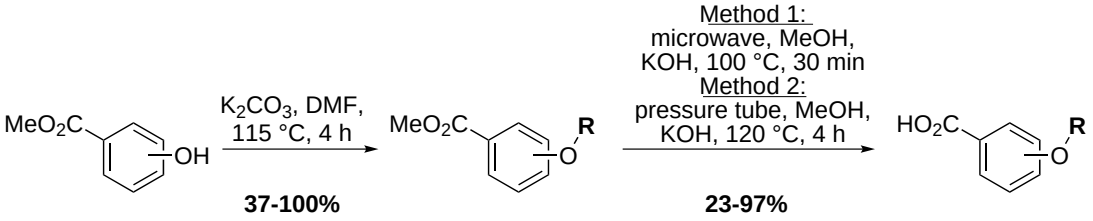
Alkyl bromides **43** and **42** (Table 3.5) were used without further purification for the next reaction steps.

Table 3.5: Yields of *n*-alkyl alcohols and *n*-alkyl bromides



	R = OH		R = Br	
n	**compound**	**yield [%]**	**compound**	**yield [%]**
5	**41**[193]	93	**43**[193]	86
4	**42**[193]	85	**44**[193]	86

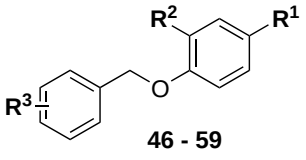
In the next reaction step the alkyl bromides were coupled with methyl hydroxybenzoates via a Williamson ether synthesis. This was performed using K_2CO_3 as a base in DMF as a solvent.[194] The products could be mostly isolated in excellent yields of higher than 80% except for compound **45** of which only 37% were formed. This could be due to impurities in the alkyl bromide which was used without any prior purification.



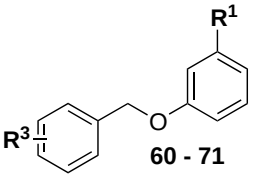
Scheme 3.12: Synthesis of benzyloxybenzoic acids and alkyloxybenzoic acids. For structures see Table 3.6 and Table 3.7.

Subsequently the protecting methyl ester was hydrolyzed yielding the desired carboxylic acid. We started to perform the reaction in the microwave oven for obvious reasons. The microwave allows an optimal energy transfer which results in short reaction times. However a big disadvantage for our purpose was, that the volume of the microwave tube was limited. We needed large amounts of benzoic acid intermediates because they should be coupled to different chromen-4-ones.

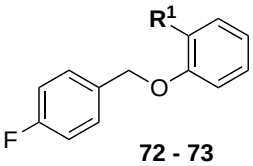
Table 3.6: Yields of methyl (benzyloxy)benzoates and (benzyloxy)benzoic acids



46 - 59



60 - 71



72 - 73

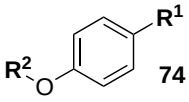
substitution pattern			$R^1 = CO_2Me$		$R^1 = CO_2H$	
R^2	R^3		compound	yield	compound	yield
	meta	*para*		[%]		[%]
H	H	F	**46**[195]	88	**47**[196]	85
H	F	F	**48**[197]	89	**49**	96
Cl	H	F	**50**[197]	86	**51**	96
Cl	F	F	**52**[198]	88	**53**[199]	92
F	H	F	**54**	92	**55**[200]	86
OCH_3	H	F	**56**[201]	90	**57**	93
Br	H	F	**58**	91	**59**	90
-	H	F	**60**[202]	86	**61**[196]	86
-	H	Br	**62**[203]	87	**63**[196]	89
-	H	Cl	**64**[204]	82	**65**[196]	94
-	Br	H	**66**	97	**67**	72
-	H	methyl	**68**	88	**69**[205]	95
-	H	H	**70**[206]	91	**71**[196]	82
			72[207]	85	**73**[196]	97

*For melting points see **chapter 5**.

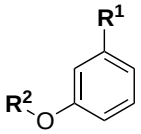
We therefore modified our method by changing the reaction device from the mircowave to a pressure tube which still allowed us to perform the reaction under pressure resulting in short reaction times. With this method we were able to hydrolyze an amount of 7 mmol instead of 2 mmol (by microwave) in one reaction. Although the reaction time increased from 30 min in the microwave up to 4 h in the pressure tube,

it was still time-saving with regard to the overall yield. The yields for both methods were comparable and mostly above 90% (Table 3.6 and Table 3.7).

Table 3.7: Yields of alkoxybenzoates and alkoxybenzoic acids



74 - 79



45, 80 - 102

R^2	$R^1 = CO_2Me$			$R^1 = CO_2H$		
	compound	**yield [%]**	**purity[a] [%]**	**compound**	**yield [%]**	**purity[a] [%]**
5-phenylpentyl	**74**[208]	92	97.7	**75**[32]	23	98.0
4-cyclohexylbutyl	**76**	77	99.1	**77**	91	100.0
5-cyclohexylpentyl	**78**	47	100.0	**79**	60	99.7
2-phenylethyl	**80**	64	97.0	**81**[196]	90	99.8
3-phenylpropyl	**82**	79	92.0	**83**	84	98.5
4-phenylbutyl	**84**	90	78.5	**85**[209]	23	98.0
5-phenylpentyl	**86**	88	90.5	**87**	86	100.0
n-hexyl	**88**[210]	67	98.2	**89**[196]	73	100.0
n-heptyl	**90**	64	63.4	**91**[196]	84	99.5
n-octyl	**92**	87	93.3	**93**[196]	72	99.0
1-cyclohexylmethyl	**94**[211]	46	98.8	**95**[212]	91	100.0
2-cyclohexylethyl	**96**	90	95.9	**97**[196]	82	99.6
3-cyclohexylpropyl	**98**	53	98.0	**99**	94	97.0
4-cyclohexylbutyl	**100**	58	94.9	**101**	63	100.0
5-cyclohexylpentyl	**45**	37	95.4	**102**	97	99.1

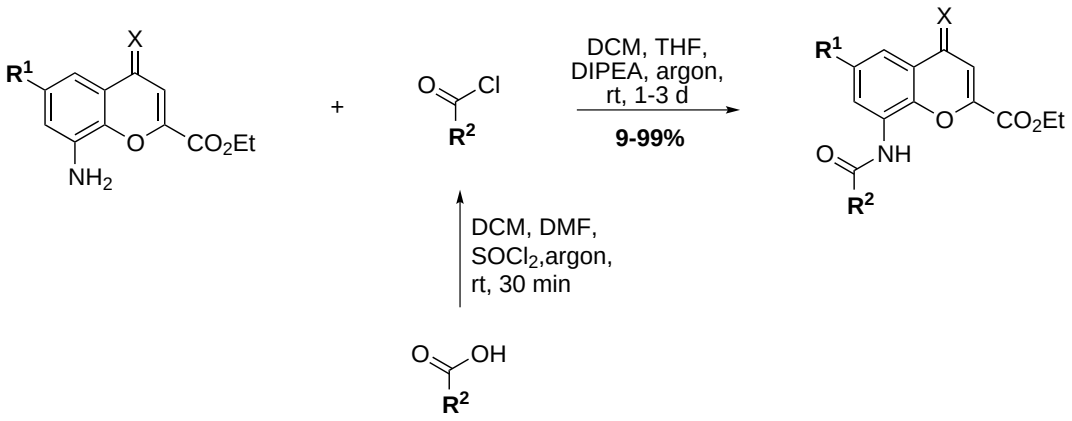
[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

*For melting points see **chapter 5**.

We synthesized 29 benzoic acid derivatives which have structural similarities to fatty acids because of their lipophilic side chains. They can now on the one hand be coupled with amines, but on the other hand they may also be tested as potential ligands for fatty acid receptors.

3.1.2.4 Synthesis of ethyl 8-benzamido-4-oxo-4*H*-chromene-2-carboxylates

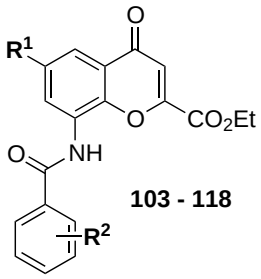
For the synthesis of the final compounds we coupled the amines with benzoic acid derivatives via an amide coupling reaction. We started by synthesizing the acid chlorides using thionyl chloride in dichloromethane (DCM) which was then directly reacted with the amine in a mixture of DCM, THF and *N*,*N*-diisopropylethylamine (DIPEA) to form the amide (Scheme 3.13).[95]



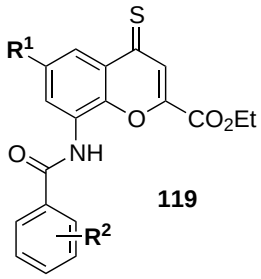
Scheme 3.13: Amide coupling reaction. For exact structures see Table 3.8, Table 3.9, Table 3.10 and Table 3.11.

The yield varied from 9 to 99% but was mostly within a good range of at least 50%. We coupled a broad range of benzoic acid derivatives with the amines **17** - **36** and finally isolated 73 amide derivatives (Table 3.8 - Table 3.11).

Table 3.8: Yields of ethyl 8-benzamido-4-oxo-4*H*-chromene-2-carboxylates



103 - 118



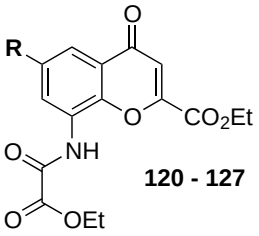
119

compound	**R^1**	**substitution pattern R^2**			**yield**	**mp** (lit.mp)	**purity**[a]
		ortho	*meta*	*para*	[%]	[°C]	[%]
103[95]	Br	H	H	OCH_3	78	(251-252)	100.0
104[65]	Br	F	H	OCH_3	64	(216-217)	100.0

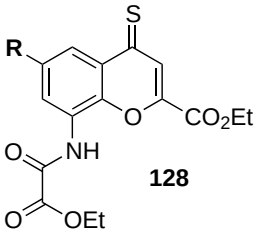
105[65]	Br	di-F	H	OCH_3	51	(262-263)	98.6
106[65]	Br	H	F	OCH_3	74	(251-252)	97.2
107[65]	methyl	H	H	OCH_3	81	(219-220)	98.2
108[65]	OCH_3	H	H	OCH_3	85	(217-218)	98.9
109	Br	OCH_3	H	OCH_3	69	284-285	99.5
110	OCF_3	H	H	OCH_3	17	176-177	100.0
111	phenyl	H	H	OCH_3	95	237-238	100.0
112	ethyl	H	H	OCH_3	90	168-170	97.9
113	Br	H	H	ethyl	91	208-210	95.9
114	*p*-chloro-phenyl	H	H	OCH_3	86	250-251	96.4
115	Br	H	H	SCH_3	50	231-232	99.9
116	phenyl	di-F	H	OCH_3	55	210-211	99.0
117	H	H	H	CO_2Et	80	201-202	99.0
118	Br	H	H	CO_2Et	88	207-208	96.0
119	Br	H	H	OCH_3	47	231-232	85.9

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

Table 3.9: Yields of ethyl 8-(2-ethoxy-2-oxoacetamido)-4-oxo-4*H*-chromene-2-carboxylates



120 - 127



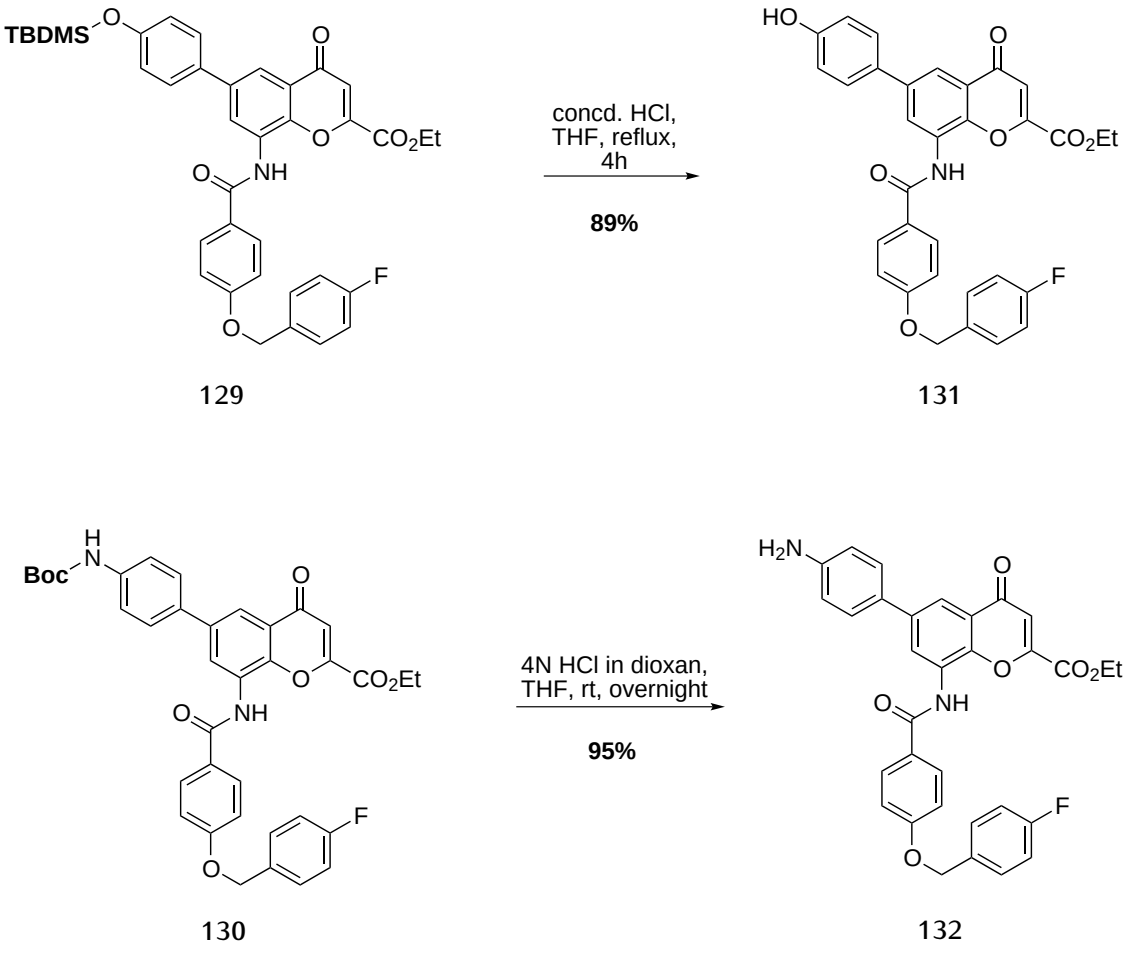
128

compound	R	yield [%]	mp [°C]	purity[a] [%]
120	Br	73	172-173	98.7
121	H	99	174-175	97.6
122	phenyl	77	158-159	99.8
123	F	74	164-166	99.3
124	Cl	23	148-150	99.4
125	methoxy	23	147-149	99.8
126	methyl	39	133-135	97.6
127	ethyl	39	133-135	99.4

128	Br	29	171-173	91.6

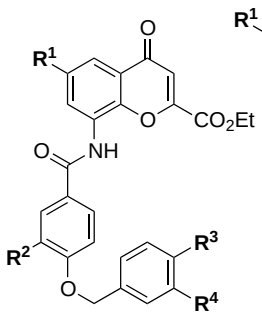
[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

Compound **129** and compound **130** were protected with TBDMS and *tert*-butyloxycarbonyl (Boc), respectively. After the amide coupling reaction we therefore had to remove the protecting groups. The TBDMS was cleaved by refluxing the compound **129** with concd. HCl in THF, and product **131** could be isolated in a good yield of 89%. The Boc group was removed by stirring compound **130** with solution of 4*N* HCl in dioxane in THF as a solvent and we isolated the amine **132** in an excellent yield of 95% (Scheme 3.14).

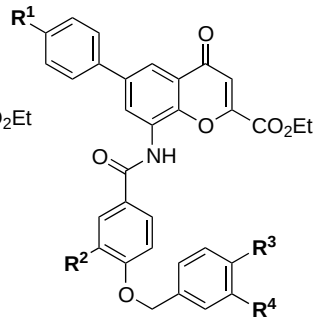


Scheme 3.14: Removal of the protecting groups TBDMS and Boc.

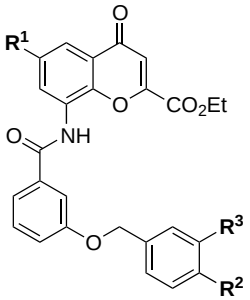
Table 3.10: Yields of ethyl 8-((benzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylates



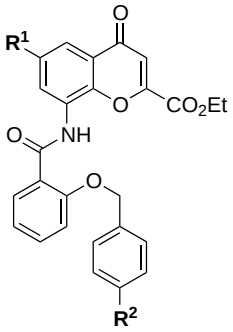
133 - 135



129 - 132, 136 - 145



146 - 152



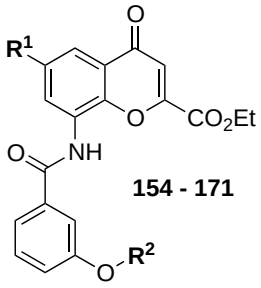
153

compound	R^1	R^2	R^3	R^4	yield [%]	mp [°C]	purity[a] [%]
133	OCH_3	H	F	H	79	253-254	100.0
134	methyl	H	F	H	78	234-235	63.0
135	ethyl	H	F	H	73	210-211	97.4
136	H	H	F	H	91	246-247	86.3
137	H	H	F	F	69	215-218	63.9
138	Cl	H	F	H	85	247-248	93.6
139	H	Cl	F	H	45	242-243	99.3
140	F	H	F	H	62	219-220	93.3
141	OCH_3	H	F	H	75	225-226	99.5
142	methyl	H	F	H	82	223-224	87.4
143	F	H	F	F	55	223-224	98.1
144	F	Cl	F	F	75	210-212	88.6
129	O-TBDMS	H	F	H	82	172-173	83.7
145	H	F	F	H	72	249-250	89.0
130	N-Boc	H	F	H	72	232-233	96.9
131	OH	H	F	H	89	291-292	89.3
132	NH_2	H	F	H	95	215-216	99.1
146	Br	F	-	-	35	186-187	100.0
147	Br	Br	-	-	46	195-196	99.3
148	Br	Cl	-	-	33	186-187	100.0
149	H	methyl	H	-	36	196-198	98.5
150	Br	methyl	H	-	14	194-196	95.8

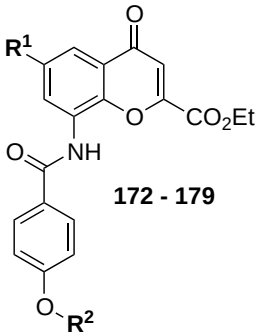
151	H	H	Br	-	63	175-176	100.0
152	Br	H	Br	-	74	198-200	96.5
153	Br	F	-	-	56	190-191	100.0

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

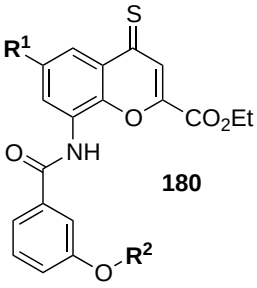
Table 3.11: Yields of ethyl 4-oxo-8-((alkylloxy)benzamido)-4*H*-chromene-2-carboxylates



154 - 171



172 - 179



180

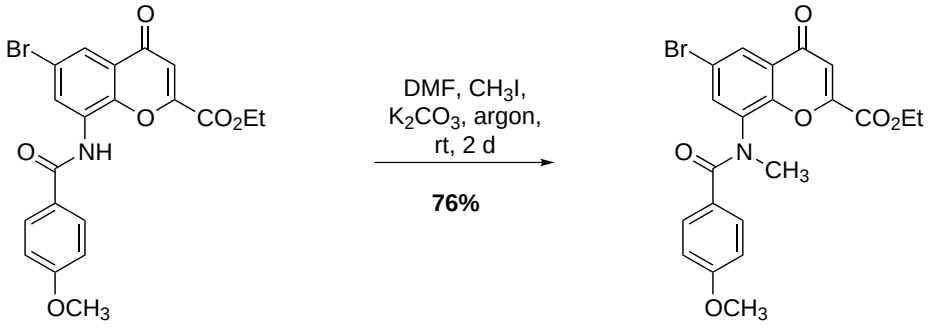
compound	**R^1**	**R^2**	**yield [%]**	**mp [°C]**	**purity[a] [%]**
154	H	1-cyclohexylmethyl	86	199-201	99.2
155	H	2-cyclohexylethyl	78	150-151	100.0
156	H	3-cyclohexylpropyl	48	129-130	85.1
157	Cl	3-cyclohexylpropyl	58	129-130	95.2
158	F	3-cyclohexylpropyl	53	147-148	98.7
159	H	4-cyclohexylbutyl	61	148-149	98.1
160	Cl	4-cyclohexylbutyl	48	147-148	94.2
161	F	4-cyclohexylbutyl	48	147-148	93.1
162	H	5-cyclohexylpentyl	85	144-145	99.4
163	Cl	5-cyclohexylpentyl	72	148-149	100.0
164	F	5-cyclohexylpentyl	63	148-149	99.6
165	Cl	hexyl	9	149-150	93.9
166	Cl	heptyl	54	153-155	97.0
165	Cl	octyl	70	151-153	97.5
167	H	1-phenylmethyl	67	195-197	99.7
168	H	2-phenylethyl	49	168-169	99.5
169	H	3-phenylpropyl	41	157-159	98.9
170	H	4-phenylbutyl	94	136-137	92.0
171	H	5-phenylpentyl	37	137-138	100.0

172	Br	3-cyclohexylpropyl	37	268-271	98.4
173	OCH_3	4-cyclohexylbutyl	40	174-175	100.0
174	methyl	4-cyclohexylbutyl	64	165-166	92.0
175	Cl	4-cyclohexylbutyl	50	62-163	99.6
176	F	4-cyclohexylbutyl	60	151-152	99.1
177	ethyl	4-cyclohexylbutyl	86	147-148	99.3
178	H	5-cyclohexylpentyl	60	186-188	100.0
179	Br	5-phenylpentyl	44	176-177	94.7
180	F	3-cyclohexylpropyl	30	145-147	78.0

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

3.1.2.5 Modification of the amide linker

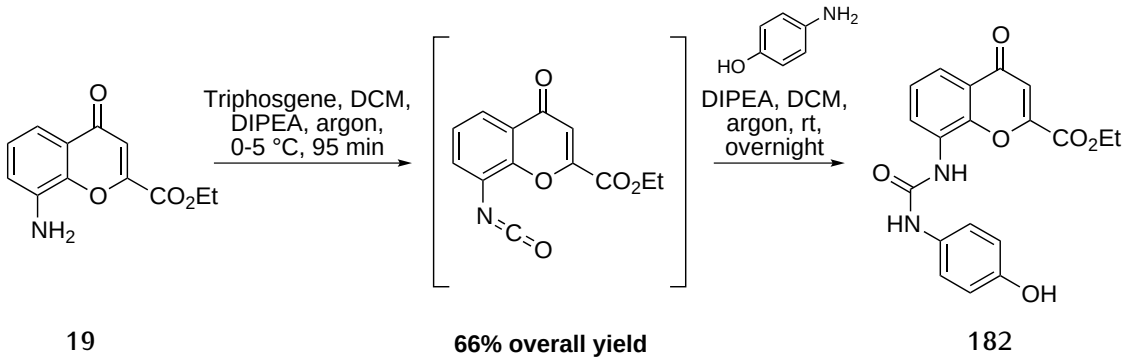
To reach a higher diversity we also modified the amide linker for some selected compounds. The first idea was to methylate the amide linker to determine wether removing the proton has any effect on the potency of the compounds. We chose the common way for methylation using methyl iodide in DMF and K_2CO_3 as a base and isolated compound **181** in a good yield of 76% (Scheme 3.15).



103 **181**

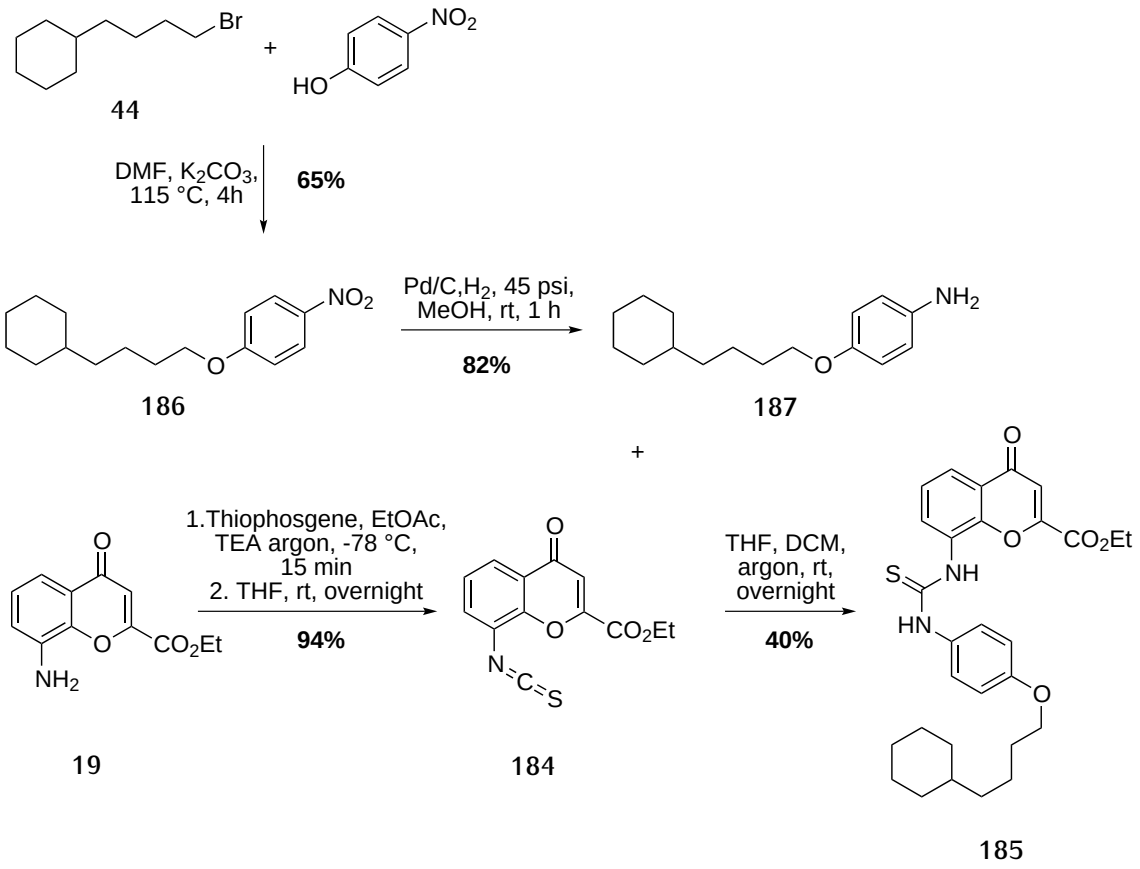
Scheme 3.15: Methylation of the amide linker.

A further linker we inserted in our products was the urea linker. Compound **19** was reacted it with triphosgene in DCM and DIPEA which formed the isocyanate. This intermediate was then further reacted without prior purification with *p*-hydroxyaniline in DCM and DIPEA yielding urea **182** in a good yield of 66% over two steps.



Scheme 3.16: Synthesis of the urea linker.

Finally we also wanted to insert a thiourea linker into a potential GPR17 ligand. To synthesize the amino side chain we performed the Williamson ether synthesis with alkyl bromide **44** and *p*-nitrophenol as previously described. The nitro group was then reduced with Pd/C and hydrogen in MeOH to the corresponding amine **183**, which was subsequently further reacted with the isothiocyanate **184** in THF obtained from compound **19** by treatment with thiophosgene in ethyl acetate (EtOAc). The thiourea derivative **185** could be isolated in a satisfactory yield of 40% (Scheme 3.17).

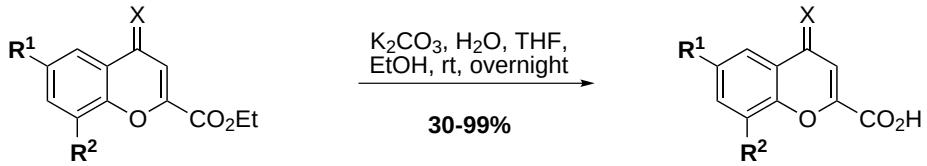


Scheme 3.17: Synthesis of thiourea derivative **185**.

In summary, we successfully modified the amide linker by synthesizing methylated amide, urea and thiourea derivatives, which extends our knowledge on the SARs of the chromenone derivatives as ligands of *orphan* GPCRs.

3.1.2.6 Synthesis of 4-oxo-4*H*-chromene-2-carboxylic acids - the final products

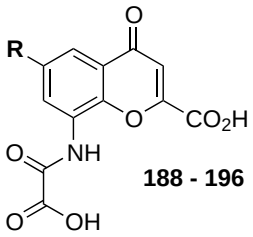
For the synthesis of our final products we had to perform the deprotection of the ethyl ester function at position 2 of the chromen-4-ones. This was achieved in a mixture of EtOH, H_2O and THF in the presence of K_2CO_3 as a base (Scheme 3.18).



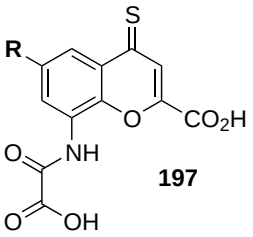
Scheme 3.18: Synthesis of free carboxylic acids. For exact structures see Table 3.12, Table 3.13, Table 3.14, Table 3.15 and Table 3.16.

For most of the compounds we reached an excellent yield of more than 80% and a purity of more than 98% (Table 3.12 - Table 3.16).

Table 3.12: Yields of 8-(2-ethoxy-2-oxoacetamido)-4-oxo-4*H*-chromene-2-carboxylic acids



188 - 196

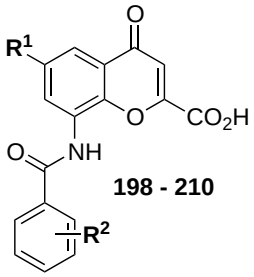


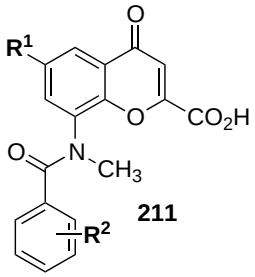
197

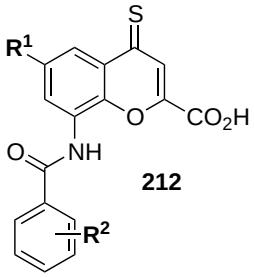
compound	R	yield [%]	mp [°C]	purity[a] [%]
188	Br	96	246-247	98.5
189	H	81	245-249	97.9
190	phenyl	79	> 300	97.6
191	F	85	254-256	99.4
192	Cl	83	242-244	98.6
193	methoxy	82	245-247	99.7
194	methyl	76	250-252	99.4
195	ethyl	93	246-248	99.4
196	butyl	78	224-225	85.1
197	Br	48	> 300	95.6

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

Table 3.13: Yields of 8-benzamido-4-oxo-4*H*-chromene-2-carboxlic acids



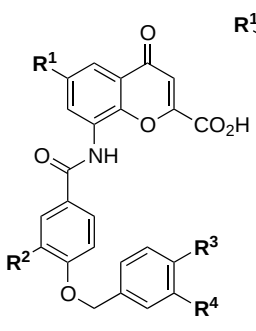




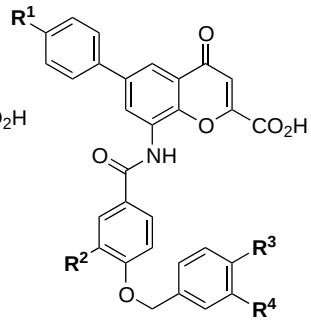
compound	R^1	substitution pattern R^2			yield[a]	mp	purity
		ortho	*meta*	*para*	[%]	[°C]	[%]
198[65]	Br	F	H	OCH_3	95	289-290	100.0
199[65]	Br	di-F	H	OCH_3	91	296-297	100.0
200[65]	Br	H	F	OCH_3	75	296-297	99.8
201[65]	methyl	H	H	OCH_3	86	283-284	99.7
202[65]	OCH_3	H	H	OCH_3	93	276-277	100.0
203	Br	OCH_3	H	OCH_3	82	284-285	99.5
204	OCF_3	H	H	OCH_3	96	270-271	99.3
205	phenyl	H	H	OCH_3	96	270-272	100.0
206	ethyl	H	H	OCH_3	86	277-278	100.0
207	Br	H	H	ethyl	94	297-298	100.0
208	*p*-chloro-phenyl	H	H	OCH_3	84	291-292	99.3
209	Br	H	H	SCH_3	80	285-286	98.7
210	phenyl	di-F	H	OCH_3	95	267-268	96.4
211	Br	H	H	OCH_3	99	146-148	100.0
212	Br	H	H	OCH_3	87	272-273	98.7

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

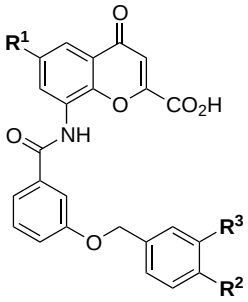
Table 3.14: Yields of 8-((benzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acids



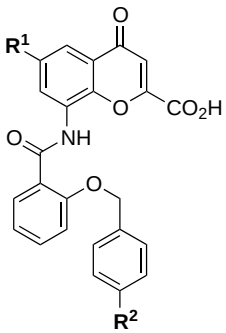
213 - 215



216 - 226



227 - 233

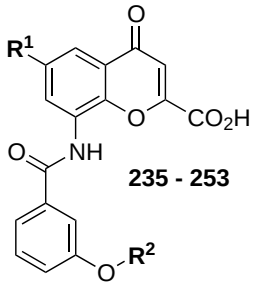


234

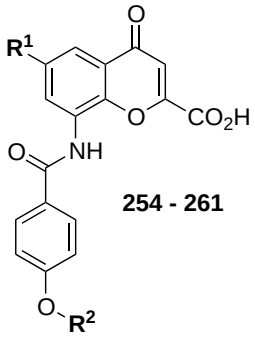
compound	R^1	R^2	R^3	R^4	yield [%]	mp [°C]	purity[a] [%]
213	OCH_3	H	F	H	87	274-278	100.0
214	methyl	H	F	H	63	260-261	100.0
215	ethyl	H	F	H	95	258-259	100.0
216	H	H	F	H	98	155-156	99.2
217	H	H	F	F	68	281-282	100.0
218	Cl	H	F	H	87	264-265	98.3
219	H	Cl	F	H	89	259-260	99.7
220	F	H	F	H	87	264-265	100.0
221	OCH_3	H	F	H	91	264-265	100.0
222	methyl	H	F	H	91	268-269	99.9
223	F	H	F	F	91	276-277	99.3
224	F	Cl	F	F	85	276-277	99.4
225	OH	H	F	H	85	> 300	99.2
226	H	F	F	H	94	262-263	97.0
227	Br	F	-	-	71	258-259	99.3
228	Br	Br	-	-	30	232-233	100.0
229	Br	Cl	-	-	72	202-203	100.0
230	H	methyl	H	-	57	229-231	99.8
231	Br	methyl	H	-	75	245-246	99.0
232	H	H	Br	-	99	256-257	99.9
233	Br	H	Br	-	85	253-254	100.0
234	Br	F	-	-	65	257-258	98.4

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

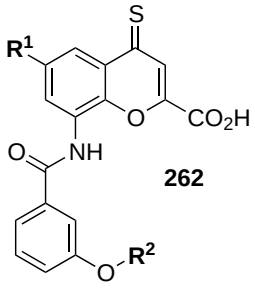
Table 3.15: Yields of 8-(3-alkylbenzamido)-4-oxo-4*H*-chromene-2-carboxylic acids



235 - 253



254 - 261



262

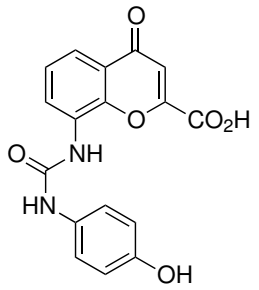
compound	R^1	R^2	yield [%]	mp [°C]	purity[a] [%]
235	H	1-cyclohexylmethyl	95	254-255	99.8
236	H	2-cyclohexylethyl	97	230-231	100.0
237	H	3-cyclohexylpropyl	62	220-221	98.9
238	Cl	3-cyclohexylpropyl	65	239-240	99.5
239	F	3-cyclohexylpropyl	58	230-231	99.6
240	H	4-cyclohexylbutyl	83	201-202	99.8
241	Cl	4-cyclohexylbutyl	80	219-220	99.7
242	F	4-cyclohexylbutyl	36	254-255	99.8
243	H	5-cyclohexylpentyl	91	239-240	99.2
244	Cl	5-cyclohexylpentyl	92	230-231	99.8
245	F	5-cyclohexylpentyl	87	227-228	99.8
246	Cl	hexyl	95	228-230	99.8
247	Cl	heptyl	50	222-224	99.8
248	Cl	octyl	95	228-230	95.0
249	H	1-phenylmethyl	87	243-244	99.9
250	H	2-phenylethyl	87	230-231	99.2
251	H	3-phenylpropyl	80	222-223	99.9
252	H	4-phenylbutyl	81	213-214	98.5
253	H	5-phenylpentyl	93	206-207	99.6
254	Br	3-cyclohexylpropyl	90	246-247	100.0
255	OCH_3	4-cyclohexylbutyl	92	223-224	99.8
256	methyl	4-cyclohexylbutyl	95	223-225	99.9
257	Cl	4-cyclohexylbutyl	94	219-220	99.7
258	F	4-cyclohexylbutyl	82	243-244	98.5

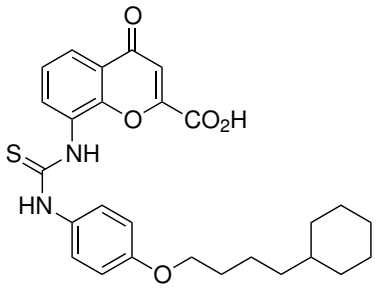
259	ethyl	4-cyclohexylbutyl	96	220-221	100.0
260	H	5-cyclohexylpentyl	79	231-232	100.0
261	Br	5-phenylpentyl	89	223-224	99.7
262	F	3-cyclohexylpropyl	56	212-214	95.0

[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

The isolation of the urea derivative **263** turned out to be difficult because it did not crystallize from the solution. This could be due to the high polarity of the compound.

Table 3.16: Yields of urea and thiourea derivatives





compound	**yield** [%]	**mp** [°C]	**purity**[a] [%]
263	8	240-241	99.0
264	56	148-150	98.0

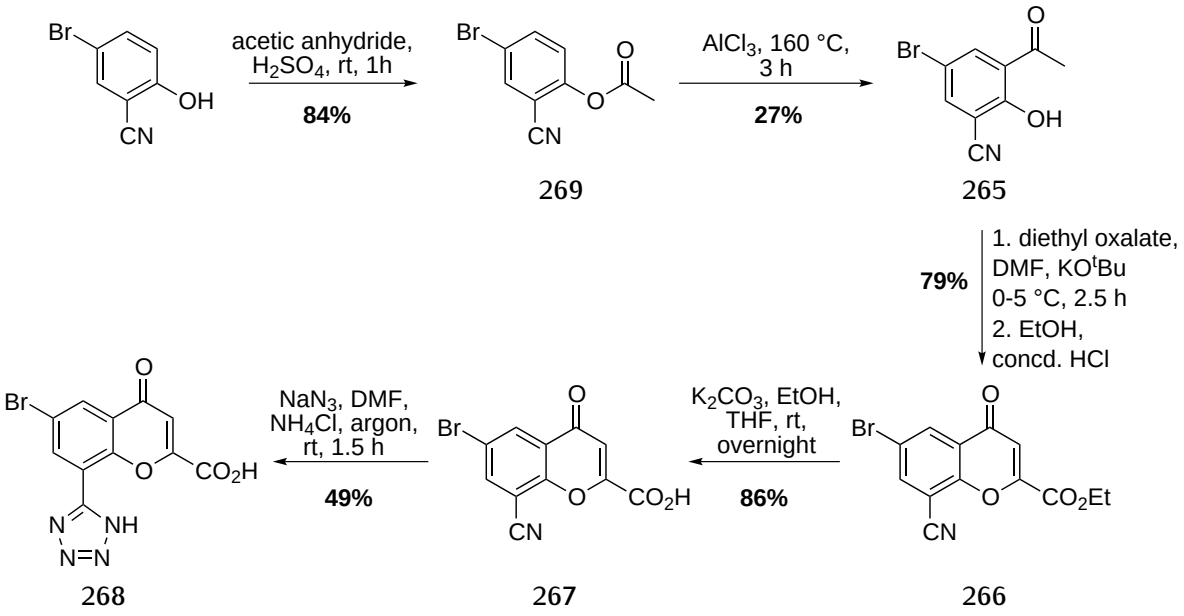
[a] Purity was determined by HPLC-UV (254 nm)-ESI-MS

All in all we synthesized 79 final products which are based on the chromen-4-one core structure representing new potential ligands for GPCRs.

3.1.2.7 Synthesis of a tetrazole derivative

To increase the water-solubility of our compounds we wanted introduce a tetrazoleate into the 8-position the chromenone. We started from commercially available 5-bromo-2-hydroxybenzonitrile which was first acetylated with acetic anhydride in the presence of a catalytic amount of concd. H_2SO_4. Then we performed a solvent-free Fries rearrangement using dry $AlCl_3$. The reaction mixture was heated to 160 °C. It was necessary to use absolutely dry and fresh $AlCl_3$ because otherwise no product was formed. We could isolate the desired acetophenone **265** in a low yield of 27% comparable to literature yields. The ring closure was carried out under the

previously described reaction conditions for the Claisen condensation, and the ester **266** was hydrolyzed as described before yielding the corresponding carboxylic acid **267**. The tetrazole was finally synthesized dissolving cyano derivative **267** in DMF and adding NH_4Cl and NaN_3. Product **268** could be isolated in a moderate yield of 49% (Scheme 3.19). The compound showed an improved water-solubility compared to the corresponding carboxylic acid **188**.

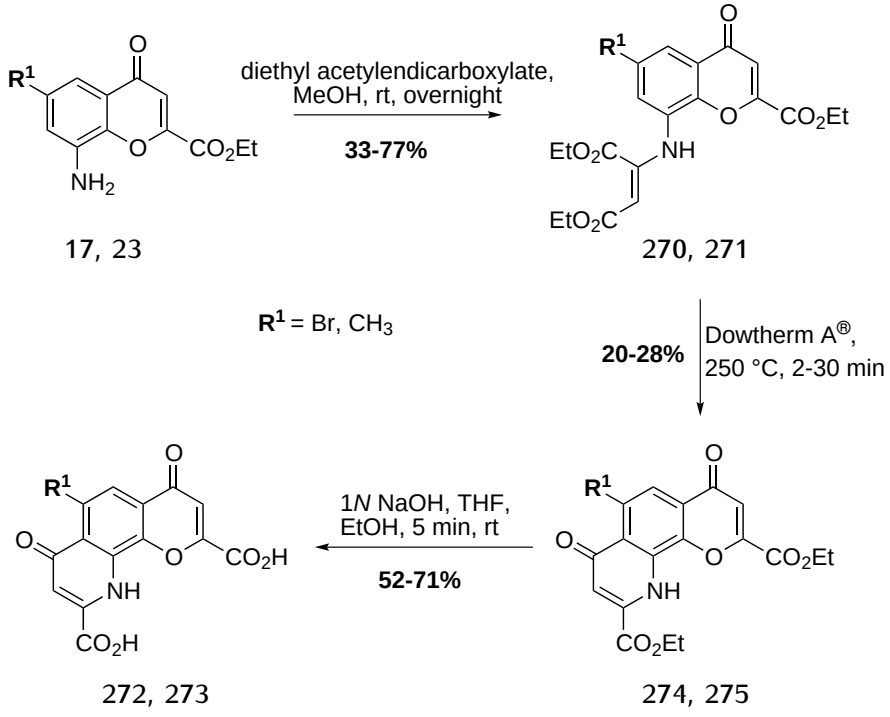


Scheme 3.19: Synthesis of the tetrazole derivative.

3.1.2.8 Synthesis of phenanthroline derivatives

To decrease the flexibility of our compounds we decided to perform a second ring closure yielding phenanthroline derivatives. Those were synthesized according a modified procedure described by Hall et. al.[213] The diethyl 2-((2-(ethoxycarbonyl)-4-oxo-4*H*-chromen-8-yl)amino)maleates **270** and **271** were synthesized using the amines **17** and **23** respectively, which were reacted with diethyl acetylene dicarboxylate in MeOH. The ring closure was performed in the high-boiling solvent Dowtherm® A (mixture of 26.5% biphenyl and 73.5% diphenyl oxide). This reaction step was critical because we observed a decarboxylation when the reaction time was too long. It was therefore necessary to permanently monitor the progress of the reaction by thin-layer chromatography (TLC) und directly stop it when the starting material was

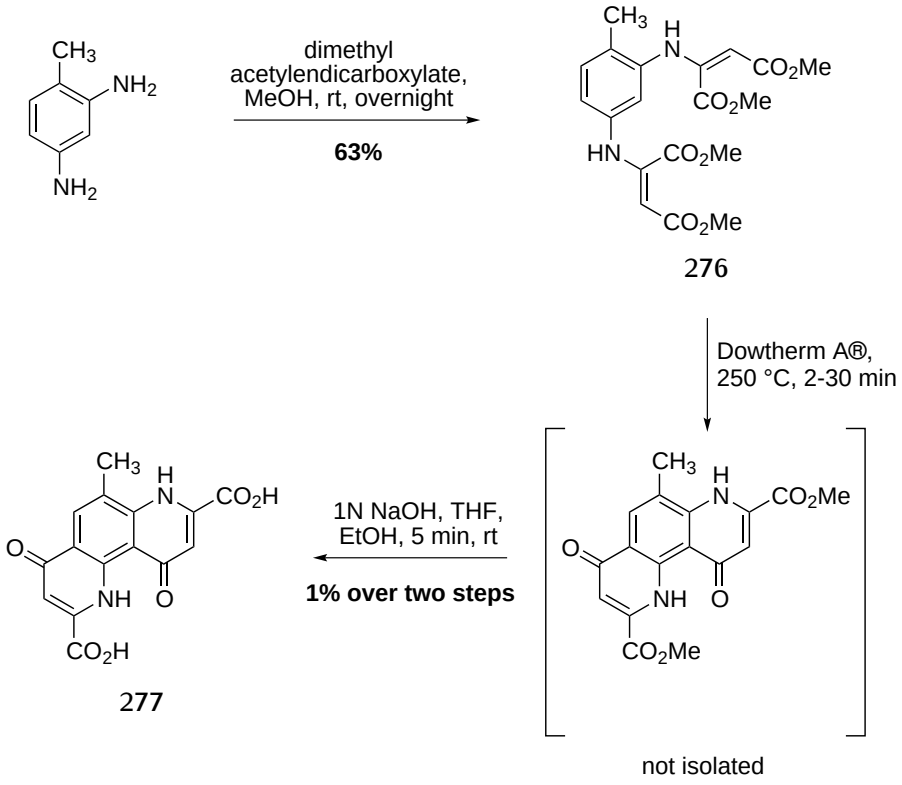
converted. Another important point was the amount of solvent. If we used too much solvent the product did not precipitate and could not be separated from the solvent. It we used too little of the solvent the product was not formed, maybe because the temperature could not be reached. An amount of 2 mL/g of starting material turned out to be optimal. Finally the ethyl ester had be hydrolyzed. Our previous method for deprotection of the carboxylic acid did not work in this case because the solubility of the phenanthroline derivatives was extremely low. The challenge was to find a method which was strong enough to cleave the ester but did not cleave the chromen-4-one structure that was also unstable under basic conditions. Using 1*N* NaOH as a base in a mixture of EtOH and THF as solvent for a reaction time of not longer than 5 min the ester was quantitatively hydrolyzed without destruction of the chromen-4-one core structure. It should be noticed that this method was superior to the previous method because of the short reaction time of only 5 min. Finally we successfully synthesized two phenanthroline derivatives **272** and **273** which will, because of their reduced flexibility, give us further SAR information (Scheme 3.20).



Scheme 3.20: Synthesis of the phenanthroline derivatives.

We decided to further synthesize a phenanthroline derivate which is not based

on a chromen-4-one but on a quinolone scaffold. We used the same synthesis procedure than described before starting from 2,4-aminotoluene which was reacted with dimethyl acetylendicarboxylate. The ring closure of product **276** was then performed in boiling Dowtherm A® for 30 min. The yield was extremely low because the second ring closure occured only after 30 min. After that reaction time some of the intermediate product (after one ring closure) was already destroyed because of the extremely high temperature that had to be applied. We isolated only a very small amount of product and therefore decided to perform the ester cleavage without further purification or analysis of the product. The quinolone is more stable than the chromen-4-one and therefore the methyl ester could be hydrolyzed in pure 1*N* NaOH. We isolated product **277** in an extremely low yield of 1% over two steps (Scheme 3.21).



Scheme 3.21: Synthesis of the phenanthroline derivative **277**.

All phenanthroline derivatives share an extremely low solubility but an increased stability towards basic condition. For drug development they are an alternative scaffold to our chromen-4-ones.

3.2 Pharmacological evaluation of chromenones at GPR35

3.2.1 Introduction

In 2013 Funke et al. published 8-benzamidochromen-4-one-2-carboxylic acids as potent and selective agonists for the human GPR35.[95] Subsequently a radioligand based on this structural class was developed, [^{3}H]PSB-13253, which is the tritiated version of 6-bromo-8-(4-methoxybenzamido)-4-oxo-4H-chromene-2-carboxylic acid.[65] However the radioligand displays a high degree of species selectivity for the human receptor being not very potent at the rat and the mouse orthologue. It is therefore necessary to further modify the structure to increase activity at the rodent receptors. This phenomenon of orthologue selectivity of GPR35 ligands was already discussed by many groups.[214]

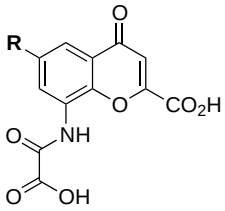
We synthesized a broad range of structurally diverse chromen-4-one derivatives that were tested regarding their potency at the different GPR35 orthologues using the Pathhunter® β-arrestin recruitment assay. This assay is a universal tool for *orphan* G protein-coupled receptor (GPCR) analysis as it is independent of second messenger pathways. For the assay a small peptide, the ProLink, is fused to the target GPCR. A larger complementing fragment, the enzyme acceptor (EA) is linked to the arrestin. When the receptor is activated by an agonist the arrestin binds to the GPCR and both peptide fragments will join forming an enzymatically active β-galactosidase. After addition of a substrate a chemiluminescent reaction product can be measured. An advantage of this technology is that we can selectively measure the activity of the fused GPCR.

We also further optimized the agonists regarding their potency at the human GPR35. As we have a radioligand available for this target we were also able to determine the binding of the ligands to the human receptor. In radioligand binding studies the ability of a compound to displace the radioligand from its binding site at the receptor is measured radiometrically.

3.2.2 Results

The pharmacological evaluation of the compounds was performed by Dr. Dominik Thimm in our group.

Table 3.17: Potency and affinity of chromen-4-one-2-carboxylic acids at human GPR35, mouse GPR35 and rat GPR35. Functional β-arrestin recruitment experiments were performed using CHO β-arrestin cells expressing human, rat or mouse GPR35. Affinity was determined in radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35. Compounds were tested in binding studies versus the radioligand [^{3}H]PSB-13253.



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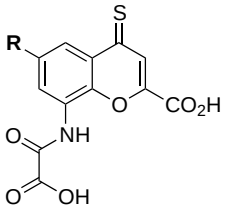

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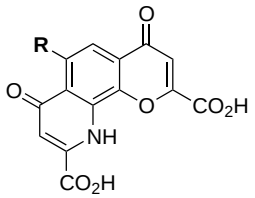

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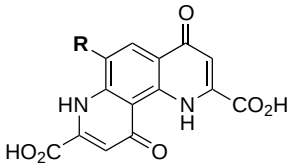

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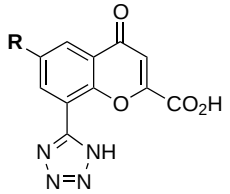

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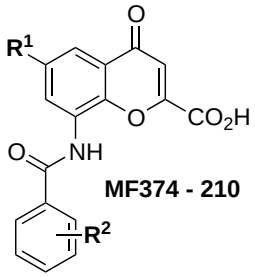
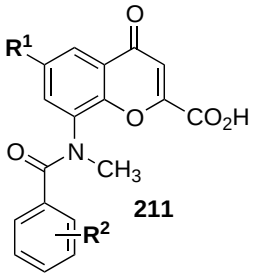
compd.	R	Human GPR35		Rat GPR35	Mouse GPR35
		EC_{50} ± SEM (μM) (% effect[a] ± SEM)	K_i ± SEM (μM) (% inhibition[b] ± SEM)	EC_{50} ± SEM (μM) (% effect[c] ± SEM)	EC_{50} ± SEM (μM) (% effect[a] ± SEM)
Standard compounds[d]					
Zaprinast		**1.96** ± 0.24[65]	**0.401** ± 0.015[172]	**0.0611** ± 0.0061[172]	**1.60** ± 0.04[172]
Cromoglicic acid		**1.26** ± 0.17[65]	**2.34** ± 0.04[65]	**0.986** ± 0.126[172]	**4.84** ± 0.60[172]
Pranlukast		> 10 (12 ± 2)[172]	**0.0407** ± 0.0073[172]	**0.480** ± 0.036[172]	> 10 (58 ± 1)[172]
PSB-13253		**0.0111** ± 0.0031[65]	**0.00518** ± 0.00033[65]	**4.17** ± 0.33[65]	> 10 (35 ± 2)[65]
188	Br	**0.0118** ± 0.0015	**0.0138** ± 0.0015	**0.0451** ± 0.0065	**1.88** ± 0.44
189	H	**0.246** ± 0.017	**0.396** ± 0.036	**0.779** ± 0.097	> 10 (37 ± 4)
190	phenyl	**0.00968** ± 0.00048	**0.00319** ± 0.00056	**0.154** ± 0.015	**0.494** ± 0.083
191	F	**0.106** ± 0.011	**0.0426** ± 0.0111	**0.263** ± 0.034	**4.76** ± 0.32
192	Cl	**0.0293** ± 0.0015	**0.00716** ± 0.00012	**0.0800** ± 0.0031	**1.71** ± 0.46
193	methoxy	**0.0938** ± 0.0087	**0.0335** ± 0.0051	**0.851** ± 0.102	**4.71** ± 0.53 (88%)
194	methyl	**0.104** ± 0.003	**0.0635** ± 0.0208	**0.406** ± 0.036	**2.38** ± 0.74 (77%)

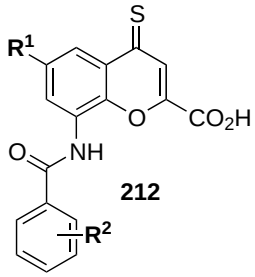
compd.	R	human GPR35		rat GPR35	mouse GPR35
		EC_{50} ± SEM (μM) (% effect[a] ± SEM)	K_i ± SEM (μM) (% inhibition[b] ± SEM)	EC_{50} ± SEM (μM) (% effect[c] ± SEM)	EC_{50} ± SEM (μM) (% effect[a] ± SEM)
195	ethyl	**0.0275** ± 0.0025	**0.0179** ± 0.0014	**0.0750** ± 0.0020	**1.00** ± 0.30
196	butyl	**0.0318** ± 0.0053	**0.00897** ± 0.0015	**0.0297** ± 0.0040	**0.399** ± 0.083
197	Br	**0.00847** ± 0.00072	**0.00271** ± 0.00032	**0.0209** ± 0.0023	**0.174** ± 0.019
272	Br	**0.037** ± 0.0021	**0.0149** ± 0.0031	**0.487** ± 0.064	**0.910** ± 0.120
273	methyl	**0.0295** ± 0.0046	**0.00836** ± 0.00075	**0.470** ± 0.042 (87%)	**0.955** ± 0.229
277	methyl	**0.0214** ± 0.0021	**0.0062** ± 0.00065	**0.0263** ± 0.0023	**0.105** ± 0.015
268	Br	**3.00** ± 0.590	**0.925** ± 0.080	**1.91** ± 0.270	**5.84** ± 0.37

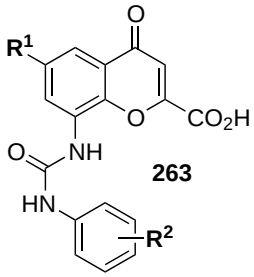
Initial screens were performed at a concentration of 10 μM. Effects were normalized to the signal induced by [a]30 μM (human and mouse) or [c]10 μM (rat) of zaprinast corresponding to a maximal response at the respective receptor. [b]Radioligand binding studies. [d]For structures of the standard compounds see Figure 1.8.

Table 3.18: Potency and affinity of chromen-4-one-2-carboxylic acids at human GPR35, mouse GPR35 and rat GPR35. Functional β-arrestin recruitment experiments were performed using CHO β-arrestin cells expressing human, rat or mouse GPR35. Affinity was determined in radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35. Compounds were tested in binding studies versus the radioligand [^{3}H]PSB-13253.







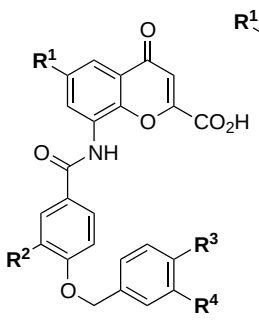


compd.	R^1	substitution pattern R^2			Human GPR35		Rat GPR35	Mouse GPR35
		ortho	*meta*	*para*	EC_{50} ± SEM (μM) (% effect[a] ± SEM)	K_i ± SEM (μM) (% inhibition[b] ± SEM)	EC_{50} ± SEM (μM) (% effect[c] ± SEM)	EC_{50} ± SEM (μM) (% effect[a] ± SEM)
MF374[65]	Cl	F	H	OCH_3	**0.00606** ± 0.00088	**0.00192** ± 0.00012	**0.374** ± 0.033	n.d.
MF338[95]	Br	H	H	OCH_3	**0.0111** ± 0.0031	**0.00518** ± 0.00033	**4.17** ± 0.33	> 10 (35 ± 2)
MF356[172]	Br	H	H	OH	**0.716** ± 0.085	n.d.	**0.349** ± 0.020	**1.07** ± 0.12
MF310[95]	F	H	H	OCH_3	**0.112** ± 0.011	**0.0254** ± 0.0023	**1.76** ± 0.08	> 10 (30 ± 4)
MF257[95]	Cl	H	H	OCH_3	**0.0168** ± 0.0021	**0.00550** ± 0.00012	**1.83** ± 0.27	> 10 (34 ± 3)
MF_AM11[95]	H	H	H	OCH_3	**0.346** ± 0.037	**0.221** ± 0.020	**2.16** ± 0.22 (85%)	**5.03** ± 0.67 (66%)
198[65]	Br	F	H	OCH_3	**0.00446** ± 0.00030	**0.00137** ± 0.00011	**0.234** ± 0.040	**2.71** ± 0.34
199[65]	Br	di-F	H	OCH_3	**0.00554** ± 0.00029	**0.000589** ± 0.000076	**0.199** ± 0.039	**2.85** ± 0.41
200[65]	Br	H	F	OCH_3	**0.00437** ± 0.00048	**0.00164** ± 0.00018	**0.769** ± 0.038 (84%)	**3.52** ± 0.76
201[65]	methyl	H	H	OCH_3	**0.0377** ± 0.0036	**0.0428** ± 0.0032	**1.20** ± 0.26 (79%)	**3.82** ± 0.70 (88%)

compd.	R^1	substitution pattern R^2			human GPR35		rat GPR35	mouse GPR35
		ortho	*meta*	*para*	EC_{50} ± SEM (μM) (% effect[a] ± SEM)	K_i ± SEM (μM) (% inhibition[b] ± SEM)	EC_{50} ± SEM (μM) (% effect[c] ± SEM)	EC_{50} ± SEM (μM) (% effect[a] ± SEM)
202[65]	OCH_3	H	H	OCH_3	**0.0255** ± 0.0027	**0.0205** ± 0.0022	**1.52** ± 0.12 (77%)	**2.69** ± 0.39
203	Br	OCH_3	H	OCH_3	**0.0162** ± 0.0009	**0.00824** ± 0.0006	**0.493** ± 0.081 (78%)	**2.52** ± 0.56 (82%)
204	OCF_3	H	H	OCH_3	**0.0104** ± 0.0017	**0.00724** ± 0.00043	**0.345** ± 0.043	**2.64** ± 0.25 (87%)
205	phenyl	H	H	OCH_3	**0.00548** ± 0.00089	**0.00172** ± 0.00009	**0.195** ± 0.010	**0.459** ± 0.104
206	ethyl	H	H	OCH_3	**0.0306** ± 0.0074	**0.0228** ± 0.0016	**0.428** ± 0.056	**1.83** ± 0.04
207	Br	H	H	ethyl	**0.0570** ± 0.0055	**0.0828** ± 0.0081	**0.857** ± 0.211 (76%)	**3.14** ± 0.23 (89%)
208	*p*-chloro-phenyl	H	H	OCH_3	**0.00821** ± 0.00189	**0.00139** ± 0.00010	**0.263** ± 0.034 (88%)	**0.724** ± 0.140
209	Br	H	H	SCH_3	**0.0150** ± 0.0009	**0.0235** ± 0.0023	**0.748** ± 0.045 (80%)	**3.29** ± 0.37 (88%)
210	phenyl	di-F	H	OCH_3	**0.00108** ± 0.00011	**0.000426** ± 0.000037	**0.157** ± 0.013	**0.293** ± 0.042
211	Br	H	H	OCH_3	> 10 (28 ± 7)	> 10 (23 ± 3)	>10 (6 ± 1)	> 10 (1 ± 1)
212	Br	H	H	OCH_3	**0.00362** ± 0.00066	**0.00125** ± 0.00006	**0.552** ± 0.042 (73%)	**1.31** ± 0.07
263	H	H	H	OH	**1.87** ± 0.08	**1.55** ± 0.126	**0.288** ± 0.034 (76%)	**1.54** ± 0.26 (89%)

Initial screens were performed at a concentration of 10 μM. Effects were normalized to the signal induced by [a]30 μM (human and mouse) or [c]10 μM (rat) of zaprinast corresponding to a maximal response at the respective receptor. [b]Radioligand binding studies.

Table 3.19: Potency and affinity of chromen-4-one-2-carboxylic acids at human GPR35, mouse GPR35 and rat GPR35. Functional β-arrestin recruitment experiments were performed using CHO β-arrestin cells expressing human, rat or mouse GPR35. Affinity was determined in radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35. Compounds were tested in binding studies versus the radioligand [^{3}H]PSB-13253.



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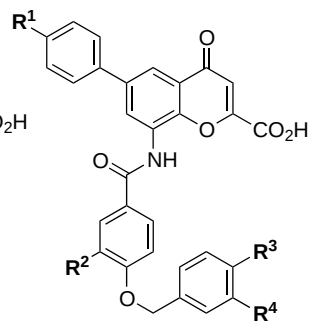

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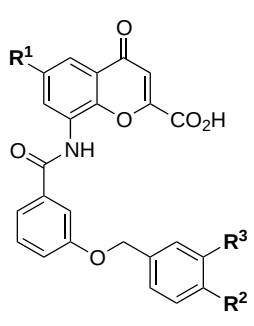

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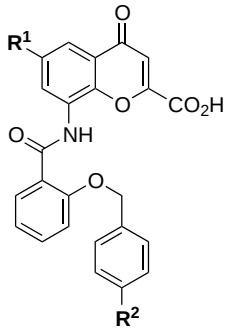

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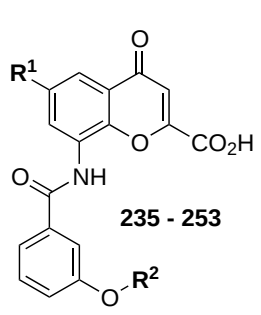
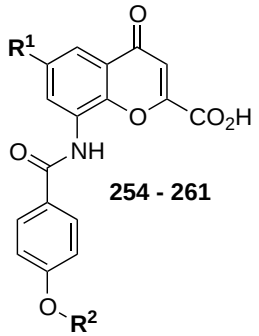
compd.	substitution pattern				Human GPR35		Rat GPR35	Mouse GPR35
	R^1	R^2	R^3	R^4	EC_{50} ± SEM (% effect[a] ± SEM)	K_i ± SEM (% inhibition[b] ± SEM)	EC_{50} ± SEM (% effect[c] ± SEM)	EC_{50} ± SEM (% effect[a] ± SEM)
213	OCH_3	H	F	H	> 10 (33 ± 6)	**1.06** ± 0.058	> 10 (52 ± 9)	**1.28** ± 0.38 (76%)
214	methyl	H	F	H	> 10 (21 ± 1)	**0.835** ± 0.058	**0.977** ± 0.051 (82%)	**1.77** ± 0.09
215	ethyl	H	F	H	**7.47** ± 1.38	**0.432** ± 0.037	**0.417** ± 0.018	**1.16** ± 0.33
216	H	H	F	H	> 10 (40 ± 4)	**0.186** ± 0.009	**0.540** ± 0.078 (64%)	**1.42** ± 0.11 (70%)
217	H	H	F	F	> 10 (8 ± 5)	**0.354** ± 0.049	**0.527** ± 0.055 (58%)	**1.61** ± 0.29 (78%)
218	Cl	H	F	H	> 10 (4 ± 2)	> 10 (30 ± 3)	> 10 (43 ± 5)	**2.77** ± 0.26 (65%)
219	H	Cl	F	H	> 10 (48 ± 7)	**0.317** ± 0.019	> 10 (50 ± 5)	**1.81** ± 0.21 (64%)
220	F	H	F	H	> 10 (13 ± 3)	> 10 (28 ± 2)	> 10 (44 ± 4)	**1.52** ± 0.28 (70%)
221	OCH_3	H	F	H	> 10 (20 ± 2)	**0.263** ± 0.050	> 10 (43 ± 4)	**1.25** ± 0.11 (64%)
222	methyl	H	F	H	> 10 (5 ± 2)	> 10 (41 ± 2)	> 10 (30 ± 3)	**3.22** ± 0.44 (60%)

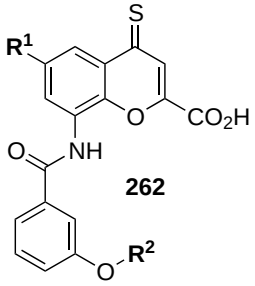
compd.	substitution pattern				human GPR35		rat GPR35	mouse GPR35
	R^1	R^2	R^3	R^4	EC_{50} ± SEM (% effect[a] ± SEM)	K_i ± SEM (% inhibition[b] ± SEM)	EC_{50} ± SEM (% effect[c] ± SEM)	EC_{50} ± SEM (% effect[a] ± SEM)
223	F	H	F	F	> 10 (1 ± 2)	> 10 (32 ± 3)	> 10 (39 ± 3)	**2.76** ± 0.46 (70%)
224	F	Cl	F	F	> 10 (2 ± 2)	> 10 (37 ± 2)	> 10 (37 ± 4)	**2.27** ± 1.05 (55%)
225	OH	H	F	H	**0.583** ± 0.086	**0.0299** ± 0.0028	**0.140** ± 0.022	**0.218** ± 0.059
226	H	F	F	H	**0.381** ± 0.021	**0.218** ± 0.022	**0.435** ± 0.036 (86%)	**1.13** ± 0.06 (86%)
227	Br	F	-	-	> 10 (7 ± 2)	**0.573** ± 0.050	**0.726** ± 0.066 (75%)	**2.36** ± 0.36
228	Br	Br	-	-	> 10 (6 ± 1)	**0.372** ± 0.031	> 10 (47 ± 3)	**2.10** ± 0.19 (88%)
229	Br	Cl	-	-	> 10 (16 ± 1)	**0.383** ± 0.027	> 10 (50 ± 5)	**2.77** ± 0.21 (79%)
230	H	methyl	H	-	> 10 (40 ± 5)	**2.11** ± 0.280	> 10 (48 ± 3)	**1.36** ± 0.21
231	Br	methyl	H	-	> 10 (35 ± 7)	**1.50** ± 0.130	> 10 (43 ± 2)	**4.27** ± 1.19 (75%)
232	H	H	Br	-	> 10 (27 ± 6)	**0.667** ± 0.032	**0.512** ± 0.020 (79%)	**0.784** ± 0.145
233	Br	H	Br	-	> 10 (4 ± 5)	**4.28** ± 0.250	**0.699** ± 0.079 (69%)	**1.41** ± 0.06 (84%)
234	Br	F	-	-	> 10 (-2 ± 1)	**1.58** ± 0.080	> 10 (53 ± 9)	**4.90** ± 0.62 (80%)

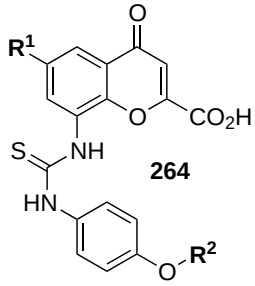
Initial screens were performed at a concentration of 10 μM. Effects were normalized to the signal induced by [a]30 μM (human and mouse) or [c]10 μM (rat) of zaprinast corresponding to a maximal response at the respective receptor. [b]Radioligand binding studies.

Table 3.20: Potency and affinity of chromen-4-one-2-carboxylic acids at human GPR35, mouse GPR35 and rat GPR35. Functional β-arrestin recruitment experiments were performed using CHO β-arrestin cells expressing human, rat or mouse GPR35. Affinity was determined in radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35. Compounds were tested in binding studies versus the radioligand [^{3}H]PSB-13253.









compd.	R^1	R^2	Human GPR35		Rat GPR35	Mouse GPR35
			EC_{50} ± SEM (% effect[a] ± SEM)	K_i ± SEM (% inhibition[b] ± SEM)	EC_{50} ± SEM (% effect[c] ± SEM)	EC_{50} ± SEM (% effect[a] ± SEM)
235	H	1-cyclohexylmethyl	> 10 (23 ± 7)	**1.26** ± 0.170	**1.25** ± 0.08 (73%)	**0.790** ± 0.058
236	H	2-cyclohexylethyl	> 10 (8 ± 5)	**0.930** ± 0.022	**1.48** ± 0.27 (58%)	**1.71** ± 0.23
237	H	3-cyclohexylpropyl	> 10 (-7 ± 1)	**0.585** ± 0.070	> 10 (41 ± 0)	**2.43** ± 0.73 (82%)
238	Cl	3-cyclohexylpropyl	> 10 (-5 ± 2)	> 10 (39 ± 6)	> 10 (17 ± 8)	**3.43** ± 0.29 (55%)
239	F	3-cyclohexylpropyl	> 10 (-4 ± 1)	**1.59** ± 0.360	> 10 (33 ± 5)	**2.98** ± 0.23 (81%)
240	H	4-cyclohexylbutyl	> 10 (-3 ± 3)	**0.474** ± 0.043	> 10 (37 ± 4)	**2.58** ± 0.31 (54%)
241	Cl	4-cyclohexylbutyl	> 10 (-6 ± 1)	**1.94** ± 0.11	> 10 (27 ± 3)	> 10 (41 ± 2)
242	F	4-cyclohexylbutyl	> 10 (-6 ± 1)	**1.32** ± 0.090	> 10 (29 ± 9)	**2.90** ± 0.52 (45%)
243	H	5-cyclohexylpentyl	> 10 (-2 ± 4)	> 10 (34 ± 10)	> 10 (8 ± 4)	**3.37** ± 1.99 (50%)
244	Cl	5-cyclohexylpentyl	> 10 (-8 ± 2)	**2.67** ± 0.590	> 10 (3 ± 4)	> 10 (30 ± 7)

compd.	R^1	R^2	human GPR35		rat GPR35	mouse GPR35
			EC_{50} ± SEM (% effect[a] ± SEM)	K_i ± SEM (% inhibition[b] ± SEM)	EC_{50} ± SEM (% effect[c] ± SEM)	EC_{50} ± SEM (% effect[a] ± SEM)
245	F	5-cyclohexylpentyl	> 10 (-9 ± 3)	**2.22** ± 0.210	> 10 (15 ± 5)	> 10 (48 ± 2)
246	Cl	hexyl	> 10 (5 ± 0)	**1.30** ± 0.210	> 10 (45 ± 4)	**2.25** ± 0.23 (78%)
247	Cl	heptyl	> 10 (-1 ± 1)	**1.13** ± 0.180	> 10 (47 ± 5)	**2.63** ± 0.23 (66%)
248	Cl	octyl	> 10 (-1 ± 1)	**1.62** ± 0.180	> 10 (37 ± 4)	> 10 (49 ± 2)
249	H	1-phenylmethyl	**4.20** ± 0.57	**2.94** ± 0.310	**0.983** ± 0.095	**1.35** ± 0.28
250	H	2-phenylethyl	> 10 (40 ± 0)	**2.39** ± 0.170	**0.561** ± 0.052 (85%)	**1.07** ± 0.19
251	H	3-phenylpropyl	> 10 (14 ± 4)	**1.08** ± 0.170	**1.72** ± 0.53 (59%)	**1.38** ± 0.04
252	H	4-phenylbutyl	> 10 (22 ± 2)	**1.00** ± 0.030	> 10 (12 ± 6)	**1.82** ± 0.10
253	H	5-phenylpentyl	> 10 (11 ± 2)	**0.490** ± 0.007	> 10 (20 ± 13)	**1.25** ± 0.21 (72%)
254	Br	3-cyclohexylpropyl	> 10 (20 ± 4)	**0.179** ± 0.036	> 10 (4 ± 2)	> 10 (21 ± 8)
255	OCH_3	4-cyclohexylbutyl	> 10 (35 ± 4)	**0.012** ± 0.005	> 10 (9 ± 1)	**2.78** ± 0.27 (63%)
256	methyl	4-cyclohexylbutyl	> 10 (31 ± 2)	**0.0172** ± 0.0002	> 10 (2 ± 3)	> 10 (18 ± 3)
257	Cl	4-cyclohexylbutyl	> 10 (3 ± 1)	**0.0646** ± 0.0063	> 10 (4 ± 1)	> 10 (23 ± 4)
258	F	4-cyclohexylbutyl	> 10 (-2 ± 1)	**0.0364** ± 0.0018	> 10 (4 ± 4)	> 10 (13 ± 2)
259	ethyl	4-cyclohexylbutyl	**0.791** ± 0.228	> 10 (39 ± 6)	> 10 (5 ± 7)	> 10 (46 ± 2)
260	H	5-cyclohexylpentyl	> 10 (12 ± 4)	**0.0155** ± 0.0012	> 10 (22 ± 3)	> 10 (21 ± 5)
261	Br	5-phenylpentyl	> 10 (22 ± 2)	**0.0833** ± 0.0040	> 10 (12 ± 6)	> 10 (39 ± 10)
262	F	3-cyclohexylpropyl	> 10 (2 ± 1)	**3.17** ± 0.180	> 10 (9 ± 2)	> 10 (36 ± 7)
264	H	4-cyclohexylbutyl	**1.87** ± 0.08	**0.132** ± 0.0012	**0.288** ± 0.034	**3.86** ± 0.91 (66%)

compd.	R^1	R^2	human GPR35		rat GPR35	mouse GPR35
			EC_{50} ± SEM (% effect[a] ± SEM)	K_i ± SEM (% inhibition[b] ± SEM)	EC_{50} ± SEM (% effect[c] ± SEM)	EC_{50} ± SEM (% effect[a] ± SEM)

Initial screens were performed at a concentration of 10 μM. Effects were normalized to the signal induced by [a]30 μM (human and mouse) or [c]10 μM (rat) of zaprinast corresponding to a maximal response at the respective receptor. [b]Radioligand binding studies.

3.2.3 Structure-activity relationships

3.2.3.1 Introduction

The phenomenon of orthologue selectivity at the *orphan* receptor GPR35 was already discussed in the introduction. It was therefore necessary to test the compounds not only at the human receptor but also at the rodent receptors because the potency of ligands at the human receptor cannot be easily translated to the rodent orthologues. In the following paragraphs the results will first be discussed for each receptor separately and then analyzed regarding orthologue selectivity.

3.2.3.2 Human GPR35

A broad range of chromen-4-one derivatives had already been synthesized in our group. In the course of these studies the side-chain at the 8-position of the chromen-4-one was modified extensively. A phenyl which was substituted in the *para*-position with a methoxy group linked to the chromen-4-one via an amide turned out to be the best substituent. It could also be observed that fluorine substitutions at the phenyl-ring were beneficial for potency.[65,95]

For the present study we wanted to further investigate the importance of the 6-position. So far it has only been substituted with hydrogen, bromine, chlorine, and fluorine. The results identified bromine as the best substituent at this position so far.[95] For the modifications of the 6-position we chose compound **MF_AM11**[95] as a lead structure. The most potent compounds contain halogens at the 6-position and show the following rank order of potency: bromine (**MF338**[95] EC_{50} = **0.0111** ± 0.0031 μM) ≈ chlorine (**MF257**[95] EC_{50} = **0.0168** ± 0.0021 μM) > fluorine (**MF310**[95] EC_{50} = **0.112** ± 0.011 μM) ≈ hydrogen (**MF_AM11**[95] EC_{50} = **0.346** ± 0.037 μM). The introduction of a methoxy group, which is also a hydrogen bond acceptor like the halogens, decreased the potency compared to bromine and chlorine substituents (**202**[65] EC_{50} = **0.0255** ± 0.0027 μM) but resulted still in a higher potency compared to fluorine and hydrogen. The replacement of the methyl ether by a trifluoromethyl ether, which has a higher electronegativity and lipophilicity, resulted in an increase in potency (**204** EC_{50} = **0.0104** ± 0.0017 μM) comparable to the one of the bromine compound **MF338**.[95] To determine whether electronegativity or lipophilicity have the higher impact on the potency we introduced alkyl chains of different lengths at the 6-position. The potency of the resulting compounds was between the one

of the fluorine and the chlorine compounds. The length of the chain did not make a significant difference. The longer ethyl chain (**206** EC_{50} = **0.0306** ± 0.0074 μM) seemed to be equallly potent as a methyl group (**278**[65] EC_{50} = **0.0377** ± 0.0036 μM). Besides the electronegativity and lipophilicity also the size of this residue could play an important role, which would be a further explanation for the good potency of the trifluoromethoxy compound **204**. We therefore decided to introduce a large phenyl substitutent in this position. The phenyl derivative showed a 2-fold higher potency compared to the bromine and the trifluoromethoxy compound (**205** EC_{50} = **0.00548** ± 0.00089 μM). To further increase the size of the compound and to increase 6-substituent's electronegativity we additionally added a chlorine in the *para*-position of the phenyl-ring. However the resulting compound (**208** EC_{50} = **0.00821** ± 0.00189 μM) was less active than the unsubstituted phenyl derivative but still more potent than the bromine and the trifluoromethyl derivatives (Table 3.18).

We designed a second series of compounds with a different side-chain where the 6-position was again modified extensively (Table 3.17). These compounds were substituted at the 8-position with a 2-amino-2-oxoacetic acid. The structure-activity relationships (SARs) of the 6-position were mostly comparable to the previously described series of compounds. The rank order of substituents was as follows: phenyl (**190** EC_{50} = **0.00967** ± 0.00048 μM) ≈ bromine (**123** EC_{50} = **0.118** ± 0.0015 μM) > ethyl (**195** EC_{50} = **0.0275** ± 0.0025 μM) ≈ chlorine (**192** EC_{50} = **0.0293** ± 0.0015 μM) > butyl (**196** EC_{50} = **0.0318** ± 0.0053 μM) > methoxy (**193** EC_{50} = **0.0938** ± 0.0087 μM) ≈ methyl (**194** EC_{50} = **0.104** ± 0.003 μM) > hydrogen (**189** EC_{50} = **0.246** ± 0.017 μM). One remarkable difference was that the ethyl and the butyl substituent are better tolerated in this series of compounds whereas methoxy is not well tolerated. Still fluorine and hydrogen as small, more polar atoms are not tolerated and the phenyl substituent is the best one. SAR results are depicted in Figure 3.2.

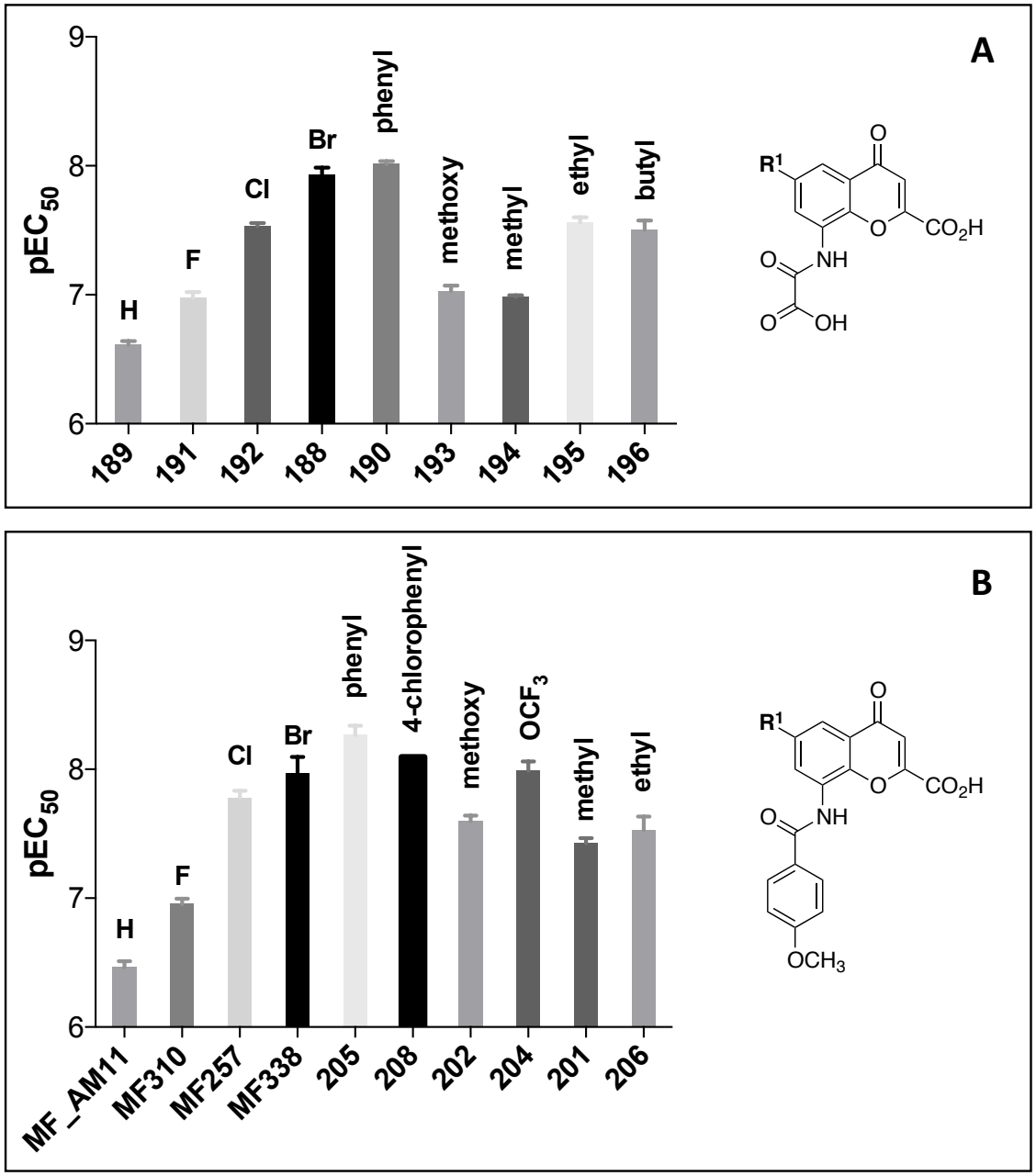


Figure 3.2: Structure-activity relationships of substituents at the 6-position of chromen-4-one derivatives determined for the human GPR35. The pEC_{50} values ± SEM were calculated from EC_{50} values that were determined by β-arrestin recruitment assay. Two scaffolds (**A** and **B**) were analyzed.

Besides the modification of the 6-position many other modifications of the linker,

the side-chain, and the chromen-4-one core structure itself were performed. The most remarkable one was the replacement of the oxygen at the 4-position of the chromen-4-one by a sulfur atom. The C=S bond is less polarized than the C=O bond and sulfur has a lower ability to form hydrogen bonds. The sulfur was well tolerated. Compound **197** (EC_{50} = **0.00847** ± 0.00072 μM) appeared to be even slightly more potent than the corresponding oxygen analogue **188** (EC_{50} = **0.0118** ± 0.0015 μM). The same was observed for the second series of compounds. Also here the sulfur analogue (**212**, EC_{50} = **0.00362** ± 0.00066 μM) was almost 3-fold more potent than the keto-derivative (**MF338**[95] EC_{50} = **0.0111** ± 0.0031 μM).

We also performed some modifications of the linker. The methylation of the primary amide to a secondary amide and thereby the abolishment of the ability to form hydrogen bonds resulted in a complete loss of potency in compound **211**. A urea linker was also not beneficial for the potency (**263** EC_{50} = **1.87** ± 0.08 μM).

Regarding the side-chain, the substitution of the phenyl ring was already investigated comprehensively.[65,95] Because a fluorine substitution seemed to be beneficial for the potency we also performed different fluorine substitutions at the methoxyphenyl group in the 8-position of the chromen-4-one. The potency of the compounds with *ortho*-substitution (**198**[65] EC_{50} = **0.00446** ± 0.0030 μM) was almost comparable to the di-*ortho*-(**199**[65] EC_{50} = **0.00554** ± 0.00029 μM) and the *meta*-substituted (**200**[65] EC_{50} = **0.00437** ± 0.00048 μM) derivatives. All compounds were about 2.5-fold more potent than the unsubstituted **MF338**. However the affinity determined in radioligand binding studies was slightly different. The affinity of **199** (K_i = **0.000589** ± 0.000076 μM) was more than 2-fold higher compared to the mono-substituted compounds (**198** K_i = **0.00137** ± 0.00011 μM and **200** K_i = **0.00164** ± 0.00018 μM).

An *ortho*-methoxy substitution (**203** EC_{50} = **0.0162** ± 0.0009 μM) as well as the replacement of the *para*-methoxy group by either ethyl (**207** EC_{50} = **0.0570** ± 0.0055 μM) or thiomethyl (**209** EC_{50} = **0.0150** ± 0.0009 μM) did not result in compounds with improved potency.

Also the compounds with the 2-amino-2-oxoacetic acid side chain and the tetrazole in the 8-position were not more potent at hGPR35 than the phenyl derivatives. The larger compounds displayed in Table 3.19 and Table 3.20, which were originally designed for other *orphan* receptors, did not activate GPR35 at all. They are too

big and too lipophilic. However there are two exceptions (Figure 3.3). Compound **225** (EC_{50} = **0.583** ± 0.086 μM) and **226** (EC_{50} = **0.381** ± 0.021 μM) were able to activate the receptor in the higher nanomolar range. Regarding **225** this could be explained by the hydroxy group in the *para*-position of the phenyl ring in the 6-position. The compound binds to the the receptor in a different binding mode and the hydroxy group might interact in the same binding pocket where the methoxy group of the side chain phenyl ring normally binds. The activity of compound **226** could be explained by the additional fluorine substituent in the side-chain. We previously identified a fluorine in the *meta*-position as an enhancer of the potency of the compounds (Figure 3.3).

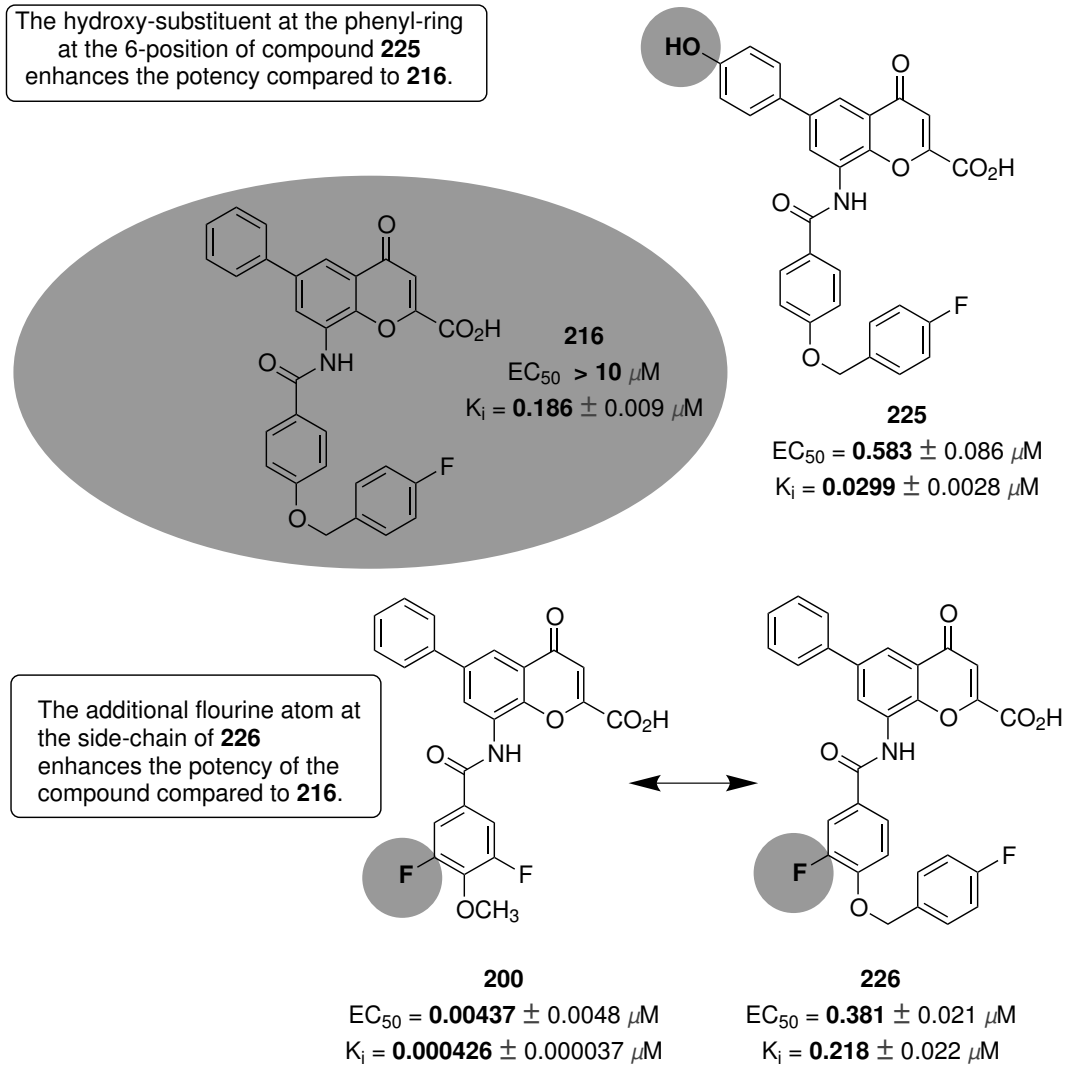
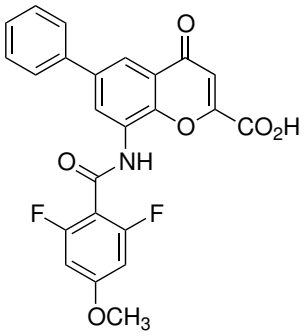


Figure 3.3: Small structural modifications convert the inactive compound **216** to GPR35 agonists with moderate potency.

Combining the *ortho*-difluoro-4-methoxyphenyl side chain with the phenyl substituted chromen-4-one core structure resulted in the most potent hGPR35 agonist known to date (**210**, EC_{50} = **0.00108** ± 0.00011 μM) (Figure 3.4).



210

EC_{50} = **1.08** ± 0.11 *n*M
K_i = **0.426** ± 0.037 *n*M

Figure 3.4: Compound **210** - the most potent agonist at human GPR35 known to date is able to activate the receptor with a high potency and binds with nanomolar affinity.

We also performed a ring closure which was inspired by the bufrolin derivative **277**. Bufrolin (Figure 3.8) had been identified as a potent agonist at human and rat GPR35.[215] Like our compound series displayed in Table 3.17 it has 2 carboxylic acids but the scaffold is a phenanthroline derivative which is built up of three rings. We therefore decided to perform a ring closure also at our chromen-4-ones instead of the side chain. However the resulting compounds (**272** EC_{50} = **0.037** ± 0.0021 μM and **273** EC_{50} = **0.0295** ± 0.0046 μM) were less active than the corresponding open form. It seems not to fit perfectly into the binding pocket and some flexibility or less planarity is required. Surprisingly the compound which has methyl in the 6-position was a little bit more active than the bromine-substituted compound. The bufrolin derivative **277** also was 20 fold less potent than the most potent compound **210**.

All SARs determined for the human GPR35 are illustrated in Figure 3.5.

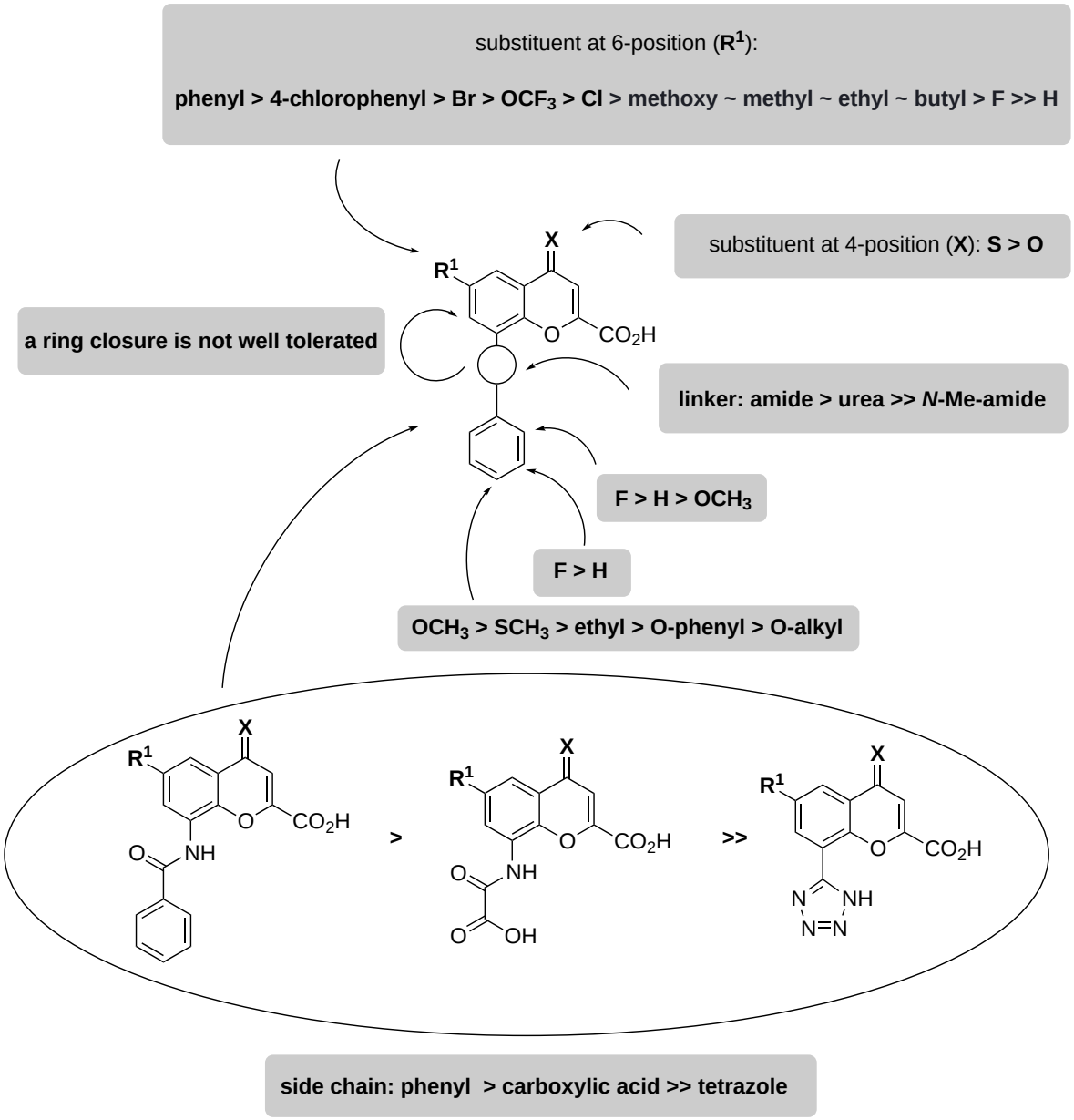


Figure 3.5: Structure-activity relationships determined for human GPR35.

We can conclude that a large, electron-rich substituent is beneficial at the 6-position. The 4-methoxyphenyl side-chain connected with an unsubstituted amide linker and substituted with fluorine atoms turned out to be optimal at the 8-position. Furthermore the replacement of the oxygen at position 4 by a sulfur atom slightly increased the potency of the compounds.

3.2.3.3 Affinity of chromenones at the human GPR35

The affinity of chromen-4-ones at the human GPR35 was determined by radioligand binding experiments using the radioligand [^{3}H]PSB-13253[65] which had been developed in our group. The affinity of GPR35 agonists mostly correlated with the potency determined in β-arrestin assays. Compounds that showed high agonistic potency at GPR35 also showed a high affinity. However we observed some notable differences.

When we analyzed the 6-position we saw that the rank order of affinity was comparable to that we observed for the previously determined potency. Earlier studies indicated that halogen atoms have a larger effect on the affinity than on the potency compared to a methoxy group or an alkyl-chain.[65] This could not be confirmed. However the chlorine atom in the 6-position increased the affinity on a higher level than the potency (**192**, EC_{50} = **0.0293** ± 0.0015 μM; K_i = **0.00716** ± 0.00012 μM).

The most potent agonist at the human GPR35, compound **210** also showed the highest affinity of all tested chromen-4-ones (EC_{50} = **0.00108** ± 0.00011 μM; K_i = **0.000426** ± 0.000037 μM).

The results of the binding studies with the larger chromenones shown in Table 3.19 and Table 3.20 were more surprising. These compounds mostly did not show agonistic activity at GPR35 in the β-arrestin recruitment assays. In the radioligand binding experiments however some of the compounds showed high affinities. To determine if they were antagonists at GPR35, they were tested in the β-arrestin recruitment assay for antagonism with the result that surprisingly none of the compounds showed antagonistic activity at GPR35. These compounds are able to displace the binding of [^{3}H]PSB-13253 at GPR35 with high affinity but they are not able to activate or block the β-arrestin signalling pathway. These compounds may be so-called "biased agonists" with a functional selectivity for the G protein. Biased agonists are interesting tools as they avoid the functional antagonism which is a result of the recruitment of arrestins which results in receptor internalization. As a consequence they could be able to activate the receptor permanently. However the hypothesis that the compounds are biased agonists, has to be confirmed by further studies with functional assays that are able to measure the activity of the G protein independently from β-arrestin. One possibility would be the performance of assays using chimeric G proteins.

The SARs for the potential biased agonists were interesting. First of all the compounds were much larger and more lipophilic than the full agonists shown in Table 3.17 and Table 3.18. Large side-chains at the 8-position were well tolerated. Thereby a substitution of the phenyl-amide at the 8-position in the *para*-position resulted in higher affinities than the substitution in the *meta*- or in the *ortho*-position. The affinity increased with the length of the side-chain. At the end of the side-chain a cyclohexyl-ring as well as a phenyl-ring were well tolerated. The compounds shown in Table 3.19, whose side-chain had a length of only one C-atom showed moderate affinities in the higher nanomolar range. However there was one exception. Compound **225**, which showed also a moderate potency at the human GPR35, showed a high affinity at the human GPR35 in the lower nanomolar range (EC_{50} = **0.583** ± 0.0086 μM; K_i = **0.0299** ± 0.0028 μM). The introduction of a hydroxy group in the *para*-position of the phenyl-ring in position 6 resulted in a 6-fold increase in affinity compared to the hydrogen-substituted compound **216** (EC_{50} > 10 μM; K_i = **0.186** ± 0.009 μM). Whereas the introduction of a fluorine atom at the first phenyl-ring of the side-chain showed a high effect on the potency, it did not have any effect on the affinity (**279**, EC_{50} = **0.381** ± 0.021 μM; K_i = **0.218** ± 0.022 μM) (Figure 3.3).

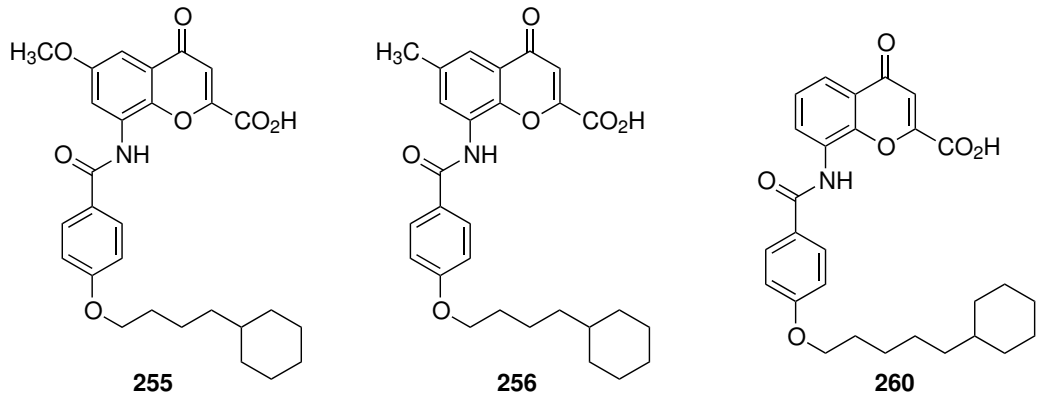


Figure 3.6: Potential biased agonists for the human GPR35 with high affinity and potential functional selectivity for the G protein.

Compounds with longer alkyl side-chains in the *meta*-position of the phenyl-amide showed only moderate affinities at the human GPR35 in the high nanomolar or micromolar range. However, when the side-chain was moved to the *para*-position

the affinity was extremely increased. The substituent in the 6-position thereby did not have a significant effect on the affinity, apart from an ethyl group which resulted in a complete loss of affinity. The effect of the length of the side-chain could not be determined due to the lack of a series of structures with comparable substituents in the 6-position.

The potential biased agonists with the highest affinity were compounds **255** (EC_{50} > 10 μM; K_i = **0.012** ± 0.005 μM), **256** (EC_{50} > 10 μM; K_i = **0.0172** ± 0.0002 μM), and **260** (EC_{50} > 10 μM; K_i = **0.0155** ± 0.0012 μM) which showed affinities in the low nanomolar range but were completely inactive in the β-arrestin recruitment assays (Figure 3.6).

3.2.3.4 Rat GPR35

The SARs determined for the rat GPR35 were different from the ones at the human receptor. When we analyzed the 6-position of the compounds we saw that the rank order of potency for the halogens and hydrogen is comparable to that observed at the human receptor. The methoxy substituent however was not tolerated and comparable to hydrogen. The next interesting point are alkyl chains of different lengths. We observed an increase in potency which is connected to an increase in the number of C-atoms. As a result we get the following rank order of substituents: butyl (**196** EC_{50} = **0.0297** ± 0.0040 μM) > bromine (**188** EC_{50} = **0.0451** ± 0.0065 μM) > ethyl (**195** EC_{50} = **0.0750** ± 0.0020 μM) ≈ chlorine (**192** EC_{50} = **0.0800** ± 0.0031 μM) > phenyl (**190** EC_{50} = **0.154** ± 0.015 μM) > fluorine (**191** EC_{50} = **0.263** ± 0.034 μM) > methyl (**194** EC_{50} = **0.406** ± 0.036 μM) > hydrogen (**189** EC_{50} = **0.779** ± 0.097 μM) ≈ methoxy (**193** EC_{50} = **0.851** ± 0.102 μM).

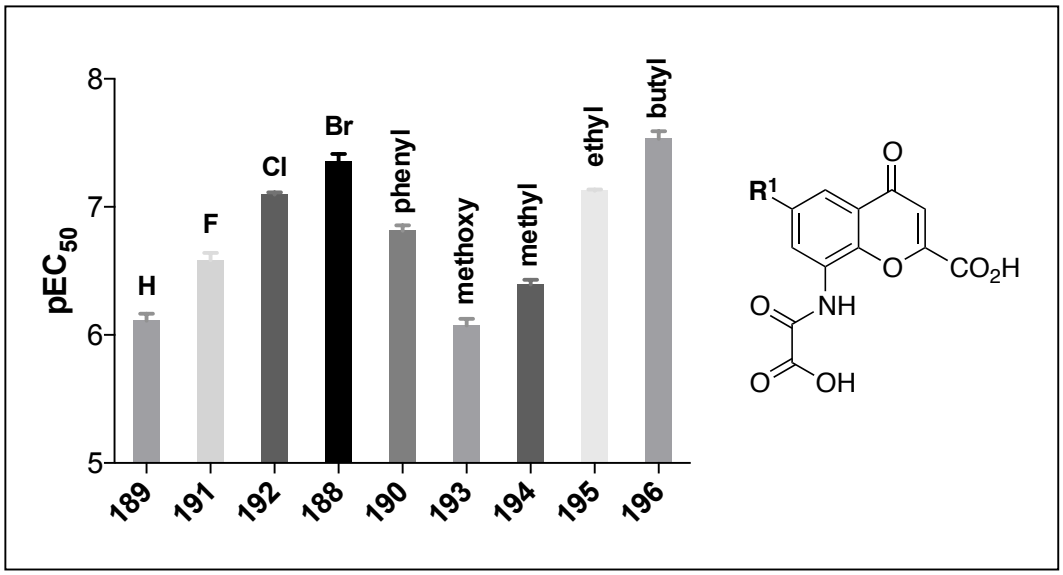


Figure 3.7: Structure-activity relationships of substituents at the 6-position of chromen-4-one derivatives determined for the rat GPR35. The pEC_{50} values ± SEM were calculated from EC_{50} values that were determined by β-arrestin recruitment assay.

The binding pocket of the rat GPR35 is different from the one of the human receptor. There seems to be a lot of space so that big groups like bromine are beneficial. However lipophilicity appears to be more important because long alkyl chains are the best substituents in this position.

When we look at the second series of compounds which contains a phenyl ring instead of the second carboxylic acid at the 8-substituent, we cannot clearly determine SARs because the potency of the compounds was very low. The bromine-substituted compound (**MF338**[95] EC_{50} = **4.17** ± 0.020 μM) only activates the receptor in the micromolar range. However phenyl (**205** EC_{50} = **0.195** ± 0.010 μM) and *p*-chlorophenyl (**208** EC_{50} = **0.263** ± 0.034 μM) are well tolerated at the 6-position. Also OCF_3 (**204** EC_{50} = **0.345** ± 0.043 μM) and ethyl (**206** EC_{50} = **0.428** ± 0.056 μM) increased the potency of the compounds compared to hydrogen (**MF_AM11**[95] EC_{50} = **2.16** ± 0.22 μM). Methyl (**201**[65] EC_{50} = **1.20** ± 0.26 μM) and methoxy (**202**[65] EC_{50} = **1.52** ± 0.12 μM) were not tolerated as well which matches to our previous results. One explanation for the differences in SARs at the 6-position could be that the compounds bind to the receptor in a different orientation. This should be further investigated using computational methods.

As we also had observed for the human receptor the replacement of the oxygen at

the 4-position of the chromen-4-one by sulfur resulted in an increase in potency. Compound **197** (EC_{50} = **0.0209** ± 0.0023 μM) was about 2-fold more potent than compound **188** (EC_{50} = **0.0451** ± 0.0065 μM) and compound **212** (EC_{50} = **0.552** ± 0.042 μM) was even almost 8-fold more potent than compound **MF338**[95] EC_{50} = **4.17** ± 0.020 μM).

It is noticeable that the introduction of fluorine atoms at the side chain significantly increased the potency of the compounds. Especially the *ortho*-substitution had an enormous effect. The mono-substituted compound **198** (EC_{50} = **0.234** ± 0.040 μM) is almost 20-fold more potent than the unsubstituted derivative (**MF338**[95] EC_{50} = **4.17** ± 0.020 μM). Di-substitution resulted in an even higher potency (**199** EC_{50} = **0.199** ± 0.039 μM) whereas *meta*-substitution had the smallest effect (**200** EC_{50} = **0.769** ± 0.038 μM). These results were corroborated by a second pair of compounds: the *ortho*-difluoro derivative **205** (EC_{50} = **0.157** ± 0.013 μM) was more potent than the unsubstituted compound **205** (EC_{50} = **0.195** ± 0.010 μM). These results correspond to the results determined for the human receptor.

Interestingly also an *ortho*-substitution with methoxy was beneficial (**203** EC_{50} = **0.493** ± 0.081 μM) and increased the potency by about 8-fold.

The effect of the replacement of *para*-methoxy by other groups was also investigated. Both, ethyl (**207** EC_{50} = **0.857** ± 0.211 μM) and thiomethyl (**209** EC_{50} = **0.748** ± 0.045 μM) were preferred by the receptor.

The modification of the amide linker also resulted in interesting SARs. The methylation of the amide was absolutely not tolerated and completely abolished the activity of the compound (compound **211**). In contrast to that the thiourea linker increased potency significantly. Compound (**264** EC_{50} = **0.288** ± 0.034 μM) showed an unexpectedly high potency in the nanomolar range although it is much larger and more lipophilic compared to the other active compounds. Due to the lack of directly corresponding compounds we could not draw clear conclusions about the urea linker, but it seems to be about equally beneficial as the amide linker as we could not observe any remarkable effect.

The most active compounds so far were the compounds which contain two carboxylic acid functions. We performed a ring closure for some compounds of this series which did not have any negative but also no positive effect on the potency of the compounds (**272** EC_{50} = **0.487** ± 0.064 μM and **273** EC_{50} = **0.470** ± 0.042 μM). The

bufrolin derivative **277** however was much more potent (EC_{50} = **0.0263** ± 0.0023 μM). If we compare the structure of the compounds it is obvious that the direction of the ketone next to the 8-position of the chromen-4-one is really important for the potency (Figure 3.8).

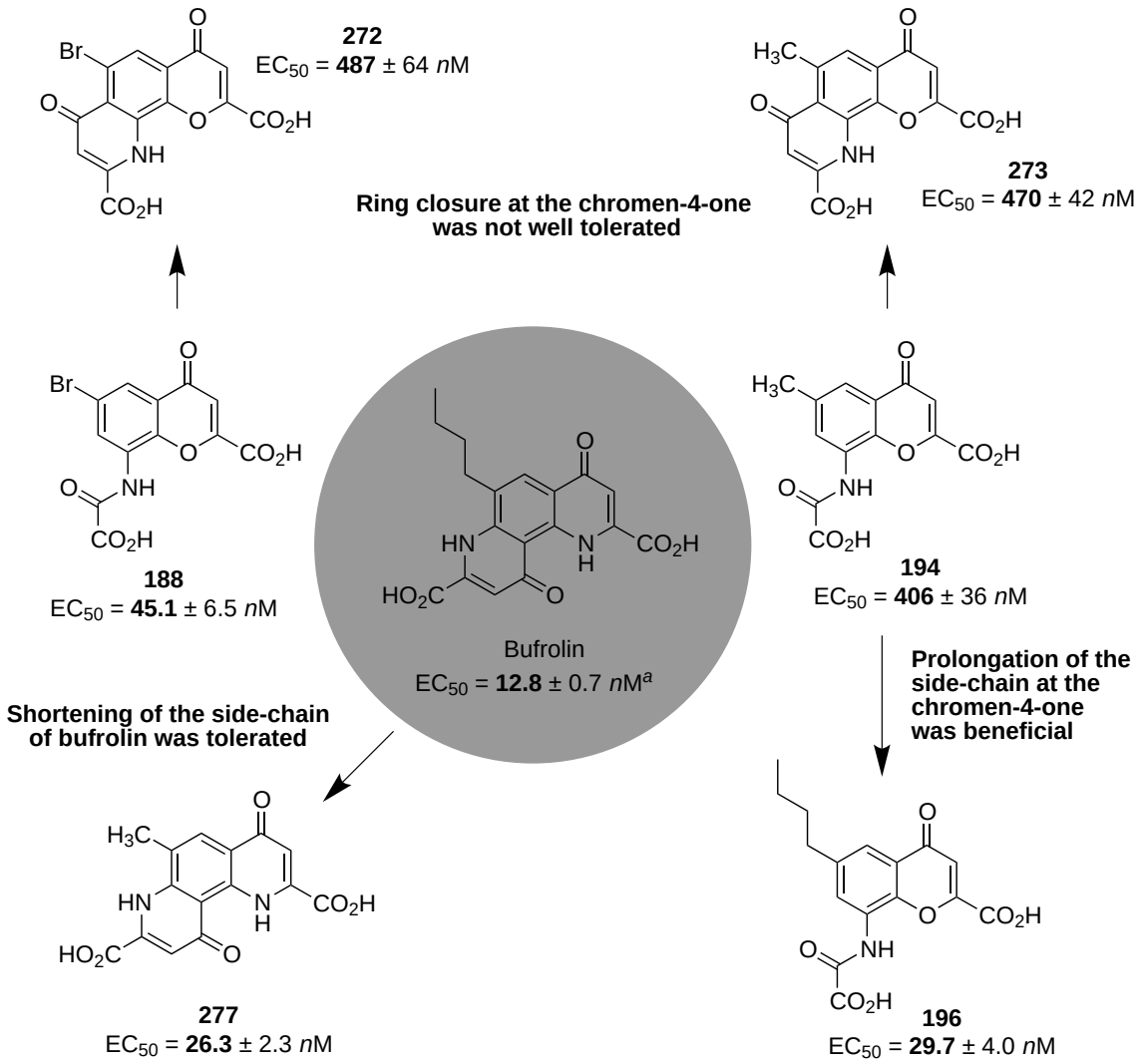


Figure 3.8: Structure-activity relationships determined for the rat GPR35 agonists that share substructures with the GPR35 agonist bufrolin. [a]Bufrolin was tested by MacKenzie et al. using the Pathhunter® β-arrestin recruitment assay.[85]

The last modification was the replacement of the acetic acid in the side chain by a tetrazole which is also acidic. However the tetrazole is not able to interact with the receptor in the same way which resulted in a loss of potency (**268** EC_{50} = **1.91** ± 0.270 μM). This could also be explained by the lack of the amide, especially

the oxygen, which was previously identified as an important factor for the potency. The 1*H*-tetrazole is a frequently used bioisosteric modification in drug design as it shows a similar clogP than the carboxylic acid.[216]

All SARs determined for rat GPR35 are illustrated in Figure 3.9. All compound which did not show agonistic activity were also tested for antagonism at the rGPR35 but none of the compounds showed any antagonistic activity in the β-arrestin recruitment assays.

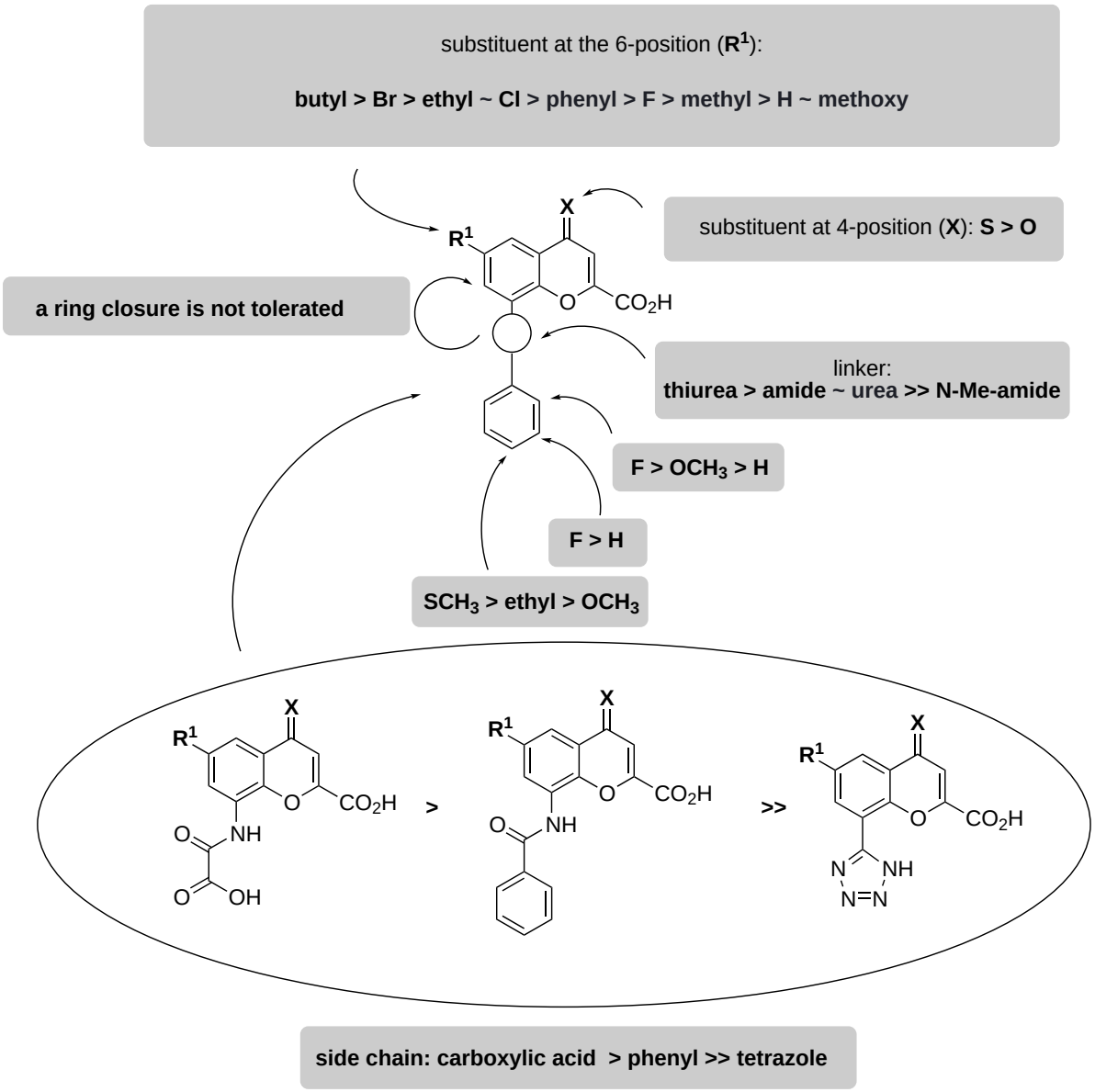
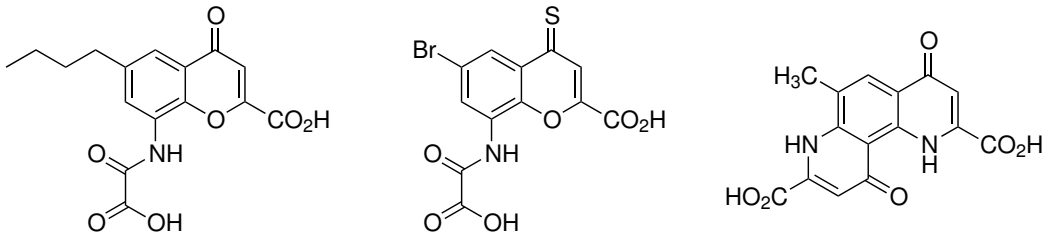


Figure 3.9: Structure-activity relationships of chromen-4-ones with different substituents determined for the rat GPR35.

In conclusion the SARs determined for rat GPR35 differ in some regions from those determined for the human receptor. This issue will be discussed in section 3.2.5..

The most potent agonist for the rat GPR35 is **197** with an EC_{50}-value of **0.0209** ± 0.0023 μM (Figure 3.10).



196 **197** **277**

EC_{50} = **29.7** ± 4.0 *n*M EC_{50} = **20.9** ± 2.3 *n*M EC_{50} = **26.3** ± 2.3 *n*M

Figure 3.10: The most potent agonists at rat GPR35.

Based on the results of this study, further optimizations can be done to increase the potency of the GPR35 agonist at the rat orthologue. The replacement of the oxygen at the 4-position of **196** by sulfur could be beneficial. A modification of the alkyl side-chain at position 6 has also to be investigated. A prolongation or the replacement by branched alkyl side-chains as well as the introduction of bromoalkyl residues would be interesting.

3.2.3.5 Mouse GPR35

The SARs of chromen-4-ones for the mouse GPR35 are hard to determine. One fact is that almost all compounds which were tested activated the receptor in the micromolar range although they were structurally completely different. Only few compounds were able to activate the receptor at lower concentrations. But what we still definetely can see is that the smaller and less lipophilic compounds from series one (Table 3.17) and series two (Table 3.18) were usually more potent.

The SARs for the 6-position corresponded well to the SARs for the rat orthologue. Long aliphatic alkyl chains were found to be beneficial, the longer the better. Also phenyl was a suitable substitutent followed by the halogens bromine and chlorine. Fluorine, methoxy and hydrogen resulted in a loss of potency. As a result we got the following rank order of potency: butyl (**196**, EC_{50} = **0.399** ± 0.083 μM) > phenyl

(**190**, EC_{50} = **0.494** ± 0.083 μM) > ethyl (**195**, EC_{50} = **1.00** ± 0.30 μM) > chlorine (**192**, EC_{50} = **1.71** ± 0.46 μM) > bromine (**188**, EC_{50} = **1.88** ± 0.44 μM) > fluorine (**191**, EC_{50} = **4.76** ± 0.32 μM) ≈ methoxy (**193**, EC_{50} = **4.71** ± 0.53 μM) > hydrogen (**189**, EC_{50} > 10 μM).

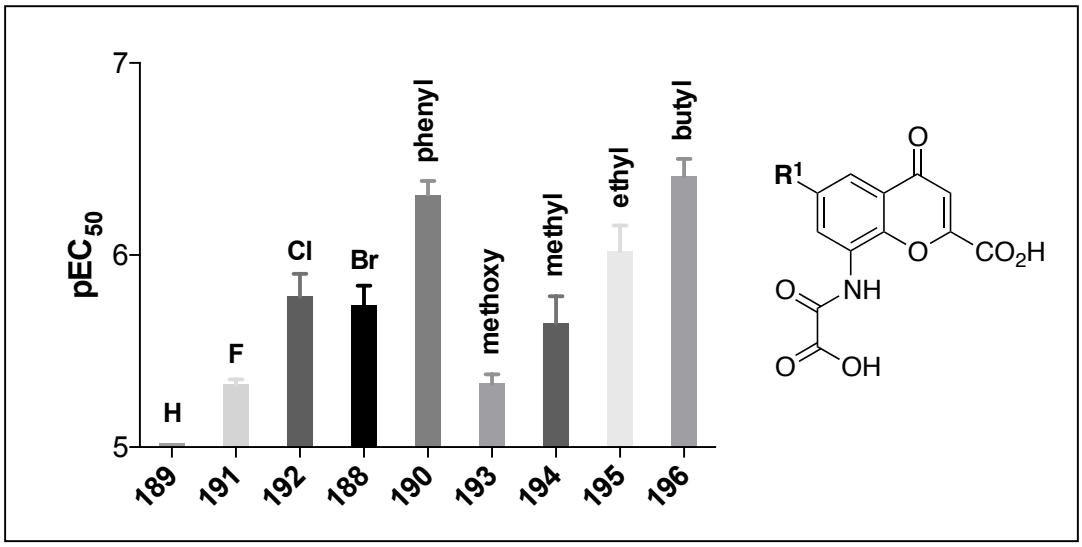


Figure 3.11: Structure-activity relationships of substituents at the 6-position of chromen-4-one derivatives determined for the mouse GPR35. The pEC_{50} values ± SEM were calculated from EC_{50} values that were determined by β-arrestin recruitment assay.

Although the the rank order of potency for the substituents corresponded to that observed for the rat GPR35 the potency of the mGPR35 agonists was on average about 10-fold lower compared to the EC_{50}-values determined at the rat GPR35 (Figure 3.11). The compounds shown in Table 3.18 were less active and SARs could hardly be determined. However, like for the rat GPR35, the phenyl-substituted compound (**205**, EC_{50} = **0.459** ± 0.104 μM), the *p*-chlorophenyl-substituted compound (**208**, EC_{50} = **0.724** ± 0.140 μM) and the ethyl derivative (**206**, EC_{50} = **1.83** ± 0.04 μM were the most active compounds.

A fluoro-substitution at the side-chain further increased potency of the compounds. Compound **280**, (EC_{50} = **0.293** ± 0.042 μM), which contains the *ortho*-difluoro-substituent was about 2-fold more potent than the non-substituted compound **205** (EC_{50} = **0.459** ± 0.104 μM). An *ortho*-substitution seemed to be preferred. The *meta*-substituted compound **200**[65] (EC_{50} = **3.52** ± 0.76 μM) was less active than the *ortho*-substituted **198**.[65] A second fluorine in the *ortho*-position did not have any effect (**199**,[65] EC_{50} = **2.85** ± 0.41 μM).

When we looked at the linker we observed again that methylation of the amide (**211**, EC_{50} = > 10 μM) resulted in a complete loss of activity. Neither the urea nor the thiourea linker resulted in a change in potency. However what we have to keep in mind is that we do not have a series of compounds that is really comparable.

As it was also found for the human and the rat receptor the replacement of the oxygen at the 4-position by sulfur had a positive effect on the potency of the mGPR35 agonists. Compound **197** (EC_{50} = **0.174** ± 0.019 μM) was about 10-fold more potent than compound **188** (EC_{50} = **1.88** ± 0.44 μM). The same applied to the compounds from series 2 (Table 3.18. Replacement of oxygen by sulfur in compound **MF338**,[95] which was completely inactive, resulted in the mGPR35 agonist **212** (EC_{50} = **1.31** ± 0.07 μM) which showed a potency in the micromolar range.

One interesting compound is **225** (EC_{50} = **0.218** ± 0.059 μM) which had an unexpectedly high potency. We already saw this phenomenon at the rat receptor. Compound **225** contains a hydroxyphenyl-group at the 6-position.

The dicarboxylic acid derivatives shown in Table 3.17 were the most active compounds so far. The ring closure did not have any effect on potency at rat receptor or even resulted in a loss of activity at the human receptor. For the mouse receptor we observed the opposite. The ring closure (**272** EC_{50} = **0.910** ± 0.120 μM and **273** EC_{50} = **0.955** ± 0.229 μM) resulted in an increase in potency compared to the open form. The position of the oxygen of the amide does not seem to play an important role. This would also explain why we do not see any difference when we change the linker.

We also synthesized a derivative of bufrolin (**277**, EC_{50} = **0.105** ± 0.015 μM), which turned out to be the most potent compound (Figure 3.13). Ring closure fixes the conformation of the compound which is no longer flexible. If a sterically fixed conformation represents the active conformation of a ligand it can be expected to be more potent than flexible analogues.

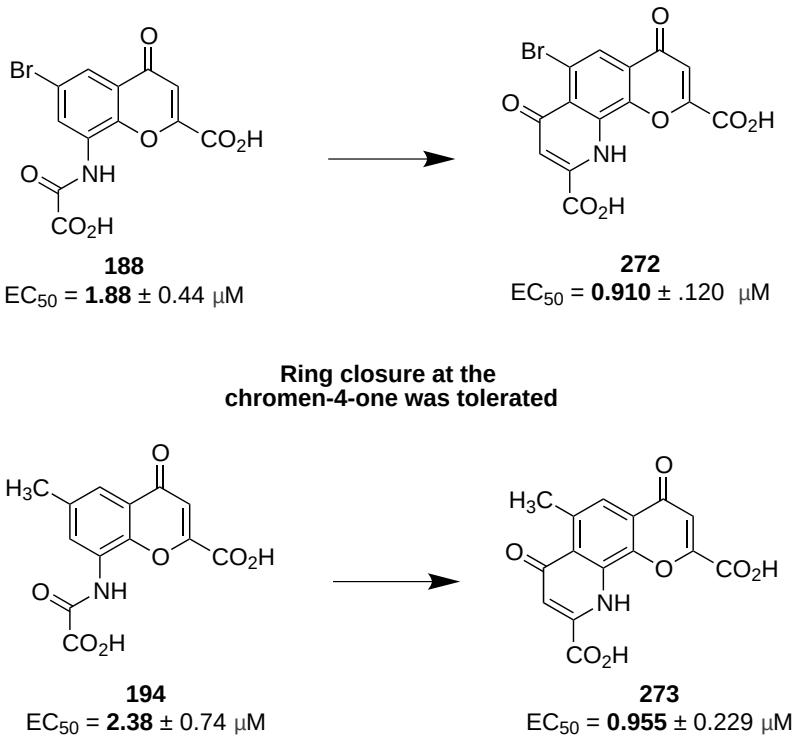


Figure 3.12: The ring closure at the chromen-4-ones enhances the potency of the mouse GPR35 agonists.

The best mouse GPR35 agonist developed so far is **277** (EC_{50} = **0.105** ± 0.015 μM) which is not based on the chromen-4-one scaffold. The best chromen-4-one based compound is **197** (EC_{50} = **0.174** ± 0.019 μM) (Figure 3.13). All compound which did not show agonistic activity were also tested for antagonism at the mGPR35 but none of the compounds showed any antagonistic activity in the β-arrestin recruitment assays.

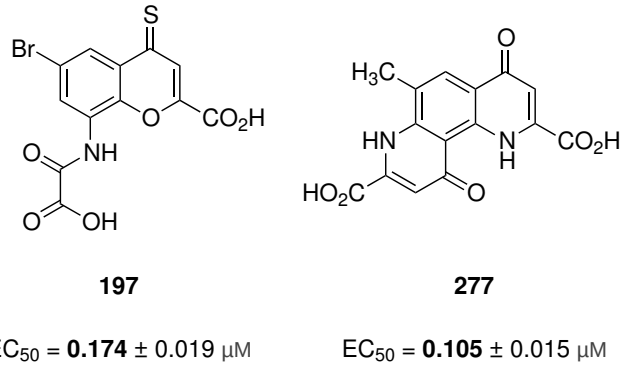


Figure 3.13: Compounds **277** and **197**-the most potent agonists for the mouse GPR35

All in all the compounds are less active at the mouse orthologue than at the rat-orthologue and the human receptor. The fact that many compounds show a low activity at the mGPR35 indicates that most of the chromen-4-ones do not perfectly fit into the binding pocket. The SARs determined for the mouse receptor are shown in Figure 3.14. However, with a potency around 100 nM our mGPR35 agonist are highly interesting tools for mouse experiments since only few GPR35 agonist for the mouse orthologue have been identified so far.

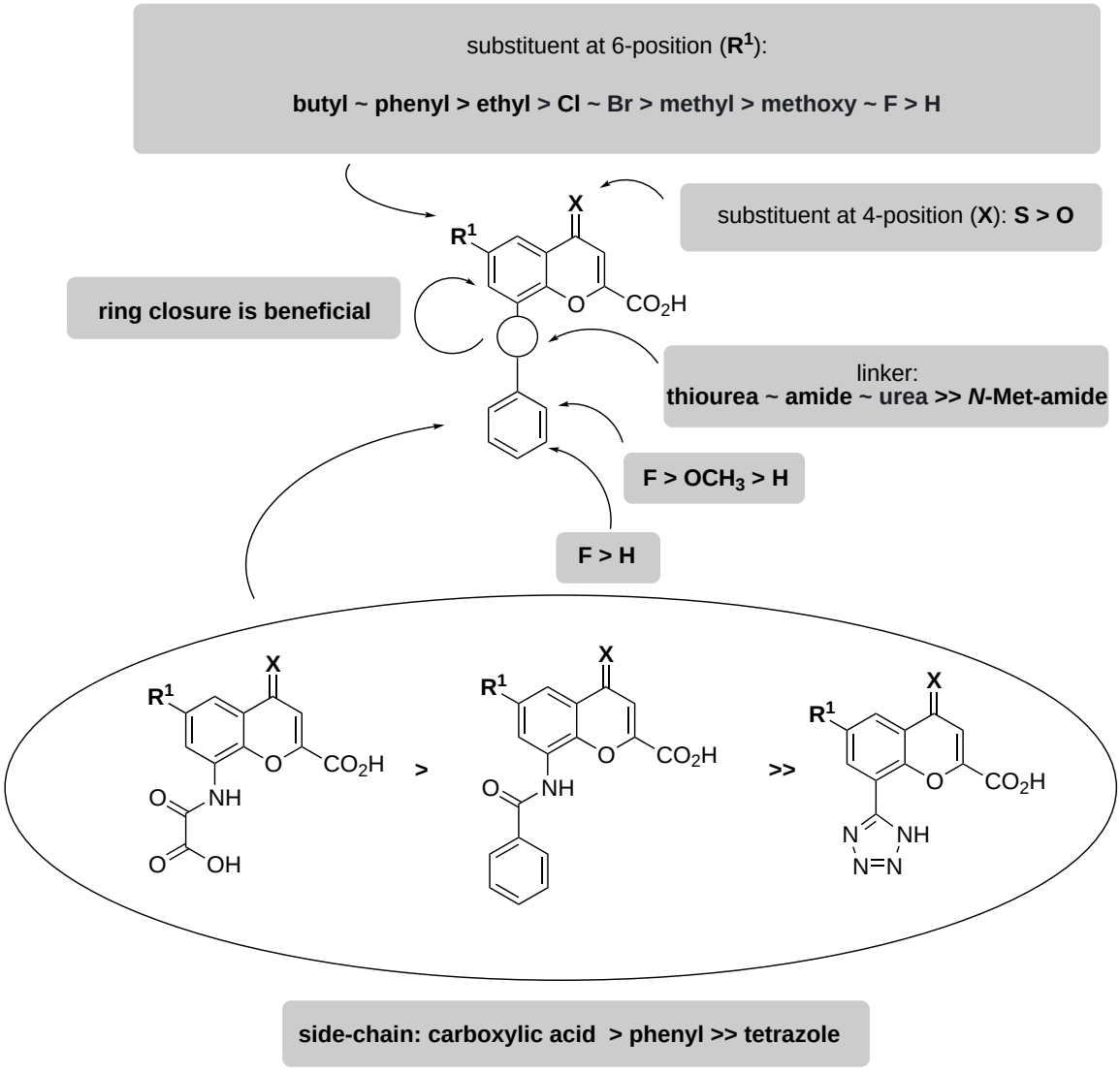


Figure 3.14: Structure-activity relationships determined for the mouse GPR35.

For further optimization it would be interesting to synthesize compounds containing a sulfur atom at the 4 position of the chromen-4-one with longer alkyl-residues or

a phenyl ring at position 6. Additionally ring closure could be beneficial.

3.2.4 GPR35 orthologue selectivity

The comparison of the SARs at the different orthologues shows that there are substantial differences in the potency of the compounds. It is obvious that on average the compounds are more potent at the human GPR35 than at the rodent receptors. The most potent compound **210** (hGPR35: EC_{50} = **0.00108** ± 0.00011 μM; rGPR35: EC_{50} = **0.157** ± 0.013 μM; mGPR35: EC_{50} = **0.293** ± 0.042 μM) is about 150-fold more potent at the human receptor than at the rodent orthologues (Figure 3.15). However, for animal studies we also need potent ligands for the mouse and rat GPR35.

Changing the substituents at the side-chain phenyl ring did not increase the potency for rodent receptors. A fluorine substituent at the phenyl side chain improved the potency at all receptors. What could also be seen in every species was the effect of the replacement of the oxygen at the 4-position of the chromen-4-one by a sulfur. Compounds containing a sulfur were more potent compared to their oxygen-analogues perhaps due to to increased lipophilicity. This indicates that the oxygen atom is not forming hydrogen-bonds since sulfur is a much weaker H-bond acceptor.

When we compare the effect of substituents at the 6-position we see differences. Bromine is only tolerated by the human receptor whereas the rodent receptors prefer long aliphatic chains or a phenyl ring. The clearest differences could be observed when we changed the side-chain at the 8-position. The introduction of a second acidic function significantly improved the potency of the compounds at the rodent receptors whereas the potency at the human receptor was decreased (**197** hGPR35: EC_{50} = **0.00847** ± 0.00072 μM; rGPR35: EC_{50} = **0.0209** ± 0.0023 μM; mGPR35: EC_{50} = **0.174** ± 0.019 μM) (Figure 3.15). The ring-closure yielding phenanthroline derivatives improved only the potency at the mouse receptor but decreased the potency at the human and the rat orthologue.

Compound **225** hGPR35: EC_{50} = **0.583** ± 0.086 μM; rGPR35: EC_{50} = **0.140** ± 0.022 μM; mGPR35: EC_{50} = **0.218** ± 0.059 μM) interestingly was about 3 folds more potent at the rodent receptors than at the human receptor (Figure 3.15). It contains a phenol and a long lipophilic side chain which might not be well tolerated by the human receptor (Figure 3.15).

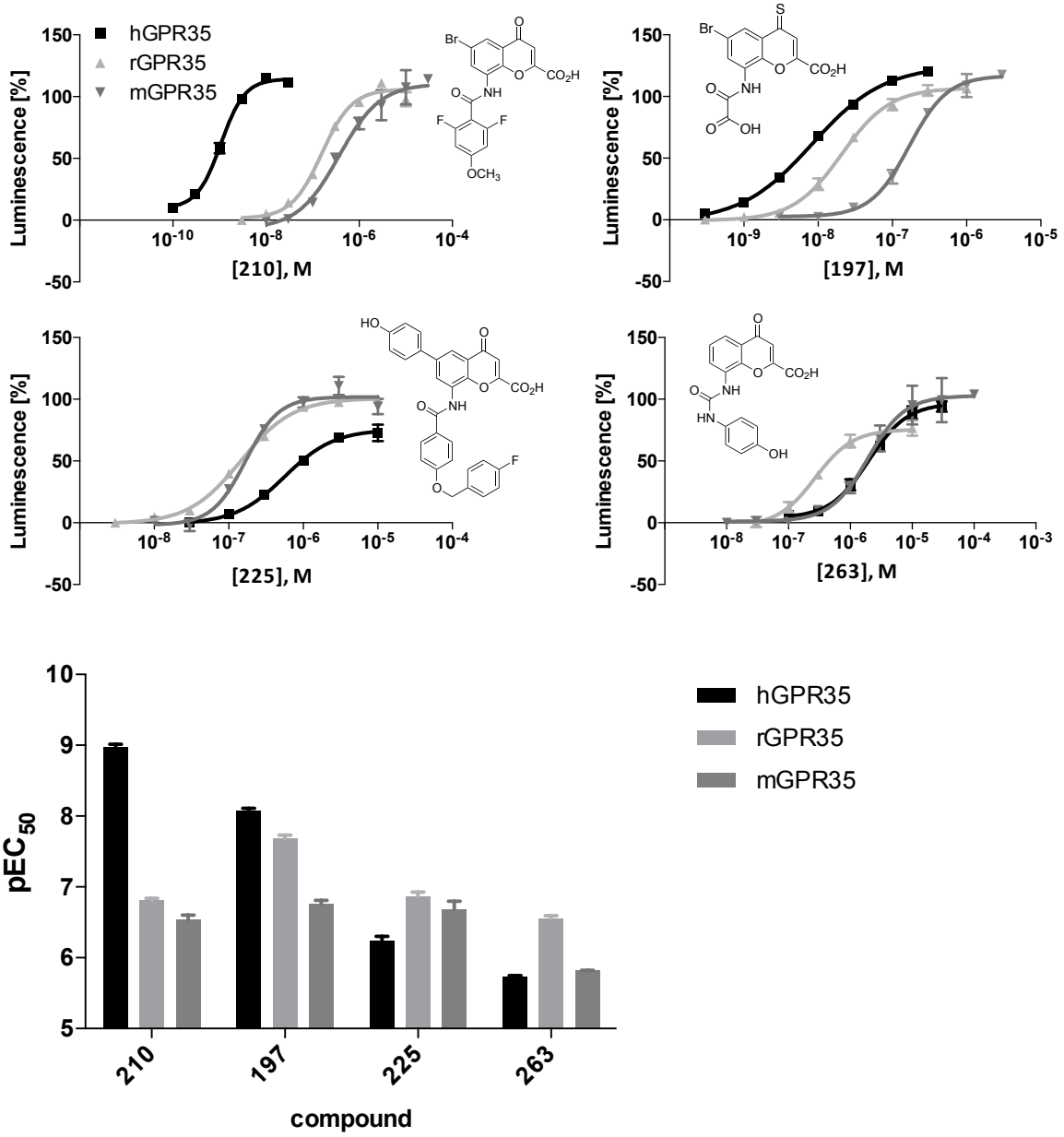


Figure 3.15: GPR35 agonists with high species selectivity. The pEC_{50} values ± SEM were calculated from the EC_{50} values that were determined in β-arrestin recruitment assay.

We also identified one compound which was selective for the rat GPR35. Compound **263** (hGPR35: EC_{50} = **1.87** ± 0.08 μM; rGPR35: EC_{50} = **0.288** ± 0.034 μM; mGPR35: EC_{50} = **1.54** ± 0.26 μM) contains an urea linker and a phenol in the

para-position of the side-chain. It is about 6-fold more potent at the rat orthologue than at the human or the mouse receptor. The compounds that show a high species selectivity are shown in Figure 3.15.

We identified also compounds that are almost equipotent at the human and the rodent orthologues. These compounds contain two carboxylic acid functions. If we compare the substituents at position 6 of the chromen-4-ones it can clearly be observed that an introduction of an alkyl side-chain at this position results in a decreased species selectivity between the human and the rat orthologue. Compound **277** (hGPR35: EC_{50} = **0.0214** ± 0.0021 μM; rGPR35: EC_{50} = **0.0263** ± 0.0023 μM; mGPR35: EC_{50} = **0.105** ± 0.015 μM) is the most balanced compound. Planarity could be a feature to design this class of GPR35 agonists (Figure 3.16).

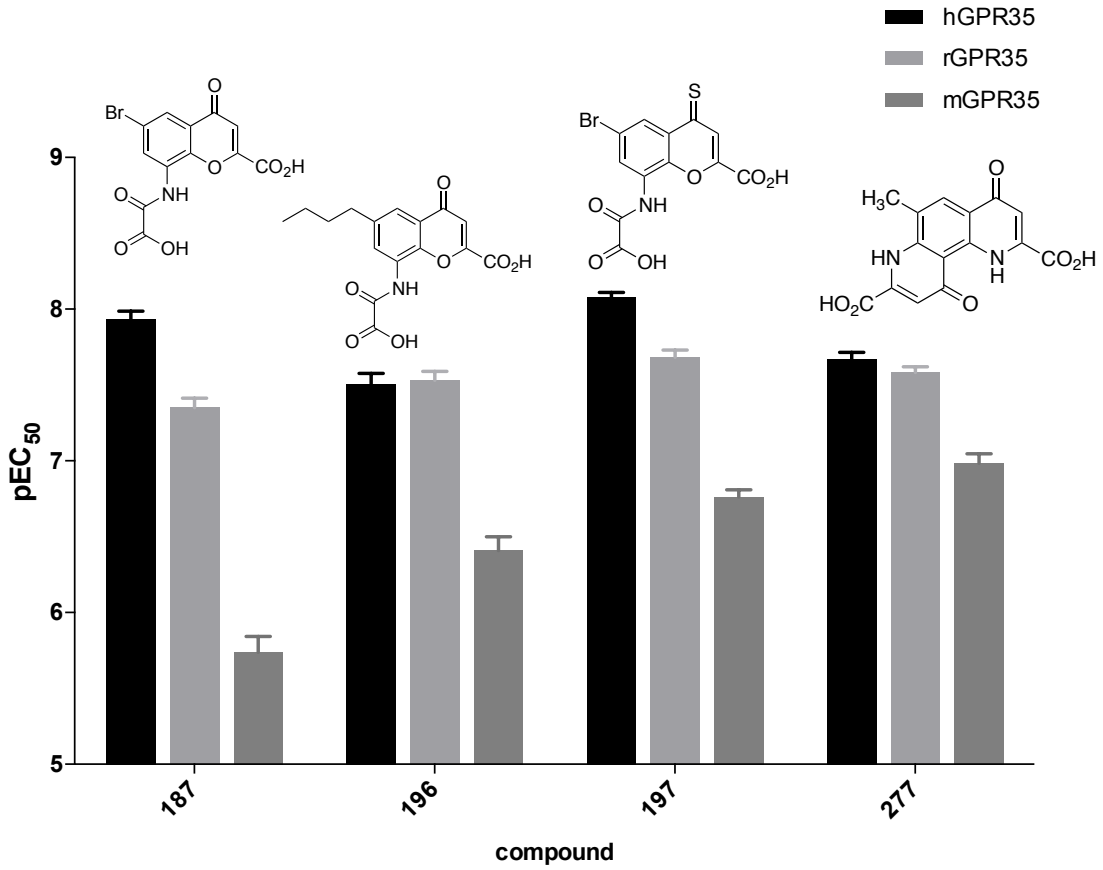


Figure 3.16: GPR35 agonists with low species selectivity

Physicochemical properties were calculated for the most potent GPR35 agonists using Stardrop® (Figure 3.17). All of the selected chromen-4-ones as well as the

bufrolin derivative **277** perfectly fit into the criteria of Lipinski's rule of five, which is a parameter for predicting the oral bioavailability of drugs.[217] Furthermore the TPSA (topological polar surface area), which is a criterion for the drug's ability to permeate cells, was below or equal to 140 Å which should allow cell permeability.

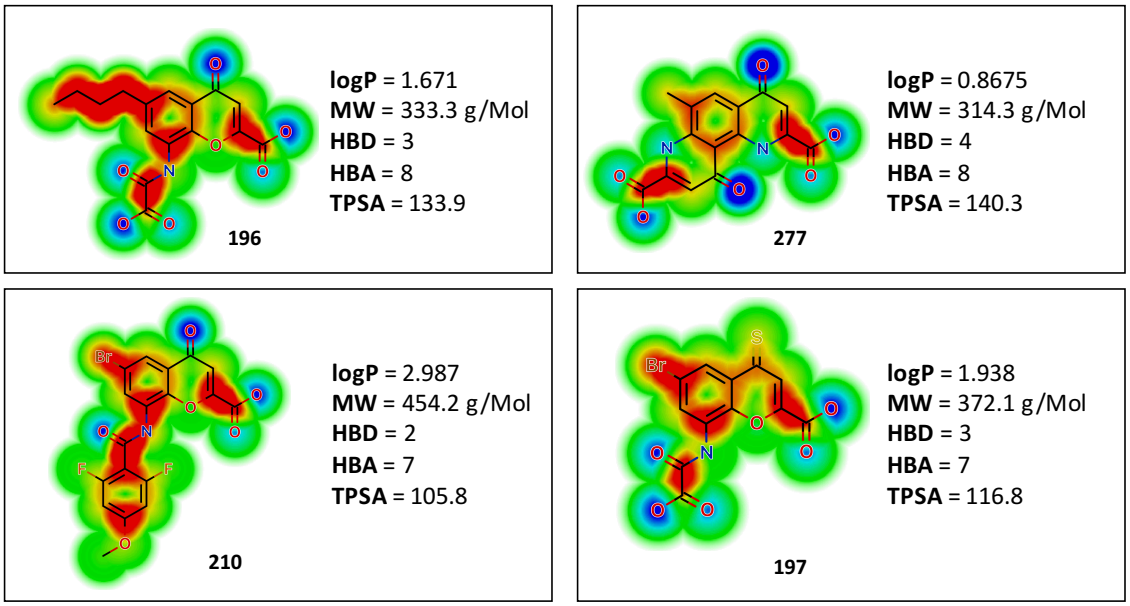


Figure 3.17: Physicochemical properties of selected GPR35 agonists. Analysis of the polarity of the structure performed with Stardrop®. Polar groups are shown in blue colour whereas unpolar parts of the molecule are shown in red colour. The logP, molecular weight (MW), hydrogen-bond donators (HBD), hydrogen-bond acceptors (HBA) and the topological polar surface area (TPSA) were calculated using Stardrop®.

In conclusion the second acidic function is the main factor that improves the potency at the rodent receptor whereas it is not needed for the potency at the human receptor. We designed GPR35 agonists with a high species selectivity as well as balanced compounds. Both give insight into the receptors' structures and can be used as pharmacological tools at human cells as well as in animal studies. The physicochemical properties indicate that the new GPR35 agonists are suitable lead compounds for the development of new drugs. Computational methods or mutagenesis studies could clarify which amino acids in the binding pocket are responsible or this species differences. It has to be kept in mind that molecular modeling is highly speculative since no X-ray structure of GPR35 is available, and only very few class A δ-branch GPCRs have been crystallized so far. In the future a radioligand with

high potency at the mouse and the rat orthologue should be developed based on our new agonists. Based on the results from the radioligand binding experiments with the human GPR35 it is likely that some of the compounds, that were inactive in functional β-arrestin assays, might be identified as biased agonists activating an alternative pathway.

3.2.4.1 Strategy for the development of GPR35 radioligands

[(^{3})H]PSB-13253 (6-bromo-8-(4-[(^{3})H]methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid) is a potent and selective radioligand for the *orphan* receptor GPR35 and was developed in our group. However, the radioligand displays a high orthologe selectivity for the human receptor.[65] We developed a series of GPR35 agonists that show high potency at the human receptor and at the murine orthologes. In the following a strategy for the development of a radioligand based on the new GPR35 agonists will be presented (Figure 3.18).

Compounds **277** which is based on bufrolin and the chromenone based compound **196** were the most balanced compounds of our series regarding their potency at the different orthologes of GPR35. They showed high potency at the human receptor as well as at the mouse and rat GPR35. Furthermore their structure offers the possibility for the introduction of a radioactive marker. The long aliphatic side-chain, which is present in both compounds, is a suitable structural element for the introduction of a tritium marker. Therefore these compounds represent lead structures for the development of a orthologe independent radioligand. The first step would be the optimization of the compounds based on the SARs we determined in our study. Therefore the ketones of the lead structures should be displaced by a thioketone which will enhance the potency. For the synthesis of a radioligand a unsaturated aliphatic chain should be introduced which could then easily be radiolabled using tritium gas.

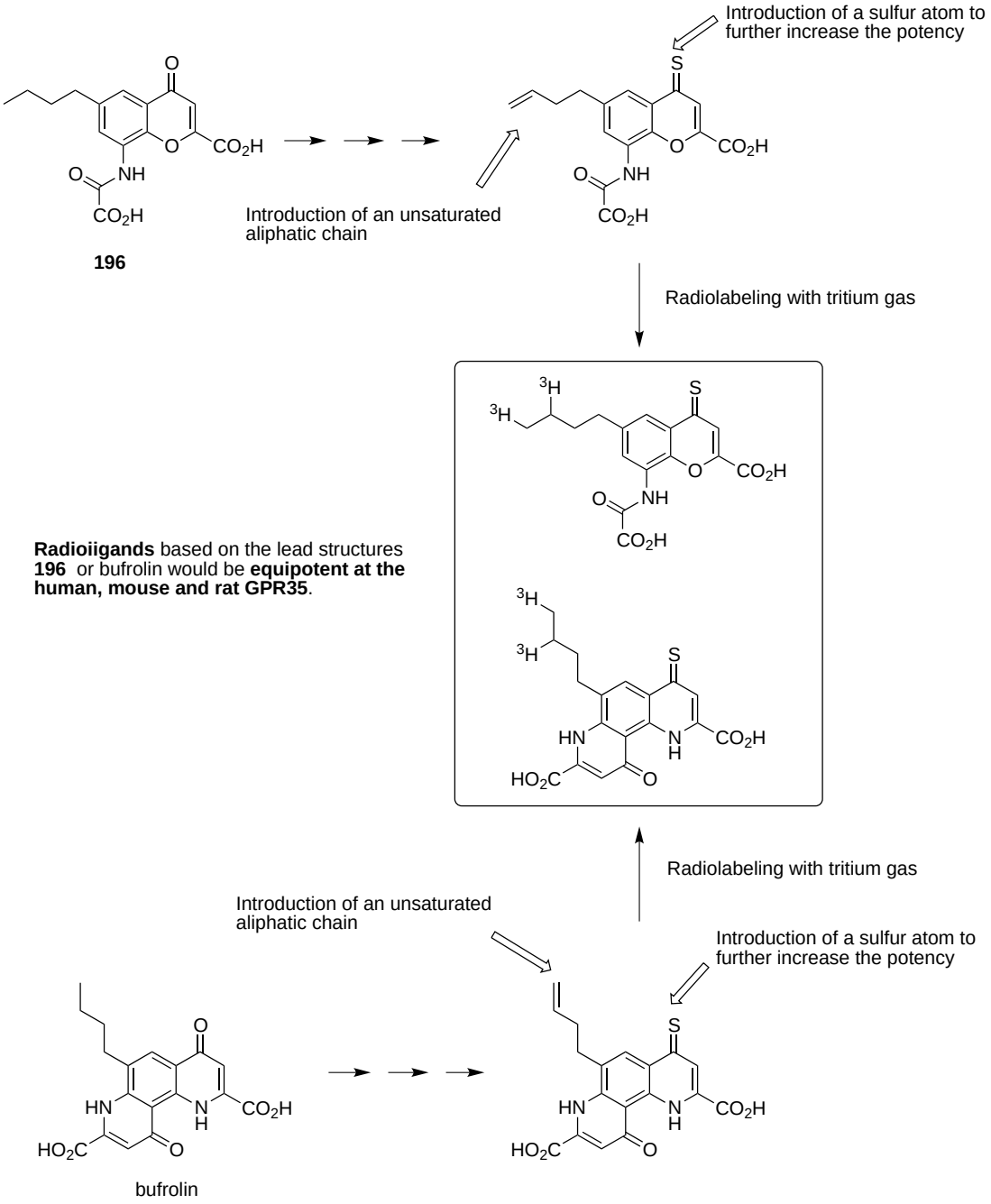


Figure 3.18: Strategy for the development of new radioligands as pharmacological tools for the human and the murine orthologes of the *orphan* receptor GPR35.

3.3 Pharmacological evaluation at the human GPR84

3.3.1 Introduction

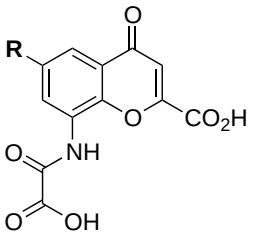
In contrast to GPR35, which was activated by chromenone derivatives, this class of compounds acted as antagonists of the (hydroxy) fatty acid-activated GPR84. The ability of chromen-4-one derivatives to block GPR84 was investigated in a radiometric cyclic adenosine monophosphate (cAMP) accumulation assay. This assay is based on the fact that activation of the G_i-coupled receptor GPR84 results in an inhibition of cAMP production. For the assay the cAMP production was stimulated by the addition of the diterpene forskolin (100 μM), which is a direct activator of adenylate cyclase. Decanoic was added as a GPR84 agonist and the cAMP accumulation was measured radiometrically in the absence and in the presence of test compounds.

3.3.2 Test results

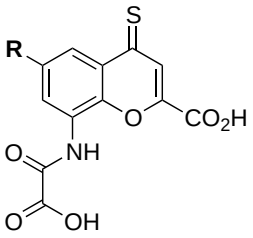
The pharmacological evaluation of the compounds was performed by Dr. Meryem Köse and Katharina Sylvester in our group.

The following compounds were originally designed as GPR35 agonists.

Table 3.21: Potency of chromen-4-one-2-carboxylic acids at GPR84 determined by cAMP accumulation assay in CHO-β-arrestin-GPR84 cells.



188 - 195



197

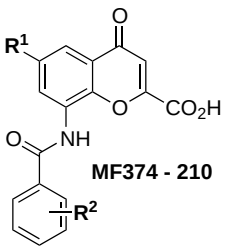
compd.	R	$IC_{50} \pm SEM^a$ (μM) (% inhibition)
188	Br	>> 3 (1%)
189	H	> 3 (0%)
190	phenyl	>> 3 (0%)
191	F	>> 3 (0%)

compd.	R	$IC_{50} \pm SEM^a$ (μM) (% inhibition)
192	Cl	>> 3 (0%)
193	methoxy	>> 3 (0%)
194	methyl	>> 3 (0%)
195	ethyl	>> 3 (0%)
197	Br	>> 3 (5%)

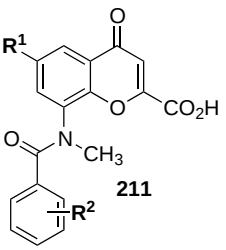
[a] Concentration-inhibition curves were performed in 3 independent experiments.

As expected none of the compounds is able to inhibit GPR84. The compounds in Table 3.22 were also optimized for GPR35.

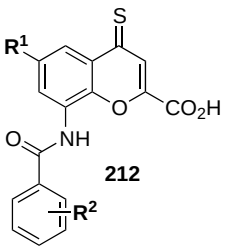
Table 3.22: Potency of chromen-4-one-2-carboxylic acids at GPR84 determined by cAMP accumulation assay in CHO-β-arrestin-GPR84 cells.



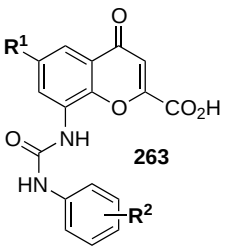
MF374 - 210



211



212



263

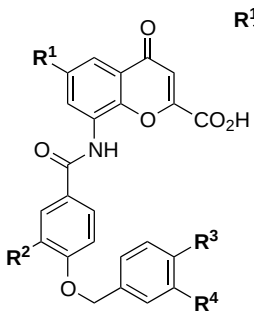
cpd	R^1	substitution pattern R^2			GPR84 $IC_{50} \pm SEM$ (% inhibition)[a] (μM)
		ortho	*meta*	*para*	
198[65]	Br	F	H	OCH_3	> 3 (16%)
199[65]	Br	di-F	H	OCH_3	>> 3 (3%)
200[65]	Br	H	F	OCH_3	> 3 (11%)
201[65]	methyl	H	H	OCH_3	>> 3 (0%)
202[65]	OCH_3	H	H	OCH_3	>> 3 (0%)
203	Br	OCH_3	H	OCH_3	> 3 (33%)
204	OCF_3	H	H	OCH_3	> 10 (21%)
205	phenyl	H	H	OCH_3	**1.52** ± 0.23
206	ethyl	H	H	OCH_3	>> 3 (0%)
207	Br	H	H	ethyl	> 3 (17%)

cpd	R^1	substitution pattern R^2			GPR84 $IC_{50} \pm SEM^a$ (μM)
		ortho	*meta*	*para*	
208	*p*-chloro-phenyl	H	H	OCH_3	**0.259** ± 0.109
209	Br	H	H	SCH_3	> 3 (0%)
210	phenyl	di-F	H	OCH_3	**6.37** ± 0.98
MF325	Cl	H	CH_3	H	**4.24** ± 0.35[172]
MF133	Br	H	CH_3	H	**2.19** ± 1.00[172]
MF298	Br	H	OCH_3	OCH_3	**1.07** ± 0.44[172]
211	Br	H	H	OCH_3	>> 3 (0%)
212	Br	H	H	OCH_3	**9.92** ± 2.27
263	H	H	H	OH	> 3 (2%)
WA26	H	H	*p*-fluo-roben-zyloxy	OCH_3	**0.457** ± 0.103[172]

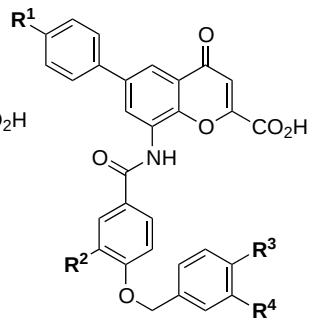
[a] Concentration-inhibition curves were performed in 3 independent experiments.

For the development of specific GPR84 antagonists we synthesized chromen-4-ones with large residues in the 8-position and position 6. The final compounds are shown in Table 3.23.

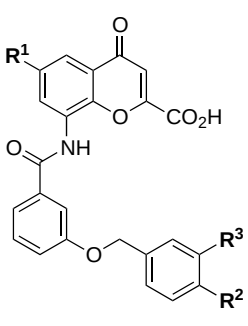
Table 3.23: Potency and affinity of chromen-4-one-2-carboxylic acids at GPR84 determined by cAMP accumulation assay in CHO-β-arrestin-GPR84 cells.



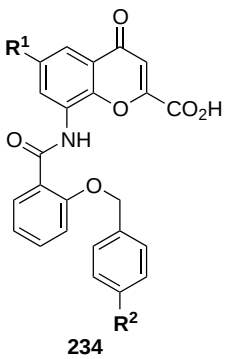
213 - 215



216 - 226



227 - 233



234

compound	substitution pattern R^1	R^2	R^3	R^4	GPR84 $IC_{50} \pm SEM^a$ (μM) (% inhibition)
213	OCH_3	H	F	H	**0.190** ± 0.059

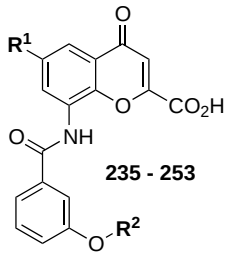
	substitution pattern				GPR84
compound	**R^1**	**R^2**	**R^3**	**R^4**	**IC_{50} ± SEM[a]** (% inhibition) (μM)
214	methyl	H	F	H	**0.171** ± 0.026
215	ethyl	H	F	H	**0.514** ± 0.157
MF320	H	H	H	H	**1.94** ± 0.36[172]
MF201	H	H	F	H	**1.33** ± 0.18[172]
MF303	H	H	Cl	H	**3.19** ± 1.42[172]
MF302	H	H	Br	H	**1.85** ± 0.94[172]
MF282	H	H	CH_3	H	**4.43** ± 0.86[172]
MF281	H	H	H	F	**1.71** ± 0.37[172]
MF311	F	H	F	H	**2.17** ± 0.12[172]
MF304	Cl	H	F	H	**0.527** ± 0.055[172]
MF329	Cl	H	Br	H	**0.331** ± 0.019[172]
MF299	Br	H	F	H	**0.391** ± 0.017[172]
MF321	Br	H	Br	H	**0.426** ± 0.053[172]
MF326	Br	H	H	Br	**1.29** ± 0.12[172]
216	H	H	F	H	**0.165** ± 0.029
217	H	H	F	F	**0.088** ± 0.018
218	Cl	H	F	H	**0.00734** ± 0.000168
219	H	Cl	F	H	**0.122** ± 0.011
220	F	H	F	H	**0.0592** ± 0.0039
221	OCH_3	H	F	H	**0.161** ± 0.076
222	methyl	H	F	H	**0.0404** ± 0.0072
223	F	H	F	F	**0.126** ± 0.001
224	F	Cl	F	F	**0.274** ± 0.044
225	OH	H	F	H	**0.264** ± 0.156
226	H	F	F	H	**0.257** ± 0.053
227	Br	F	H	-	**1.99** ± 0.37
228	Br	Br	H	-	**0.541** ± 0.122
229	Br	Cl	H	-	**1.46** ± 0.31
MF316	H	F	H	-	**1.85** ± 0.94[172]
230	H	methyl	H	-	**7.27** ± 3.74
231	Br	methyl	H	-	**4.91** ± 0.30
232	H	H	Br	-	> 3 (19%)
233	Br	H	Br	-	**2.50** ± 0.23

	substitution pattern				GPR84
compound	R^1	R^2	R^3	R^4	IC_{50} ± SEM[a] (% inhibition) (μM)
234	Br	F	-	-	**3.25** ± 0.78

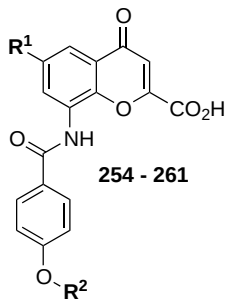
[a] Concentration-inhibition curves were performed in 3 independent experiments

The following compounds were optimized for GPR17 and GPR55. They contain long alkyl side-chains at position 8 (Table 3.24).

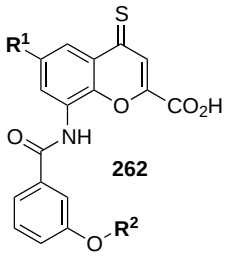
Table 3.24: Potency of chromen-4-one-2-carboxylic acids at GPR84 determined by cAMP accumulation assay in CHO-β-arrestin-GPR84 cells.



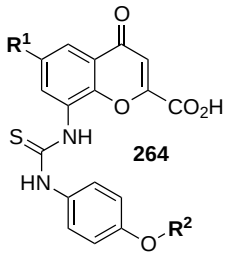
235 - 253



254 - 261



262



264

	substitution pattern		GPR84
compound	R^1	R^2	IC_{50} ± SEM[a] (% inhibition) (μM)
Pranlukast			**1.40** ± 0.05[218]
235	H	1-cyclohexylmethyl	> 3 (48%)
236	H	2-cyclohexylethyl	**4.12** ± 0.67
237	H	3-cyclohexylpropyl	> 3 (29%)
238	Cl	3-cyclohexylpropyl	> 3 (43%)
239	F	3-cyclohexylpropyl	**1.68** ± 0.57
240	H	4-cyclohexylbutyl	**6.36** ± 1.94
241	Cl	4-cyclohexylbutyl	**0.757** ± 0.05
242	F	4-cyclohexylbutyl	**1.03** ± 0.04
243	H	5-cyclohexylpentyl	> 3 (21%)
244	Cl	5-cyclohexylpentyl	> 3 (33%)
245	F	5-cyclohexylpentyl	**15.7** ± 3.2
246	Cl	hexyl	**2.09** ± 0.82
247	Cl	heptyl	**1.66** ± 0.61
248	Cl	octyl	**1.04** ± 0.28
249	H	1-phenylmethyl	>> 3 (4%)
250	H	2-phenylethyl	>> 3 (4%)

	substitution pattern		GPR84
compound	**R^1**	**R^2**	**IC_{50} ± SEM[a] (% inhibition) (μM)**
251	H	3-phenylpropyl	> 3 (29%)
252	H	4-phenylbutyl	**2.00** ± 0.30
253	H	5-phenylpentyl	**3.16** ± 0.43 (*efficacy* 58%)
MF191	H	2-cyclohexylethoxy	**2.21** ± 0.75[172]
254	Br	3-cyclohexylpropyl	> 10 (20%)
255	OCH_3	4-cyclohexylbutyl	>> 3 (0%)
256	methyl	4-cyclohexylbutyl	**3.45** ± 0.60 (65%)
257	Cl	4-cyclohexylbutyl	>> 3 (0%)
258	F	4-cyclohexylbutyl	**17.5** ± 2.7 (66%)
259	ethyl	4-cyclohexylbutyl	> 10 (7 ± 4)
260	H	5-cyclohexylpentyl	>> 3 (0%)
MF192	H	2-phenylethyl	**2.00** ± 0.30[172]
MF230	Br	3-phenylpropyl	**1.41** ± 0.14[172]
261	Br	5-phenylpentyl	
262	F	3-cyclohexylpropyl	**5.14** ± 0.88
264	H	4-cyclohexylbutyl	**0.564** ± 0.127

[a] Concentration-inhibition curves were performed in 3 independent experiments

3.3.3 Structure-activity relationships

GPR84 is a receptor which binds medium-chain free fatty acids.[67] So it is no surprise that the most polar compounds of our chromen-4-one library, compounds **188** - **197**, which contain two carboxylic acid functions, were not able to block the receptor (Table 3.21). The compounds shown in Table 3.22, which contain only a small side-chain with one aromatic ring substituted with small groups, did not show antagonistic activity at GPR84 as well, with a few exceptions that will be discussed later. Previous results showed, that compounds with a halogen in 6-position combined with a benzyloxyphenyl side-chain, *para*-substituted with bromine or fluorine, were able to block the receptor.[172]

We synthesized a series of compounds containing a *p*-fluorobenzyloxyphenyl residue, the best side chain so far, and modified the 6-position extensively. We already knew from previous results that compounds with chlorine and bromine in this posi-

tion (**MF304** IC_{50} = **0.527** ± 0.055 μM[172] and **MF299** IC_{50} = **0.391** ± 0.017 μM,[172] respectively) were more potent antagonists than compounds substituted with hydrogen or fluorine (**MF201** IC_{50} = **1.33** ± 0.18 μM[172] and **MF311** IC_{50} = **2.17** ± 0.12 μM,[172] respectively). We started our series of compounds by inserting small groups at the 6-position. Substitution with ethyl (**215** IC_{50} = **0.514** ± 0.157 μM) resulted in a decreased activity whereas substitution with methyl (**214** IC_{50} = **0.171** ± 0.026 μM) and methoxy (**213** IC_{50} = **0.0.190** ± 0.0.059 μM) resulted in a 2-fold higher potency compared to the halogen-substituted compounds.

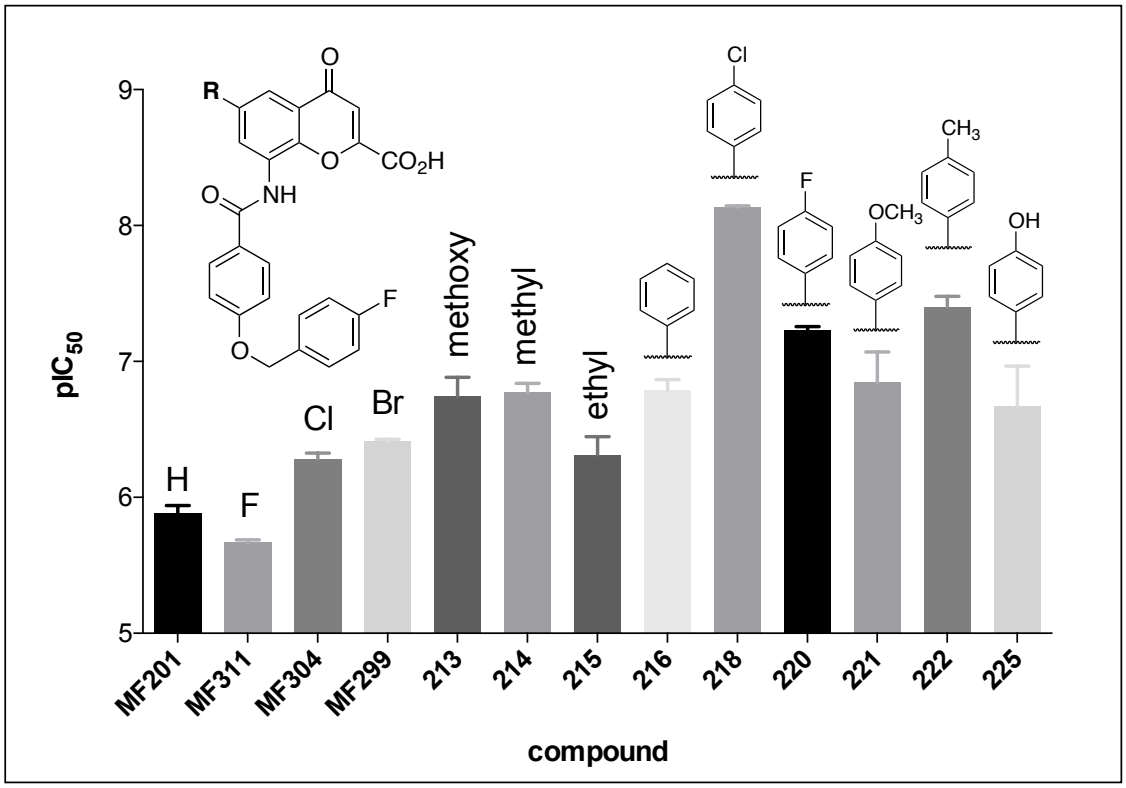


Figure 3.19: Optimization and structure-activity relationships of the substituent at the 6-position of the chromen-4-one. The pIC_{50} values ± SEM were calculated from IC_{50} values determined by cAMP-accumulation assays. The inhibition of forskolin-activated GPR84 expressed in CHO cells was measured.

We assumed that bulky substituents are beneficial at the 6-position and as a consequence we developed compounds with a phenyl group at that position. Compound **216** (IC_{50} = **0.165** ± 0.029 μM), which contains a phenyl ring, showed comparable activity to the methyl- and methoxy-substituted compounds. Substitution of the phenyl ring in the *para*-position further increased the potency. The best substituent

was chlorine (**218**, IC_{50} = **0.00734** ± 0.000168 μM) followed by methyl (**222**, IC_{50} = **0.0404** ± 0.0072 μM) and fluorine (**220**, IC_{50} = **0.0592** ± 0.0039 μM). Compound **221** showed a comparable activity to the unsubstituted derivative **216** with an IC_{50} of **0.161** ± 0.076 μM and the hydroxy-substituted compound **225** showed the lowest potency of the phenyl derivatives (IC_{50} = **264** ± 0.156 μM). This results confirms our hypothesis that large and unpolar substitutents at the 6-position increase the inhibitory potency of our compounds. This is thought to be due to a large lipophilic binding pocket in that domain of the receptor. This is also supported by the fact that the very few potent compounds with a short side chain (Table 3.22), **205**, **208** and **210**, are substituted with a phenyl ring at the 6-position. The most potent compound of this series, compound **208**, contains a *para*-chlorophenyl-substituent and displayed an IC_{50} value of **0.252** ± 0.111 μM. That substituent appears to fill the binding pocket in this area of the receptor optimally, and thus the nature of the side-chain becomes less important. SARs for the 6-position are illustrated in Figure 3.19.

Besides the variation of the 6-position many variations of the side-chain were performed.

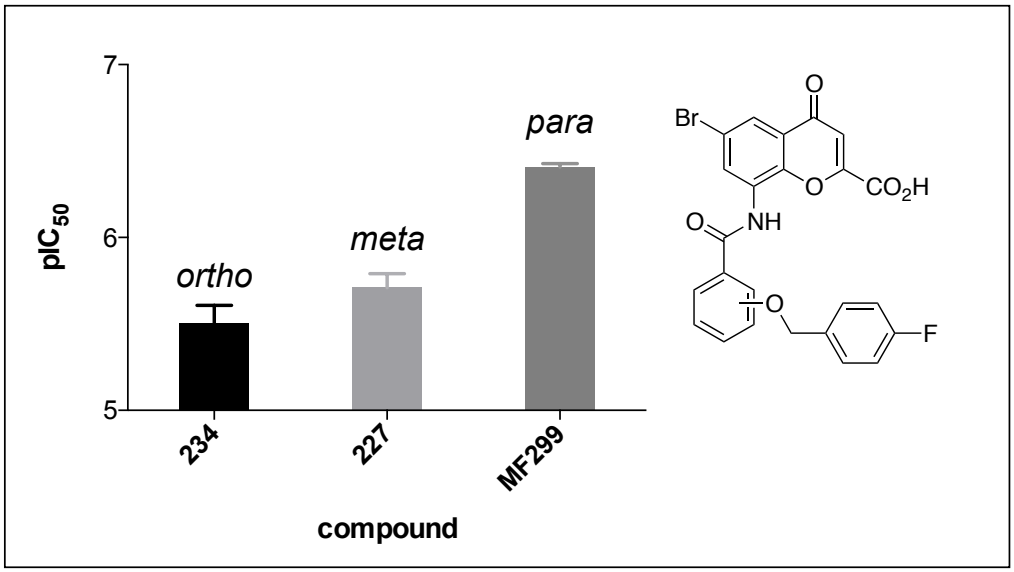


Figure 3.20: Variation of the position of the *p*-fluorobenzyloxy-residue. The pIC_{50} values ± SEM were calculated from IC_{50} values determined by cAMP-accumulation assays. The inhibition of forskolin-activated GPR84 expressed in CHO cells was measured.

Shifting the *p*-fluorobenzyl-part of the side-chain from the *para*- to the *meta*- or

the *ortho*-position resulted in a decrease in potency. The rank order of potency was *para* (**MF299**, IC_{50} = **0.391** ± 0.017 μM[172]) > *meta* (**227**, IC_{50} = **1.99** ± 0.37 μM) ≈ *ortho* (**234**, IC_{50} = **3.25** ± 0.78 μM) as shown in Figure 3.20.

The effects of the introduction of further halogen atoms on the phenyl rings of the side chain were diverse. An introduction of chlorine in the *meta*-position of the amide linker at the first phenyl ring of the side-chain (**219**, IC_{50} = **0.122** ± 0.011 μM) slightly increased the potency compared to the unsubstituted compound **216** (IC_{50} = **0.165** ± 0.029 μM) when there was an unsubstituted phenyl in the 6-position whereas a fluorine substituent was less well tolerated at this position (**226**, IC_{50} = **0.257** ± 0.053 μM). However there was almost not difference in inhibitory potency observed. A slightly increased potency was observed when inserting a second fluorine atom in the *meta*-position of the second phenyl ring of the side-chain (**139**, IC_{50} = **0.088** ± 0.018 μM) which resulted in a 2-fold higher potency compared to the mono-fluoro derivative **216**. However when there was a *p*-fluoro-phenyl substituent in the 6-position a second fluorine decreased the potency (**223**, IC_{50} = **0.126** ± 0.001 μM) by about 2-fold. A combination of 2 fluoro substituents and the chloro substitutent resulted in an even lower potency (**224** IC_{50} = **0.274** ± 0.044 μM). Ultimately the introduction of further halogen atoms had no major effect on the inhibitory potency.

Compounds with larger alkyl side chains (Table 3.24), which were originally designed for GPR17 and GPR55, did not show high potency at GPR84. Only compounds which contained a halogen or a methyl group at the 6-position turned out to be weak antagonists at GPR84 with IC_{50} values in the micromolar range. Interestingly the most potent compound of that series was **247** with a heptyl residue in the *meta*-position (IC_{50} = **1.03** ± 0.01 μM) which seemed to contradict all previously determined SARs. However the potency was still about 100-fold lower compared to the most potent antagonist.

The SARs determined for GPR84 are illustrated in Figure 3.21.

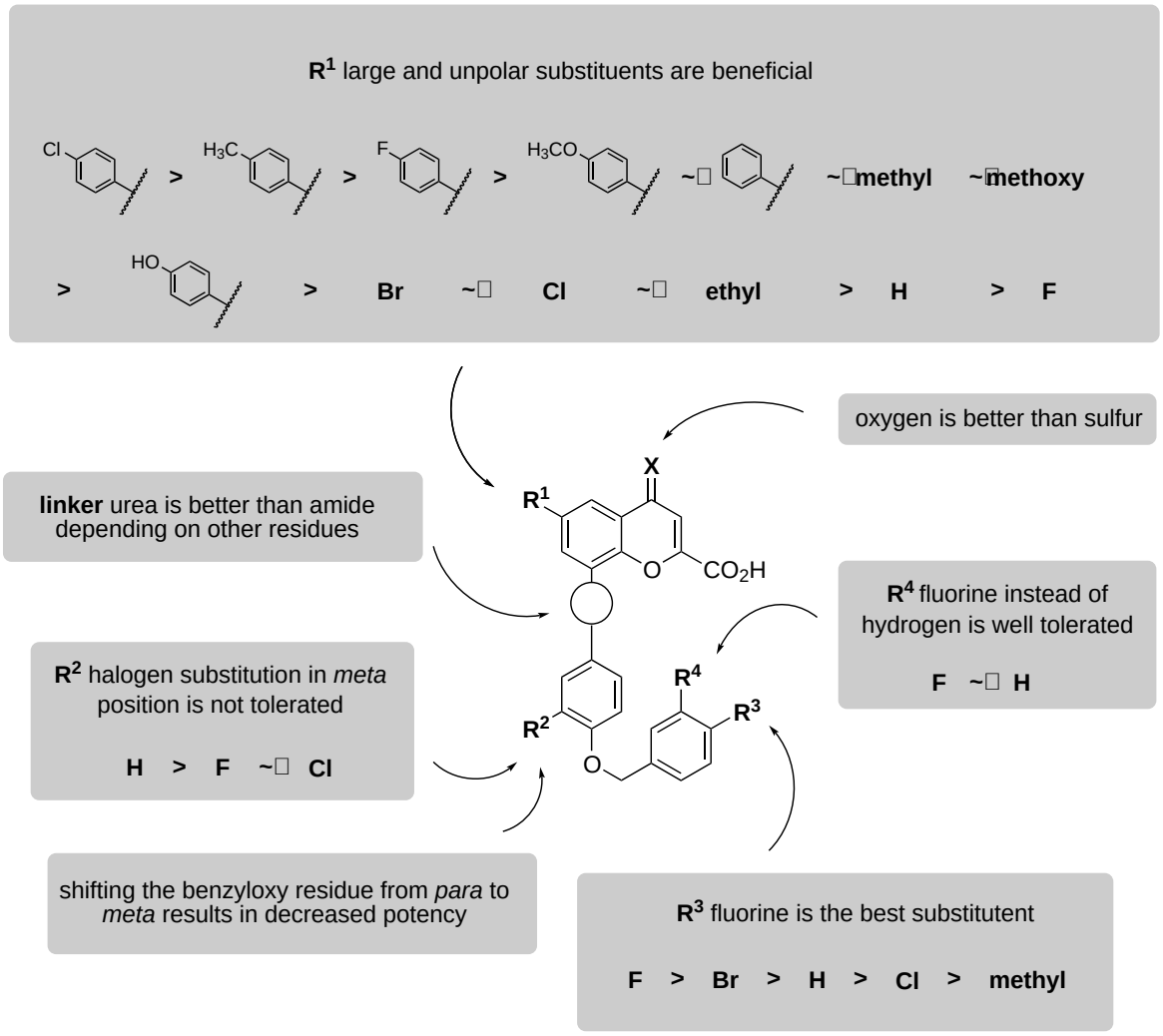
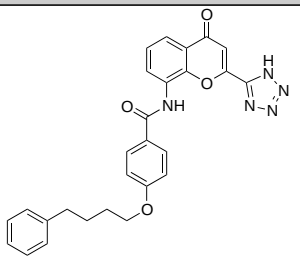
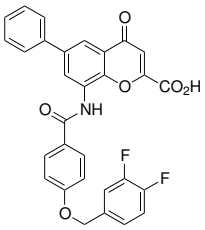
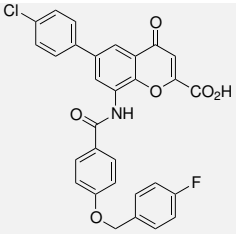
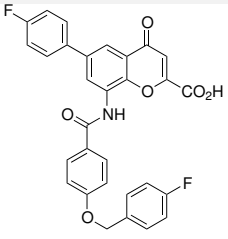


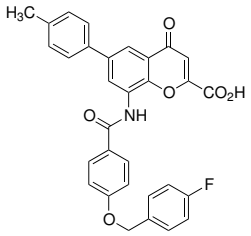
Figure 3.21: Structure-activity relationships determined for chromen-4-ones as GPR84 antagonists.

We successfully optimized the chromen-4-ones regarding their potency at GPR84. The best GPR84 antagonist of the present series was **218** with a IC_{50} value in the low nanomolar range (Figure 3.22). It is at the same time the most potent antagonist known to date for GPR84.

The most potent compounds were further analyzed in β-arrestin recruitment assays using CHO-β-arrestin-GPR84 cells and in radioligand binding experiments (Table 3.25).

Table 3.25: Potency and affinity of selected chromen-4-one-2-carboxylic acids at GPR84 determined by cAMP accumulation assays in CHO-β-arrestin-GPR84 cells (%inhibition of Yazh-365 (30nM) response at 10 μM) and β-arrestin assay in CHO-β-arrestin-GPR84 cells (% inhibition of the effect of 10 μM Yazh-365) and radioligand binding experiments in CHO-β-arrestin-GPR84 cells (% inhibition of 5 nM [^{3}H]PSB-1584 at 10 μM).

compd.	**structure**	**cAMP assay** IC_{50} ± SEM[a] [μM]	**β-arrestin assay** IC_{50} ± SEM[a] [μM]	**radioligand-binding experiments** IC_{50} ± SEM[a] [μM] (% inhibition)
Pranlukast		**1.40** ± 0.05[218]	**1.23** ± 0.344	**40.7** ± 7.4
217		**0.088** ± 0.018	**0.112** ± 0.048	**3.61** ± 0.64
218		**0.00734** ± 0.000168	**0.00302** ± 0.00095	**0.047** ± 0.016
220		**0.0592** ± 0.0039	**0.00708** ± 00060	**0.241** ± 0.043

222		**0.0404** ± 0.0072	**0.0137** ± 0.0025	**0.258** ± 0.157

[a] Concentration-inhibition curves were performed in 3 independent experiments.

The selected compounds were able to block GPR84 in all performed pharmacological assays in a comparable rank order of potency. The IC_{50}-values in β-arrestin assays were slightly lower than the ones determined in the cAMP assay, except for compound **217** which showed about the same potency in the cAMP-assay. Interestingly also in the radioligand binding experiments **217** showed a high IC_{50}-value in the micromolar range whereas **218**, **220** and **222** showed IC_{50}-values in the nanomolar range. A large lipophilic group at position 6 seems to be important for a high affinity.

Some selected compounds that were inactive in the cAMP assay were also tested for agonistic activity at GPR84 (**255**, **260**, **257**, **232**, **243**, **244**, **235**, **249**, **251**, **250**, **238**, and **237**). None of the tested compounds was able to activate the receptor at a high concentration of 10 μM.

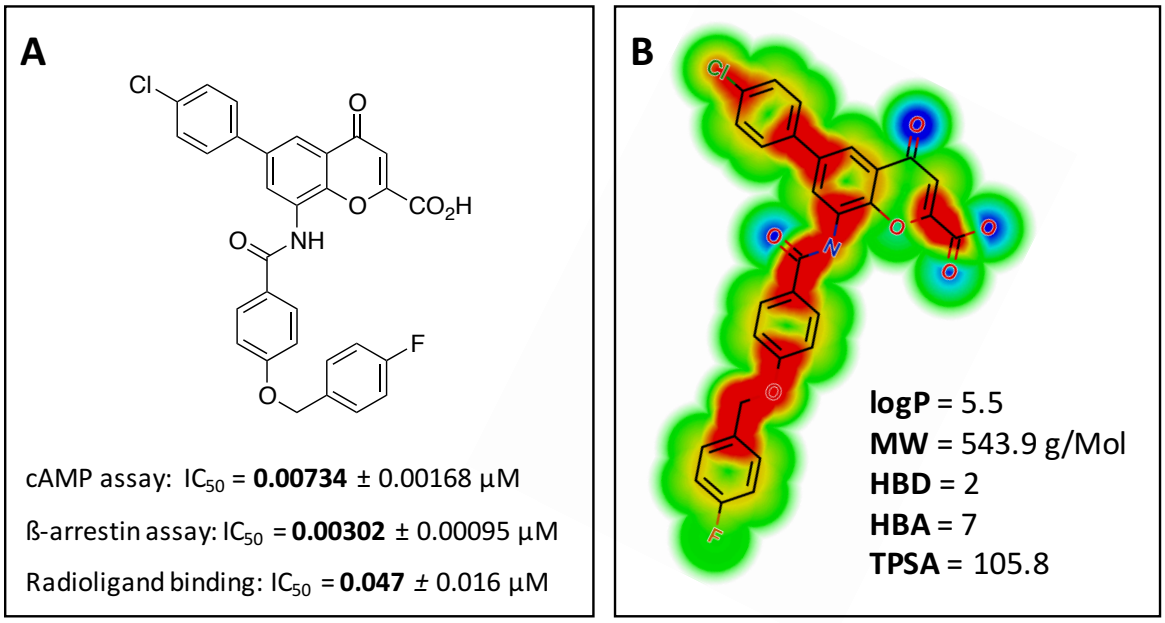


Figure 3.22: Structure and physicochemical properties of compound **218**, the most potent GPR84 antagonist known to date. **A** Structure and potency of **218**; **B** Analysis of the polarity of the structure performed with Stardrop®. Polar groups are shown in blue colour whereas unpolar parts of the molecule are shown in red colour. The logP, molecular weight (MW), hydrogen-bond donator (HBD), hydrogen-bond acceptor (HBA) and the topological polar surface area (TPSA) were calculated using Stardrop®.

We analyzed the physicochemical properties of the most potent compound of our series of chromenones using Stardrop®. Compound **218** fulfills the criteria of the Rule of Five with two exceptions. The logP value, which describes the lipophilicity of the compound and the molecular weight are slightly too high. On the other hand the TPSA is below 140 Å which indicates a potentially good cell permeability. The compound represents an important pharmacological tool for further investigation of GPR84. The physicochemical properties can be improved and so the compound could also function as a lead structure for the development of future drugs.

3.4 Pharmacological evaluation at the human GPR17

3.4.1 Introduction

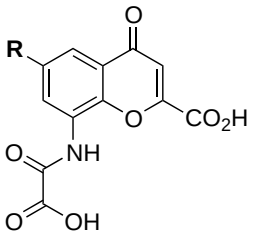
Some of the chromenon derivatives were found to block GPR17. None of the investigated compounds appeared to activate the receptor. The inhibitory potency

of the potential GPR17 antagonists was determined by the calcium ion mobilization assay. In this assay system the intracellular calcium, which is released via the signal transduction cascade of the G_q-coupled receptor, is measured fluorimetrically. A cell-permeable form of the calcium fluorescence indicator Oregon Green®, Oregon Green®BAPTA-1/acetoxylmethyl ester (AM), was incubated with 1321N1 astocytoma cells, that recombinantly express the receptor. Upon receptor activation by the agonist MDL29,951,[40] a calcium-dependend fluorescence signal could be measured. When the cells were preincubated with the antagonist the signal was decreased in a concentration-depend manner. For screening the compounds were initially tested at a concentration of 10 μM. In case the inhibition was higher than 50%, concentration-inhibition curves were determined to measure the IC_{50} values. Concentration-inhibition curves were performed in 3 independent experiments each in duplicates.

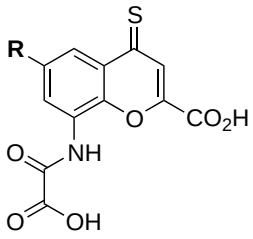
3.4.2 Results

The pharmacological evaluation of GPR17 antagonists was performed by Dr. Aliaa Abdelrahman.

Table 3.26: Potency of chromen-4-one-2-carboxylic acids at GPR17 determined by calcium ion mobilization assay in 1321N1 astrocytoma cells.



188 - 195



197

compd.	R	IC_{50} ± SEM[a] (μM)	inhibition (%)[b]
188	Br	> 10	-5 ± 16
189	H	> 10	4 ± 2
190	phenyl	> 10	-19 ± 12
191	F	> 10	-6 ± 4
192	Cl	> 10	-9 ± 4
193	methoxy	> 10	-15 ± 7
194	methyl	> 10	-4 ± 5

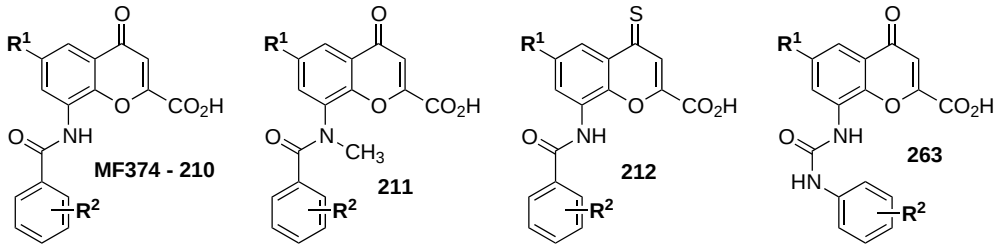
cpd	R	IC_{50} ± SEM[a] (μM)	inhibition (%)[b]
195	ethyl	> 10	-9 ± 4
197	Br	> 10	-5 ± 5

[a] Concentration-inhibition curves were performed in 3 independent experiments.

[b] at 10 μM of the test compound

Small compounds shown in Table 3.26 were not able to block GPR17. They were originally designed as GPR35 agonists.

Table 3.27: Potency of chromen-4-one-2-carboxylic acids at GPR17 determined by calcium ion mobilization assay in 1321N1 astrocytoma cells.



compd.	R^1	substitution pattern R^2			GPR17 IC_{50} ± SEM[a] (μM)	GPR17 inhibition[b] (%)
		ortho	*meta*	*para*		
198[65]	Br	F	H	OCH_3	> 10	10 ± 8
199[65]	Br	di-F	H	OCH_3	> 10	0 ± 1
200[65]	Br	H	F	OCH_3	> 10	0 ± 5
201[65]	methyl	H	H	OCH_3	> 10	-12 ± 10
202[65]	OCH_3	H	H	OCH_3	> 10	-3 ± 8
203	Br	OCH_3	H	OCH_3	> 10	0 ± 2
204	OCF_3	H	H	OCH_3	> 10	-5 ± 11
205	phenyl	H	H	OCH_3	> 10	10 ± 12
206	ethyl	H	H	OCH_3	> 10	5 ± 1
207	Br	H	H	ethyl	> 10	8 ± 4
208	*p*-chloro-phenyl	H	H	OCH_3	**5.44** ± 0.68	95 ± 4[c]
209	Br	H	H	SCH_3	> 10	10 ± 3
210	phenyl	di-F	H	OCH_3	> 10	18 ± 7
211	Br	H	H	OCH_3	> 10	0 ± 8
212	Br	H	H	OCH_3	> 10	37 ± 15

cpd	R^1	substitution pattern R^2			GPR17 $IC_{50} \pm SEM^a$ (μM)	GPR17 inhibition[b] (%)
		ortho	*meta*	*para*		
263	H	H	H	OH	> 10	5 ± 2
MF203	H	H	H	4-phenylbutoxy	**0.748** ± 0.076[172]	-

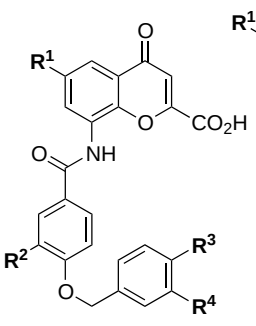
[a] Concentration-inhibition curves were performed in 3 independent experiments.

[b] at 10 μM of the test compound

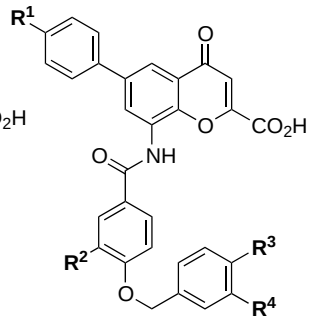
[c] at 100 μM of the test compound

The compounds shown in Table 3.27 were still too small to interact with GPR17.

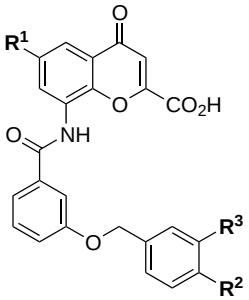
Table 3.28: Potency of chromen-4-one-2-carboxylic acids at GPR17 determined by calcium ion mobilization assay in 1321N1 astrocytoma cells.



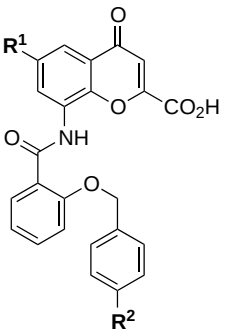
213 - 215



216 - 226



227 - 233



234

compound	substitution pattern R^1	R^2	R^3	R^4	GPR17 $IC_{50} \pm SEM^a$ (μM)	GPR17 inhibition[b] (%)
213	OCH_3	H	F	H	> 10	30 ± 6
214	methyl	H	F	H	> 10	15 ± 1
215	ethyl	H	F	H	**2.62** ± 1.05	95 ± 7[c]
MF282	H	H	methyl	H	**13.8** ± 0.3[172]	-
MF301	H	H	CF_3	H	**7.03** ± 0.75[172]	-
MF299	Br	H	F	H	**4.84** ± 0.19[172]	-
MF321	Br	H	Br	H	**2.32** ± 0.24[172]	-
216	H	H	F	H	≥ 10	42 ± 3
217	H	H	F	F	> 10	34 ±2
218	Cl	H	F	H	> 10	5 ± 5

	substitution pattern				GPR17	
compound	**R^1**	**R^2**	**R^3**	**R^4**	**IC_{50} ± SEM[a]** (μM)	**inhibition[b]** (%)
219	H	Cl	F	H	**10.2** ± 0.8	103 ± 2[c]
220	F	H	F	H	> 10	26 ± 8
221	OCH_3	H	F	H	**16.2** ± 6.1	70 ± 5[c]
222	methyl	H	F	H	≥ 10	39 ± 7
223	F	H	F	F	> 10	11 ± 5
224	F	Cl	F	F	> 10	26 ± 4
225	OH	H	F	H	≥ 10	33 ± 6
226	H	F	F	H	≥ 10	34 ± 5
227	Br	F	-	-	> 10	10 ± 6
228	Br	Br	-	-	≥ 10	40 ± 16
229	Br	Cl	-	-	> 10	35 ± 16
230	H	methyl	H	-	> 10	11 ± 7
231	Br	methyl	H	-	> 10	7 ± 4
232	H	H	Br	-	> 10	26 ± 4
233	Br	H	Br	-	> 10	26 ± 4
234	Br	F	-	-	> 10	11 ± 4

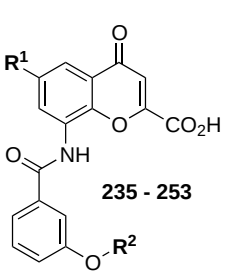
[a] Concentration-inhibition curves were performed in 3 independent experiments.

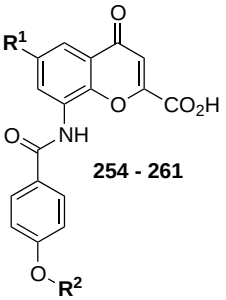
[b] at 10 μM of the test compound

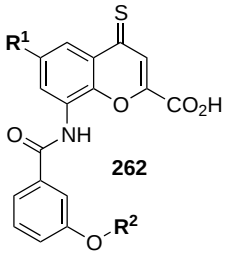
[c] at 100 μM of the test compound

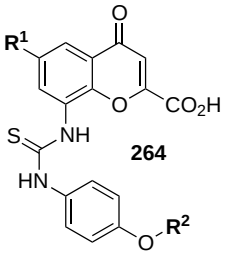
Compounds that contain an aromatic side-chain were not able to interact with GPR17 (Table 3.28).

Table 3.29: Potency of chromen-4-one-2-carboxylic acids at GPR17 determined by calcium ion mobilization assay in 1321N1 astrocytoma cells.









	substitution pattern		GPR17	
compound	**R^1**	**R^2**	**IC_{50} ± SEM[a] (μM)**	**inhibition[b] (%)**
Pranlukast			**2.69** ± 0.12[218]	-
235	H	1-cyclohexylmethyl	> 10	30 ± 14
236	H	2-cyclohexylethyl	≥ 10	45 ± 3
237	H	3-cyclohexylpropyl	**4.28** ± 0.49	100 ± 8[c]
238	Cl	3-cyclohexylpropyl	**3.05** ± 0.21	93 ± 3[c]
239	F	3-cyclohexylpropyl	**4.63** ± 0.60	94 ± 1[c]
240	H	4-cyclohexylbutyl	**1.71** ± 0.04	98 ± 1
241	Cl	4-cyclohexylbutyl	**2.81** ± 0.72	81 ± 7[c]
242	F	4-cyclohexylbutyl	**2.67** ± 0.49	82 ± 4[c]
243	H	5-cyclohexylpentyl	**3.26** ± 1.04	80± 8
244	Cl	5-cyclohexylpentyl	**1.58** ± 0.49	96 ± 1
245	F	5-cyclohexylpentyl	**6.37** ± 0.58	81 ± 9[c]
246	Cl	hexyl	> 10	27 ± 20
247	Cl	heptyl	**1.58** ± 0.63	95 ± 6
248	Cl	octyl	**2.15** ± 0.66	103 ± 2[c]
249	H	1-phenylmethyl	> 10	-4 ± 7
250	H	2-phenylethyl	> 10	8 ± 10
251	H	3-phenylpropyl	> 10	0 ± 4
252	H	4-phenylbutyl	**14.1** ± 3.1	101 ± 5[c]
253	H	5-phenylpentyl	**4.97** ± 0.85	74 ± 8[c]
MF191	H	2-cyclohexylethoxy	**11.8** ± 0.5[172]	-
MF248	H	3-cyclohexylpropyl	**0.945** ± 0.58[172]	-
254	Br	3-cyclohexylpropyl	**2.59** ± 0.74	95 ± 2

	substitution pattern		GPR17	
compound	**R^1**	**R^2**	**IC_{50} ± SEM[a]** (μM)	**inhibition[b]** (%)
WA24	H	4-cyclohexylbutyl	**0.583** ± 0.202[172]	-
WA22	Br	4-cyclohexylbutyl	**1.31** ± 0.26[172]	-
255	OCH_3	4-cyclohexylbutyl	**0.445** ± 0.108	100 ± 1[c]
256	methyl	4-cyclohexylbutyl	**0.943** ± 0.277	100 ± 3
257	Cl	4-cyclohexylbutyl	**1.34** ± 0.24	97 ± 4
258	F	4-cyclohexylbutyl	**1.15** ± 0.18	90 ± 11
259	ethyl	4-cyclohexylbutyl	**0.666** ± 0.144	96 ± 5
260	H	5-cyclohexylpentyl	**1.40** ± 0.40	95 ± 3
MF192	H	2-phenylethyl	**24.9** ± 3.8[172]	-
MF190	H	3-phenylpropyl	**10.9** ± 2.9[172]	-
WA09	F	3-phenylpropyl	**4.65** ± 0.46[172]	-
MF300	Cl	3-phenylpropyl	**5.94** ± 0.78[172]	-
MF230	Br	3-phenylpropyl	**3.30** ± 0.95[172]	-
MF150	H	4-phenylbutyl	**7.78** ± 1.72[172]	-
261	Br	5-phenylpentyl	**4.30** ± 0.79	88 ± 5[c]
MF259	H	*n*-heptyl	**1.63** ± 0.05[172]	-
MF200	H	4-methylpentyl	**3.90** ± 0.76[172]	-
262	F	3-cyclohexylpropyl	**7.74** ± 0.36	110 ± 3[c]
264	H	4-cyclohexylbutyl	**9.45** ± 0.50	93 ± 4[c]
MF208	H	4-phenylbutyl	**2.74** ± 0.94[172]	-

[a] Concentration-inhibition curves were performed in 3 independent experiments.

[b] at 10 μM of the test compound

[c] at 100 μM of the test compound

Table 3.29 shows compounds that were identified as GPR17 antagonists. The SARs will be further discussed.

3.4.3 Structure-activity relationships

Compounds **188** - **197** which include two carboxylic acids were completely inactive at GPR17 (Table 3.26). This also applied to compounds **198** - **212** and **263** which have only small side-chains consisting of one aromatic ring with a small substituent. An exception was compound **208** with an IC_{50} value of **5.44** ± 0.68 μM. In contrast

to the other compounds it contains a *p*-chlorophenyl substituent at the 6-position which is very large and lipophilic (Table 3.27).

The introduction of an additional phenyl ring at the side-chain further increased the lipophilicity and also the size of the compounds. However also in this series of compounds (compounds **213** - **234**) we identified only few compounds with moderate inhibitory potency at GPR17. The best compounds were **215** (IC_{50} = **2.62** ± 1.05 μM) and **MF321** (IC_{50} = **2.32** ± 0.24 μM[172]). Compound **215** has an ethyl substituent at the 6-position, which increases the lipophilicity, compound **MF321** has a Br at the 6-position and also at the *para*-position of the second aromatic ring of the side-chain. What both compounds have in common is the position of the second aromatic ring at the side chain. It appears to be important that the ether linker is in the *para*-position to the amide linker. The structurally related compounds **MF282**,[172] **MF301**[172] and **MF299**,[172] which were already published as weak GPR17 antagonists, are also substituted in *para*-position (Table 3.28).

It was previously described that long alkyl side-chains instead of phenyl side-chains are beneficial for the inhibitory potency at GPR17.[172] The best compound so far based on the chromenone scaffold was compound **WA24** (Table 3.29) with an IC_{50} of **0.583** ± 0.202 μM.[172] It is about 5-fold more potent than the lead structure Pranlukast (IC_{50} = **2.69** ± 0.12 μM[218]).

A series of compounds which differ only in the number of C-atoms between the ether and the cyclohexyl ring of the side-chain were tested to find the optimal chain-length. Compound **WA24** which contains a butyl linker between the ether and the cyclohexyl ring showed the best inhibition. Introduction of the longer pentyl (compound **260**) or the shorter propyl- or ethyl-chain (compounds **MF248** and **MF191** respectively) resulted in a decreased potency. This effect could be observed when the side-chain was in the *para*- as well as in the *meta*-position. However the *para*-substituted compounds were more potent than the corresponding *meta*-substituted derivatives (Figure 3.23).

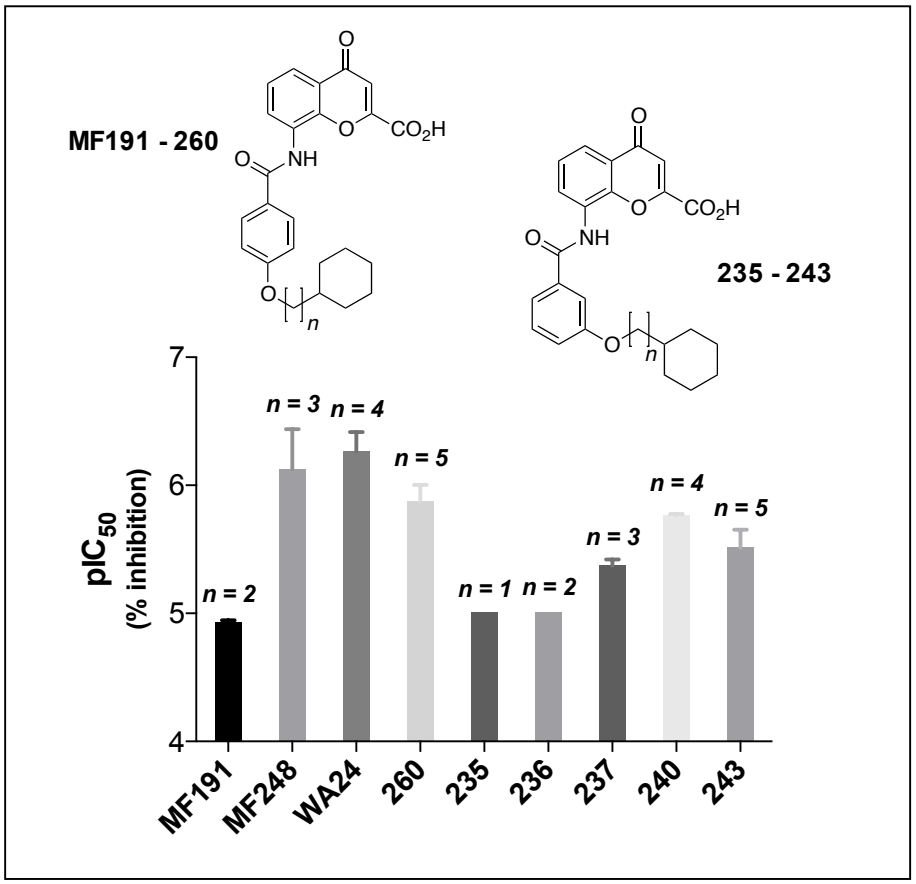


Figure 3.23: Influence of the chain length of GPR17 antagonists on the inhibitory potency. The pIC_{50} values ± SEM were calculated from IC_{50} values determined by calcium ion mobilization assay in 1321N1 astrocytoma cells.

We could also confirm that a cyclohexyl ring at the end of the side-chain is better than a phenyl ring and better than *n*-alkyl-chains. Testing of a series of 3 compounds with increasing *n*-alkyl chain-lengths from hexyl to octyl in the *meta*-position showed that a number of 7 C-atoms is the optimum. However the best compound of this series, compound **247**, showed only an IC_{50} value of **1.58** ± 0.49 μM. Interestingly the inhibitory potency of the corresponding *para*-substituted compound **MF259** is in the same range.

The compound with the optimal chain-length of 4 C-atoms in the *para*-position connected to a cyclohexyl ring was now further modified at the 6-position of the chromen-4-one. The best substituent was methoxy followed by hydrogen, ethyl and methyl. Halogen substituents resulted in a lower potency regardless of its nature, and chlorine, fluorine or bromine derivatives were in the same range (Figure 3.24).

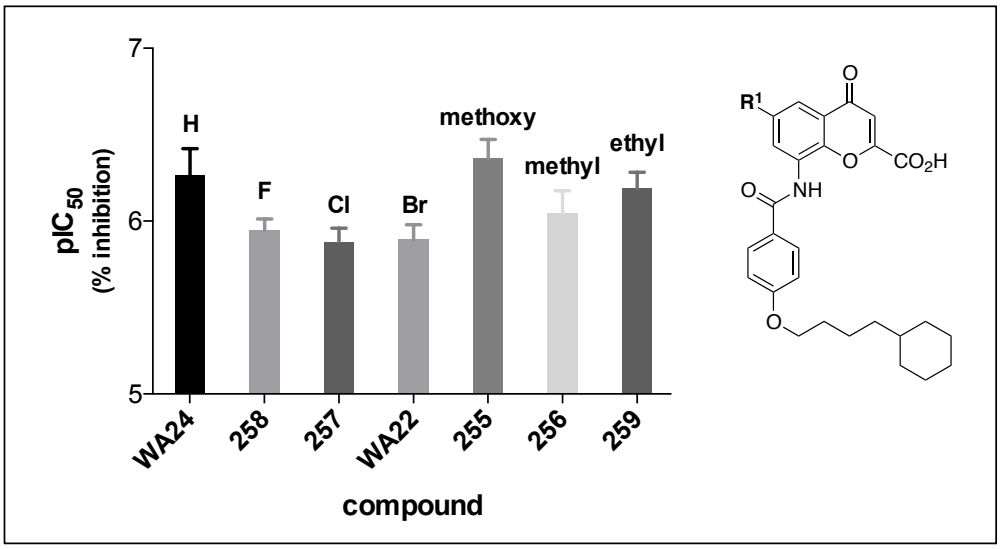


Figure 3.24: Different substituents at the 6-position of chromen-4-ones and their effect on the inhibitory potency at human GPR17. The pIC_{50} values ± SEM were calculated from IC_{50} values determined by calcium ion mobilization assay in 1321N1 astrocytoma cells.

The results show that unpolar substituents are beneficial at the 6-position and that halogen atoms were already too polar. **For the future an introduction of longer alkyl chains also at the 6-position connected via an ether linker could be interesting.**

A further modification was the replacement of the oxygen atom at the 4-position of the chromen-4-one by a sulfur atom (compound **262**, IC_{50} = **7.74** ± 0.36 μM). This modification resulted in a slightly lower potency compared to the corresponding oxygen compound **239** (IC_{50} = **4.63** ± 0.60 μM).

Previous results indicated that the replacement of the amide linker by a thiourea linker could increase the inhibitory potency.[172] This could not be confirmed in the present new series of compounds. The replacement of the amide linker by a thiourea linker was not well tolerated. Compound **264** with the thiourea linker had a 20-fold lower potency (IC_{50} = **9.45** ± 0.50 μM) than **WA24** (IC_{50} = **0.583** ± 0.202 μM) with the amide linker.

A summary of the determined SARs at GPR17 is shown in Figure 3.25.

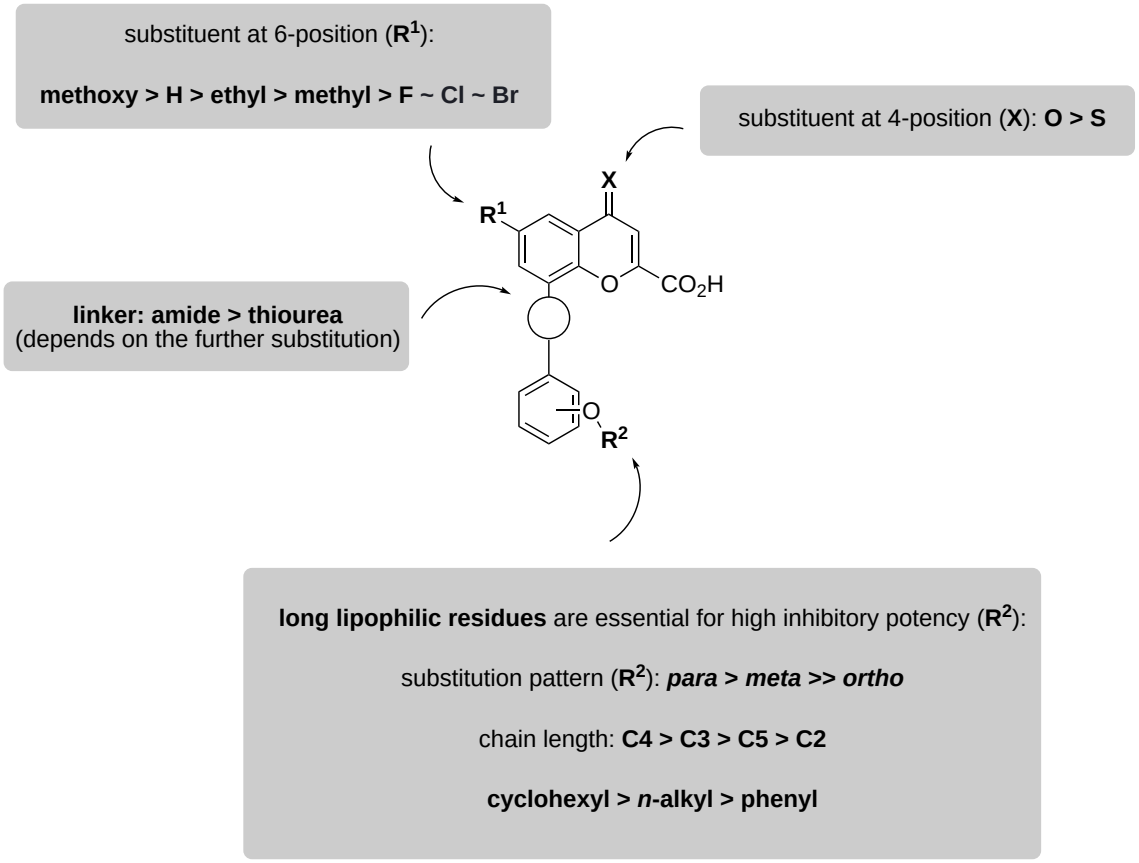


Figure 3.25: Structure-activity-relationships determined for GPR17.

Many modifications of the side-chain were performed. The introduction of longer residues as well as the change of the substitution pattern was not tolerated. The cyclohexybutyl side-chain in the *para*-position was still the best substituent. Also a modification of the linker by replacing it with thiourea as well as the replacement of the oxygen atom at the 4-position of the chromen-4-one by a sulfur atom was not tolerated and resulted in lower potencies. An improvement of the inhibitory potency could be achieved by the modification of the substituent at the 6-position. Methoxy and also ethyl are both well tolerated and resulted in potent compounds.

The best GPR17 antagonist based on the chromen-4-one scaffold was compound **255** with an IC_{50} value of **0.445** $\pm$ 0.108 μM (Figure 3.27).

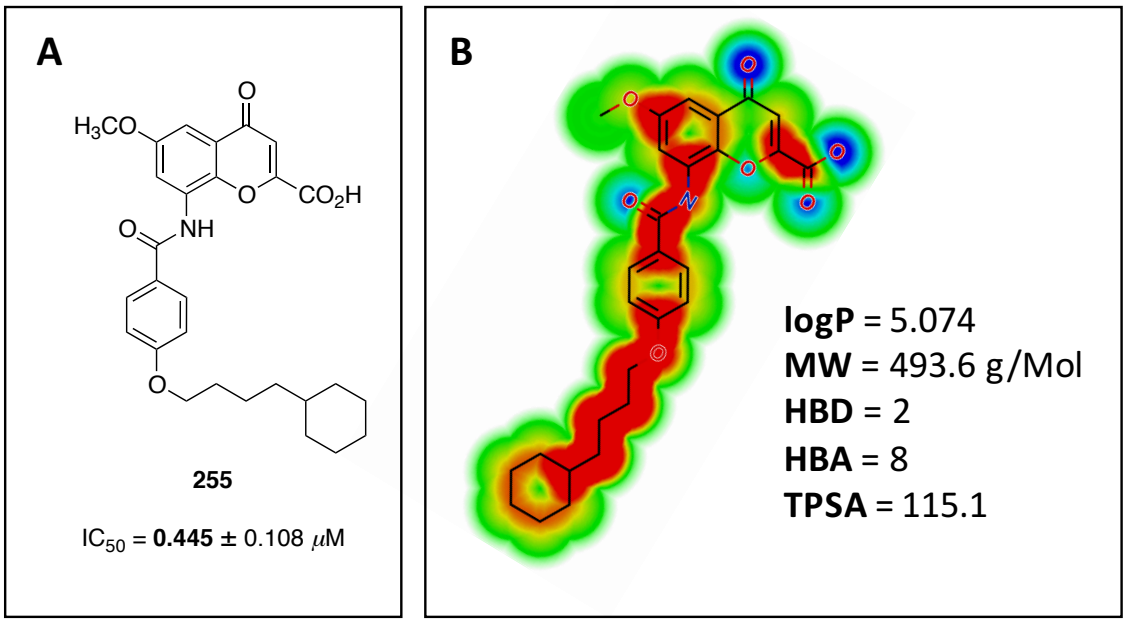


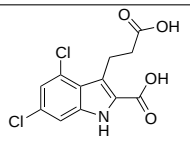
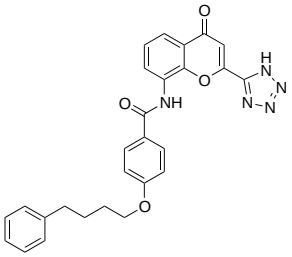
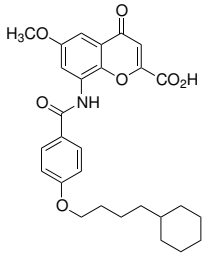
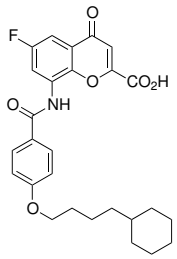
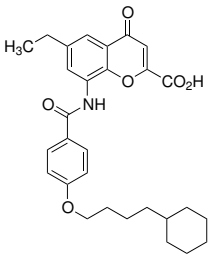
Figure 3.26: Structure and physicochemical properties of compound **255**, the most potent chromen-4-one antagonist at GPR17. **A** Structure and potency of **255**; **B** Analysis of the polarity of the structure performed with Stardrop®. Polar groups are shown in blue colour whereas unpolar parts of the molecule are shown in red colour. The logP, molecular weight (MW), hydrogen-bond donators (HBD), hydrogen-bond acceptors (HBA) and the topological polar surface area (TPSA) were calculated using Stardrop®.

Compound **255** fits into the criteria of Lipinski's rule of five, except for the logP value, which is slightly too high.

The most potent GPR17 antagonist of the series and Pranlukast were also evaluated in radioligand binding experiment with the radioligand [^{3}H]PSB12150, which is the tritiated form of the standard agonist MDL29.951. The results are shown in in Table 3.30.

Table 3.30: Potency and affinity of selected chromen-4-one-2-carboxylic acids at GPR17 determined in calcium ion mobilization assay using 1321N1 astrocytoma cells (% inhibition of calcium-dependend fluorimetric signal upon activation by GPR17 agonist MDL29,951) and radioligand binding experiments in CHO-hGPR17 cells (% Inhibition of 25 nM [^{3}H]PSB-12150 at 10 μM)

compd.	structure	calcium assay	radioligand-binding experiments
		IC_{50} ± SEM[a] [μM]	K_i ± SEM[a] [μM] (% inhibition)

MDL29.951		EC_{50} = **1.41**[218] (agonist)	
Pranlukast		**2.69** ± 0.12[218]	**4.34** ± 0.910 (79% at 30 μM)
255		**0.445** ± 0.108	> 10 (26% at 10 μM)
258		**1.15** ± 0.18	**2.20** ± 0.68 (62% at 30 μM)
259		**0.666** ± 0.144	> 10 (37% 6 at 10 μM)

[a] Concentration-inhibition curves were performed in 3 independent experiments.

Surprisingly the most potent GPR17 antagonists showed only weak or even no affinity to GPR17 in the radioligand binding experiments. Since the radioligand and the agonists that were used in the calcium ion mobilization assays contain the same structure we could not explain this result with different binding modes in both assays. We suspect that our antagonists are weak negative allosteric modulators of the receptor. They could bind in a different region and thereby affect a conformational change of the receptor which results in a decreased activity of the agonists

in the calcium assay and impacts binding of the agonist, but only with some of the antagonists. This is highly speculative and has to be further investigated.

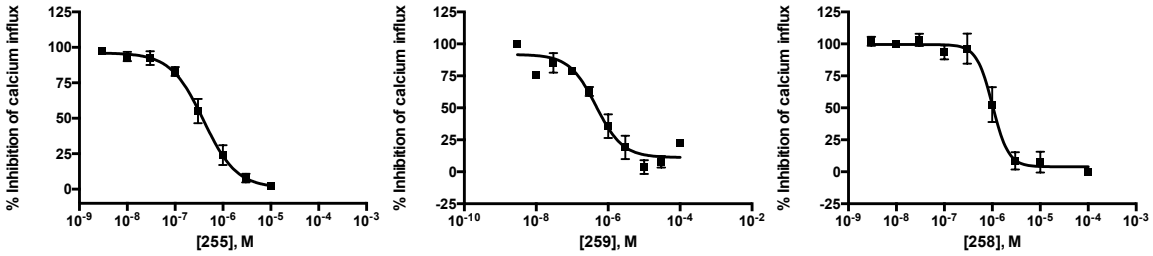


Figure 3.27: Dose-response curves of the most potent GPR17 antagonists **255**, **258** and **259**.

Compound **258** and Pranlukast could be confirmed to interact with GPR17 also in the radioligand binding experiments.

3.5 Pharmacological evaluation at human GPR55

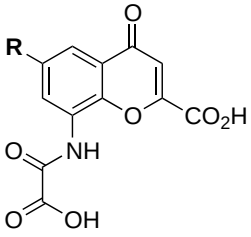
3.5.1 Introduction

L-α-lysophosphatidylinositol (LPI), which was proposed to be the endogenous ligand of GPR55, consists of a long lipophilic tail connected to a polar head. Some of our compounds share this structural properties and therefore were tested at GPR55. Additionally, the set of compounds which was active at the closely related GPR35, was tested for selectivity versus GPR55. All compounds were evaluated in the β-arrestin recruitment assay, which had already been described previously, with CHO-GPR55 cells for agonistic activity and also for antagonistic potency versus 1 μM LPI as an agonist.

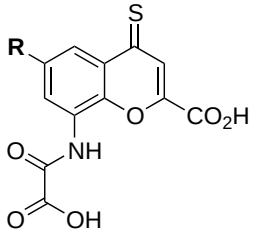
3.5.2 Test results

Testing was performed by Clara Schoeder in our group.

Table 3.31: Potency of chromen-4-one-2-carboxylic acids at GPR55 determined by β-arrestin recruitment assay using GPR55 CHO cells.



188 - 195



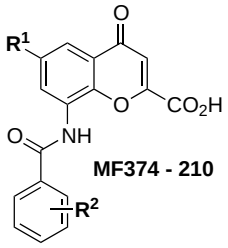
197

cpd	R	IC_{50} ± SEM (μM)[a]	EC_{50} ± SEM (μM)[a]
188	Br	> 10 (1 ± 5)	> 10 (3 ± 5)
189	H	> 10 (-8 ± 17)	> 10 (-14 ± 14)
190	phenyl	>10 (17 ± 8)	> 10 (56 ± 8)
191	F	> 10 (9 ± 11)	> 10 (34 ± 10)
192	Cl	> 10 (6 ± 9)	> 10 (27 ± 6)
193	methoxy	> 10 (-1 ± 7)	> 10 (27 ± 6)
194	methyl	> 10 (11 ± 9)	> 10 (29 ± 8)
195	ethyl	> 10 (27 ± 16)	> 10 (19 ± 12)
197	Br	> 10 (34 ± 9)	> 10 (15 ± 5)

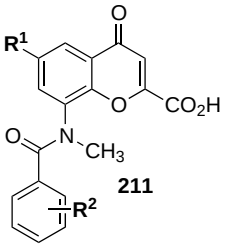
[a] Curves were performed in 3 independent experiments.

The GPR35 agonists shown in Table 3.31 and Table 3.32 were not able to interact with GPR55 although both receptors are closely related.

Table 3.32: Potency of chromen-4-one-2-carboxylic acids at GPR55 determined by β-arrestin recruitment assay using GPR55 CHO cells.



MF374 - 210



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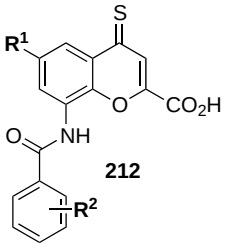
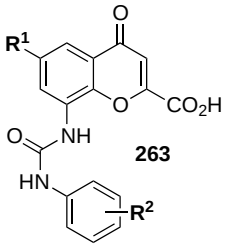


212



263

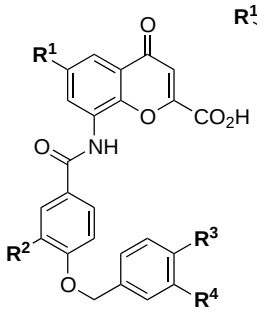
cpd	R^1	substitution pattern R^2			Antagonist IC_{50} ± SEM[a]	Agonist EC_{50} ± SEM[a]
		ortho	*meta*	*para*	(μM)	(μM)
198[65]	Br	F	H	OCH_3	> 10 (45 ± 11)	> 10 (9 ± 9)
199[65]	Br	di-F	H	OCH_3	> 10 (14 ± 9)	> 10 (8 ± 11)

cpd	R^1	substitution pattern R^2			Antagonist IC_{50} ± SEM[a]	Agonist EC_{50} ± SEM[a]
		ortho	*meta*	*para*	(μM)	(μM)
200[65]	Br	H	F	OCH_3	> 10 (35 ± 11)	> 10 (14 ± 10)
201[65]	methyl	H	H	OCH_3	> 10 (18 ± 11)	> 10 (7 ± 8)
202[65]	OCH_3	H	H	OCH_3	> 10 (4 ± 12)	> 10 (12 ± 10)
203	Br	OCH_3	H	OCH_3	> 10 (44 ± 9)	> 10 (-9 ± 11)
204	OCF_3	H	H	OCH_3	> 10 (17 ± 12)	> 10 (-14 ± 5)
205	phenyl	H	H	OCH_3	> 10 (-16 ± 2)	> 10 (7 ± 5)
206	ethyl	H	H	OCH_3	> 10 (8 ± 11)	> 10 (-8 ± 12)
207	Br	H	H	ethyl	**17.4** ± 6.5	> 10 (10 ± 8)
208	*p*-chloro-phenyl	H	H	OCH_3	> 10 (46 ± 4)	> 10 (-5 ± 7)
209	Br	H	H	SCH_3	**12.9** ± 1.7	> 10 (-18 ± 8)
210	phenyl	di-F	H	OCH_3	> 10 (28 ± 14)	> 10 (0 ± 10)
211	Br	H	H	OCH_3	> 10 (-6 ± 13)	> 10 (13 ± 9)
212	Br	H	H	OCH_3	n.d.	n.d.
263	H	H	H	OH	> 10 (11 ± 8)	> 10 (18 ± 14)

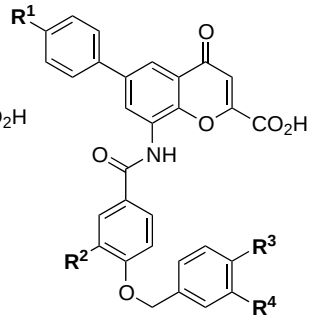
[a] Curves were performed in 3 independent experiments.

GPR55 did not tolerate aromatic side-chains. The GPR84-antagonists shown in Table 3.33 though were not able to activate GPR55.

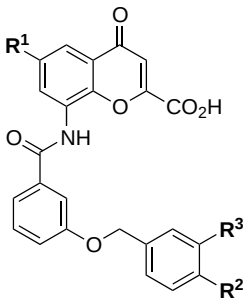
Table 3.33: Potency of chromen-4-one-2-carboxylic acids at GPR55 determined by β-arrestin recruitment assay using GPR55 CHO cells.



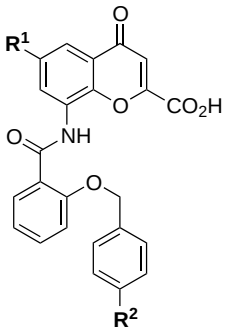
213 - 215



216 - 226



227 - 233



234

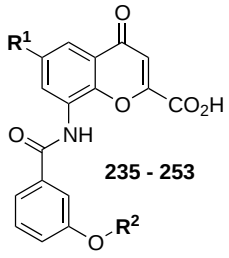
		substitution pattern			Antagonist	Agonist
compound	R^1	R^2	R^3	R^4	IC_{50} ± SEM (μM)[a]	EC_{50}± SEM (μM)[a]
213	OCH_3	H	F	H	> 10 (5 ± 8)	> 10 (-3 ± 6)
214	methyl	H	F	H	> 10 (6 ± 6)	> 10 (11 ± 12)
215	ethyl	H	F	H	> 10 (29 ± 11)	> 10 (0 ± 6)
216	H	H	F	H	> 10 (1 ± 11)	> 10 (-1 ± 10)
217	H	H	F	F	> 10 (0 ± 7)	> 10 (8 ± 12)
218	Cl	H	F	H	> 10 (-4 ± 5)	> 10 (3 ± 4)
219	H	Cl	F	H	> 10 (21 ± 7)	> 10 (10 ± 5)
220	F	H	F	H	> 10 (35 ± 8)	> 10 (-2 ± 12)
221	OCH_3	H	F	H	> 10 (1 ± 12)	> 10 (4 ± 8)
222	methyl	H	F	H	> 10 (13 ± 14)	> 10 (16 ± 13)
223	F	H	F	F	> 10 (13 ± 8)	> 10 (-15 ± 6)
224	F	Cl	F	F	> 10 (5 ± 11)	> 10 (7± 12)
225	OH	H	F	H	> 10 (31 ± 6)	> 10 (4 ± 14)
226	H	F	F	H	**16.2** ± 6.0	> 10 (-13 ± 13)
227	Br	F	-	-	**10.6** ± 1.2	> 10 (-2 ± 3)
228	Br	Br	-	-	> 10 (-87 ± 34)	**0.597** ± 0.137 (*efficacy* 98%)
229	Br	Cl	-	-	> 10 (36 ± 13)	**0.507** ± 0.033 (*efficacy* 39%)
230	H	methyl	H	-	> 10 (45 ± 10)	> 10 (20 ± 5)
231	Br	methyl	H	-	> 10 (-15 ± 5)	**0.481** ± 0.85 (*efficacy* 31%)

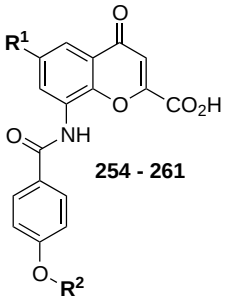
compound	substitution pattern R1	R2	R3	R4	Antagonist IC50 ± SEM (μM)[a]	Agonist EC50± SEM (μM)[a]
232	H	H	Br	-	> 10 (36 ± 13)	> 10 (-3 ± 7)
233	Br	H	Br	-	> 10 (51 ± 11)	> 10 (16 ± 7)
234	Br	F	-	-	**8.54** ± 0.55	> 10 (6 ± 7)

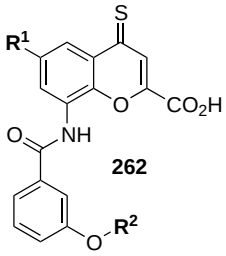
[a] Curves were performed in 3 independent experiments.

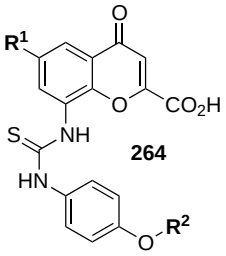
Compounds with long alkyl side-chains, shown in Table 3.34 were identified as agonists at GPR55. The SARs will be further discussed in detail.

Table 3.34: Potency of chromen-4-one-2-carboxylic acids at GPR55 determined by β-arrestin recruitment assay using GPR55 CHO cells.









compound	substitution pattern R1	R2	Antagonist IC50 ± SEM (μM)[a]	Agonist EC50 ± SEM (μM)[a]
Pranlukast			> 10 (40 ± 7)	> 10 (9 ± 2)
235	H	1-cyclohexylmethyl	> 10 (58 ± 9)	> 10 (16 ± 7)
236	H	2-cyclohexylethyl	> 10 (38 ± 13)	> 10 (25 ± 5)
237	H	3-cyclohexylpropyl	> 10 (46 ± 11)	**0.111** ± 0.042 (*efficacy* 29%)
238	Cl	3-cyclohexylpropyl	> 10 (-23 ± 23)	**0.323** ± 0.121 (*efficacy* 40%)
239	F	3-cyclohexylpropyl	> 10 (8 ± 19)	**0.117** ± 0.034 (*efficacy* 29%)
240	H	4-cyclohexylbutyl	> 10 (-51 ± 25)	**0.474** ± 0.099 (*efficacy* 73%)

	substitution pattern		Antagonist	Agonist
compound	R^1	R^2	IC_{50} ± SEM (μM)[a]	EC_{50} ± SEM (μM)[a]
241	Cl	4-cyclohexylbutyl	> 19 (-115 ± 57)	**0.342** ± 0.039 (*efficacy* 57%)
242	F	4-cyclohexylbutyl	> 10 (-93 ± 51)	**0.373** ± 0.038 (*efficacy* 71%)
243	H	5-cyclohexylpentyl	> 10 (-21 ± 20)	**0.202** ± 0.048 (*efficacy* 52%)
244	Cl	5-cyclohexylpentyl	> 10 (-39 ± 6)	**0.196** ± 0.041 (*efficacy* 86%)
245	F	5-cyclohexylpentyl	> 10 (36 ± 7)	**0.263** ± 0.074 (*efficacy* 58%)
249	H	1-phenylmethyl	**6.88** ± 1.70	> 10 (10 ± 6)
250	H	2-phenylethyl	> 10 (65 ± 5)	> 10 (14 ± 14)
251	H	3-phenylpropyl	> 10 (38 ± 8)	> 10 (18 ± 5)
252	H	4-phenylbutyl	**4.41** ± 0.28	> 10 (2 ± 9)
253	H	5-phenylpentyl	> 10 (21 ± 14)	> 10 (45 ± 6)
254	Br	3-cyclohexylpropyl	> 10 (18 ± 11)	> 10 (6 ± 11)
WA24	H	4-cyclohexylbutyl	> 10 (2 ± 7)	> 10 (-66 ± 27)
WA24	Br	4-cyclohexylbutyl	> 10 (31 ± 13)	> 10 (-27 ± 16)
255	OCH_3	4-cyclohexylbutyl	> 10 (31 ± 9)	> 10 (-5 ± 7)
256	methyl	4-cyclohexylbutyl	**4.54** ± 1.63	> 10 (2 ± 9)
257	Cl	4-cyclohexylbutyl	**23.6** ± 10.6	> 10 (-2 ± 12)
258	F	4-cyclohexylbutyl	**3.26** ± 0.14	> 10 (0 ± 13)
259	ethyl	4-cyclohexylbutyl	> 10 (48 ± 10)	> 10 (1 ± 6)
260	H	5-cyclohexylpentyl	**8.34** ± 1.16	> 10 (7 ± 5)
WA09	F	3-phenylpropyl	**1.87** ± 0.56	> 10 (1 ± 7)
261	Br	5-phenylpentyl	**11.7** ± 0.8	> 10 (-1 ± 11)
262	F	3-cyclohexylpropyl	> 10 (-70 ± 17)	> 10 (-9 ± 4)
264	H	4-cyclohexylbutyl	**8.14** ± 1.88	> 10 (9 ± 11)

[a] Curves were performed in 3 independent experiments.

3.5.3 Structure-activity relationships

The compounds listed in Table 3.31 and Table 3.32 which are agonists of the closest relative of GPR55, GPR35, were mostly not active at GPR55. However, there were two exceptions. Compound **207**, which has a bromine atom in the 6-position and an ethyl substituent in the *para*-position of the phenyl ring of the side-chain, as well as compound **209**, which is also substituted in the 6-position with a bromine atom and in the *para*-position of the phenyl side-chain with a thiomethyl group were weak antagonists at GPR55 (IC_{50} = **17.4** ± 6.5 μM and IC_{50} = **12.9** ± 1.7 μM, respectively).

The series of compounds that were originally synthesized for GPR84 is much more lipophilic as the compounds contain one more phenyl ring in the side-chain. However also in this series we only identified three weak GPR55 antagonists (**226**, IC_{50} = **16.2** ± 6.0 μM and **227**, IC_{50} = **10.6** ± 1.2 μM and **234**, IC_{50} = **8.54** ± 0.55 μM). They were structurally diverse and therefore no conclusions about SARs could be drawn. Interestingly in this series of compounds we also identified three GPR55 agonists with EC_{50}-values in the nanomolar range. All of them share a substitution in the *meta*-position of the side-chain as well as a bromine substitution in the 6-position of the chromen-4-one. Furthermore they contained a *para*-substitution at the second phenyl ring of the side-chain. The most potent compound of this class was **231**, which showed an EC_{50} value of **0.481** ± 0.85 μM. However the efficacy of the agonist was only 31%. Compound **229**, in which the methyl group in the 4-position of the side-chain was replaced by a chlorine atom showed a slightly higher efficacy of 39% but a lower EC_{50} value of **0.507** ± 0.033 μM. Substitution of the 4-position of the side-chain with a bromine atom resulted in the full agonist **228** which showed a slightly optimized EC_{50} of **0.597** ± 0.137 μM. In conclusion these results show, that a larger substituent in this position increased the efficacy but decreased the potency of the agonists.

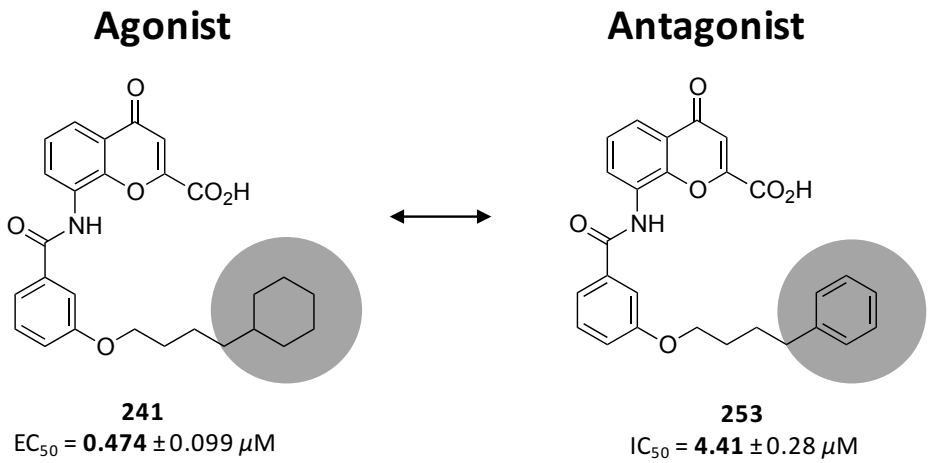
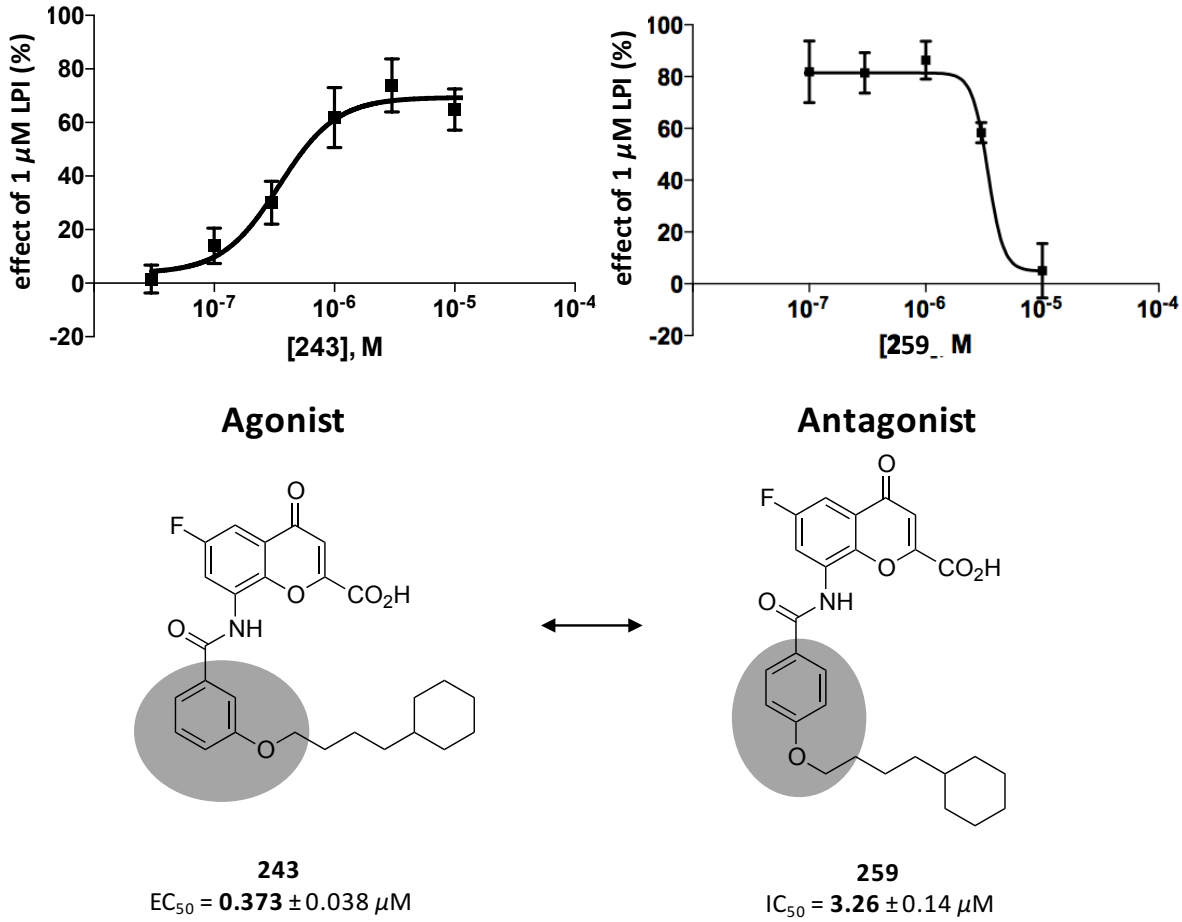


Figure 3.28: Agonist-antagonist-switch of chromen-4-one derivatives at GPR55 by aromatization of the side-chain.

This phenomenon of agonist-antagonist switch within a class of structurally very similar compounds could be observed also in the next series of compounds. These compounds were originally designed for GPR17 and contain a longer lipophilic side-chain consisting of long *n*-alkyl-chains terminated by a cyclohexyl ring or phenyl ring. Compounds with a terminal cyclohexyl ring mainly showed agonistic activity whereas compounds with a terminal phenyl ring were antagonists as shown in Figure 3.28

Compound **240** is a partial agonist with an efficacy of 73% and an EC_{50} value of **0.474** ± 0.099 μM, in contrast to **252** which is a weak antagonist with an IC_{50} value of **4.41** ± 0.28 μM. From these results we conclude that for activation of GPR55 a completely nonpolar side chain is beneficial. This is also a structural element of the potential endogenous agonist LPI.



Moving the 4-cyclohexylbutyloxy side-chain from the *meta*- to the *para* position results in an agonist-antagonist-switch.

Figure 3.29: Agonist-antagonist-switch of chromen-4-one derivatives at GPR55 moving the 4-cyclohexylbenzyloxy side-chain from the *meta*- to the *para* position.

We could also observe the antagonist-agonist switch when we changed the position of the 4-cyclohexylbutyl side-chain.

Compounds which were substituted in the *meta*-position to the amide linker were **agonists** whereas compounds which were substituted in the *para*-position were **antagonists**. Thus the angle of the lipophilic chain is very important for the functional activity of the ligand. All compounds that are substituted in the *meta*-position with an alkyl side-chain were agonists, except for the aromatic compounds as discussed before.

This interconversion of GPCR agonists and antagonists by only minor structural

changes is not an individual case. In 2016 Dosa et al. discussed it as a possibility to switch from a potent GPCR ligand with the undesired functional activity to the desired one.[219] However this phenomenon also shows us that we cannot deduce from SARs of structurally similar compounds their functional activity. It is also problematic to assume a given functional activity for a published compound if only binding data are reported, even if functional data for related ligands are available. The agonists we developed are able to stabilize GPR55 in in its active conformation. The antagonists still have affinity to the receptor but they were not able to induce the conformational change of the GPCR which is needed to activate the receptor's signal cascade.

When we analyze the optimal length of the side-chain we need a minimum of three C-atoms between the ether linker and the cyclohexyl ring to get a potent agonist. The prolongation of the alkyl chain did not result in higher potency. The most potent compound was compound **237** which contains the 3-cyclohexylpropyl chain. It was highly potent with an EC_{50} value of **0.111** $\pm$ 0.042 μM. However it was only a partial agonist with a low efficacy of only 29%. If we look now on the influence of the length of the side-chain on the efficacy we can clearly conclude that an extension resulted in an increase in efficacy. However the optimal length was 4 C-atoms, longer chains resulted in a decrease in efficacy (Figure 3.30).

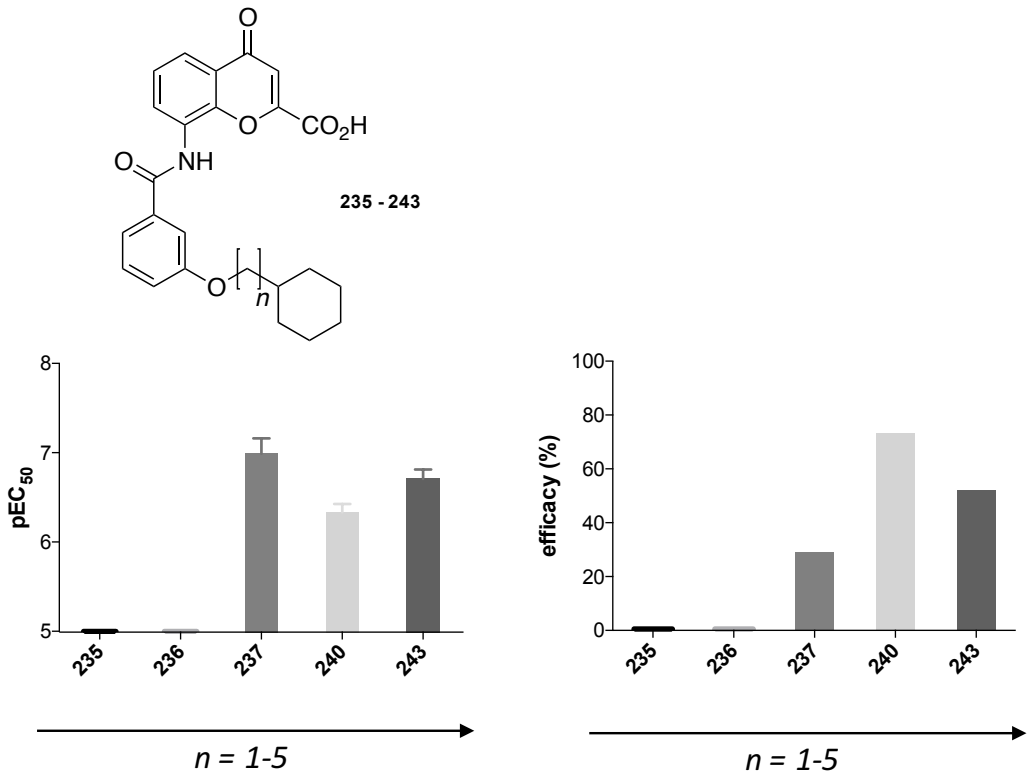


Figure 3.30: Influence of the length of the side chain on the potency and efficacy of GPR55 agonists.

The substituent at the 6-position of the chromen-4-one did not display remarkable effects on the potency of the GPR55 agonists. Chlorine, fluorine and hydrogen atoms did not make a difference. However a chlorine substituent increased the efficacy of the compounds.

Analyzing the effects of substituents at the 6-position on the potency of GPR55 antagonists we also could not observe any preferences.

To further identify important structural elements of GPR55 agonists we compared our chromen-4-one-based agonist **244** with the potential endogenous GPR55 agonist LPI. We found that **244** and LPI can be divided into three structural elements. Both compounds consist of a lipophilic tail, which is connected via a linker to an extremely polar head. The linker thereby includes a polar oxygen atom. An analysis of the polarity of the compound using Stardrop® shows that **281** and LPI share a similar polarity profile (Figure 3.31). We conclude from this analysis that our

chromen-4-one-based GPR55 agonists bind in the same binding pocket as the potential endogenous agonist LPI.

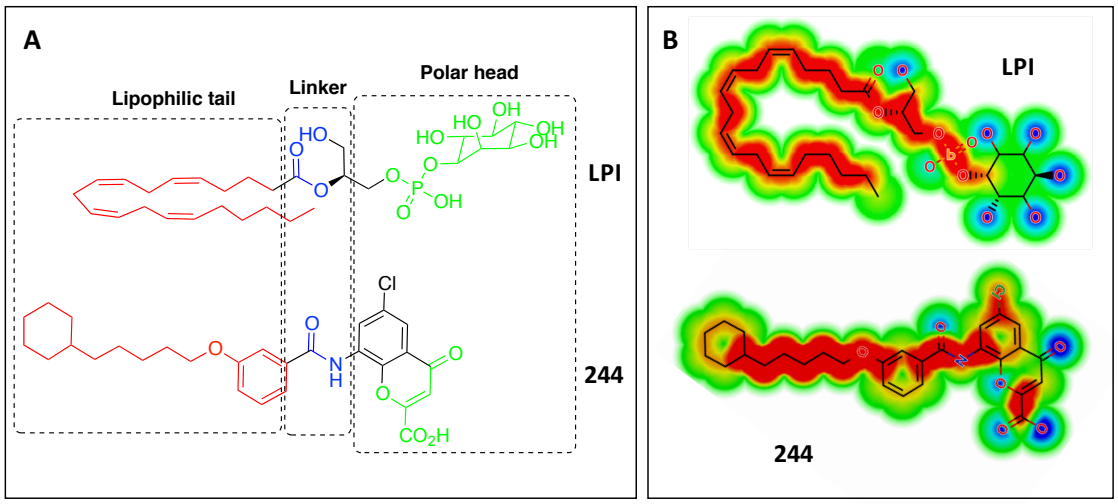


Figure 3.31: Comparison of the structures of the potential endogenous GPR55 agonist LPI and the potent GPR55 agonist **244** from our series of chromen-4-ones. **A** Both agonists share structural elements: the lipophilic tail shown in red, the linker shown in blue, the polar head shown in green. **B** An analysis of the polarity of the structure was performed using Stardrop®. Polar groups are shown in blue colour whereas unpolar parts of the molecule are shown in red colour.

In conclusion we successfully identified potent GPR55 agonists as well as GPR55 antagonists which are based on the chromen-4-one core structure. Minor structural modifications completely changed the functionality of our compounds. Compound **237** was the most potent GPR55 agonist of our series with an EC_{50} value of **0.111** ± 0.042 μM but is behaves as a partial agonist. Compound **244**, which was half as potent compared to **237** showed a much higher efficacy of 86% and is nearly a full agonist. We also developed GPR55 **antagonists** which showed potencies in the micromolar range. However our compounds were less potent than the previously published **WA09**.[172] The best compounds are shown in Figure 3.32.

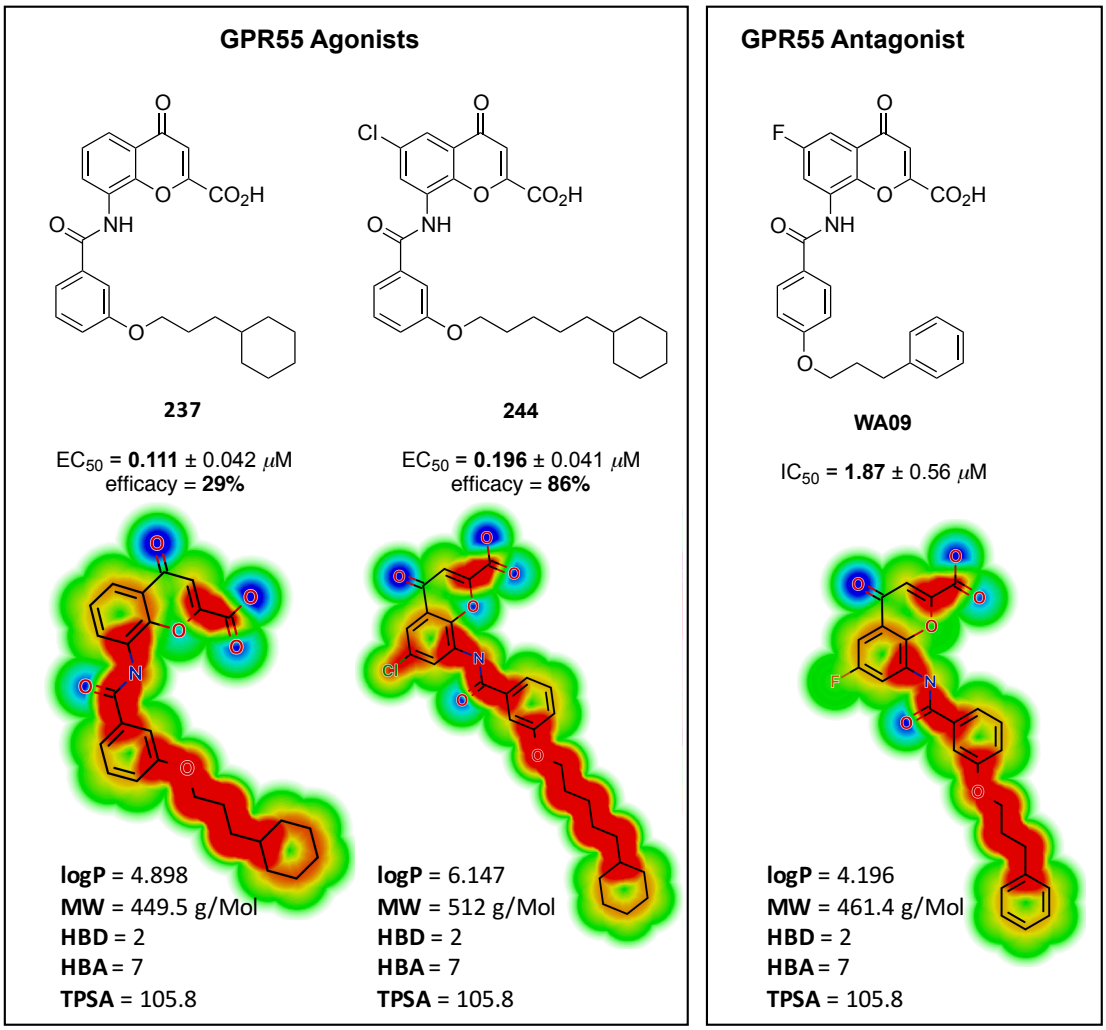


Figure 3.32: Potency and physicochemical properties of the most potent GPR55 ligands of the series of chromen-4-ones. An analysis of the polarity of the structure and of physicochemical parameters was performed using Stardrop®. Polar groups are shown in blue colour whereas unpolar parts of the molecule are shown in red colour.

All GPR55 agonists are very lipophilic and have logP values around 5. Compound **244** does not fit into Lipinki's rule of five with a logP value of 6.147 and a molecular weight of 512 g/mol. However few GPR55 synthetic GPR55 agonists have been identified so far and therefore our compound is a promising lead structure for further development of GPR55 agonists as pharmacological tools. Optimization of the physicochemical properties could result in a suitable lead structure for the development of drugs.

3.6 Selectivity of the compounds

In this chapter the selectivity of the most potent compounds will be analyzed and further discussed. What we can conclude is that the SARs were highly specific for each receptor. GPR35 agonists have to be small and polar. The most potent compounds have logP values below 3 (**197**, logP = 0.34, **210** logP = 2.95). GPR55, GPR84 and GPR17 ligands are more lipophilic and have higher molecular weights. A long lipophilic residue at the 8-position is important for their potency. Although the receptors share this SAR feature, the compounds are highly selective.

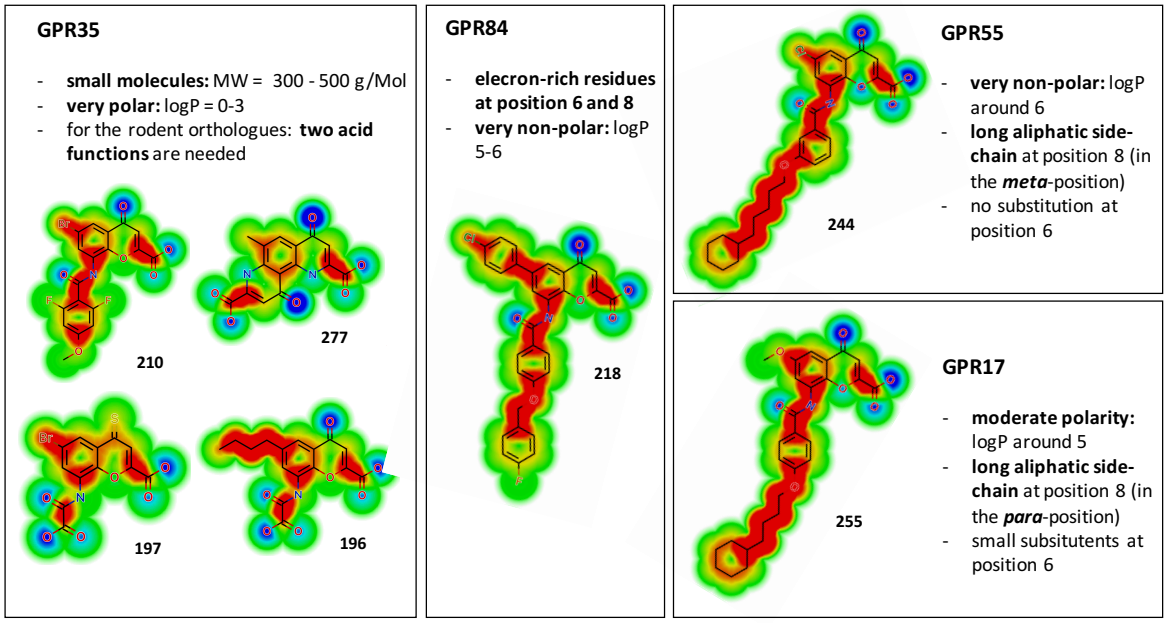


Figure 3.33: Comparison of the individual structural characteristics of the *orphan* GPCR ligands. An analysis of the polarity of the structure and of physicochemical parameters was performed using Stardrop®. Polar groups are shown in blue colour whereas unpolar parts of the molecule are shown in red colour.

GPR84 antagonists need to have a big electron-rich substituent at the 6-position. Phenyl or *p*-chlorophenyl are the best substituents for this position. In contrast to that GPR17 antagonists only tolerate aliphatic residues. Highly electronegative substituents like bromine result in a decreased potency. The same applies to GPR55 ligands. GPR55 antagonists as well as agonists prefer small groups like a fluorine or hydrogen atom at the 6-position.

When we look at the side-chain in the 8-position of the chromenone core the pref-

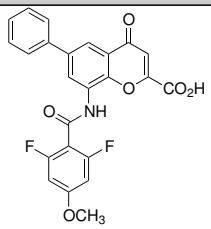
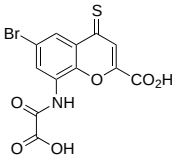
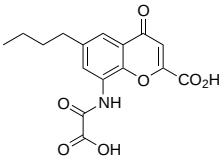
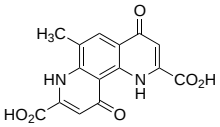
erences of the different receptors are highly diverse. All potent GPR84 antagonists contain a 4-fluorobenzyloxyphenyl side-chain which is aromatic and rich in electrons. GPR17 in contrast to that does not tolerate aromatic side-chains. Potent GPR17 antagonist contain a long alkyl side-chain which has to be in the *para*-position to the amide linker. The same applies to GPR55 agonists whereby the alkyl side-chain needs to be in the *meta*-position for potent ligands.

The most potent compounds were tested for their selectivity at phylogenetically related receptors.

3.6.1 Selectivity of the GPR35 agonists

The most potent GPR35 agonists **210**, **197**, **196**, and **277** were tested at further GPCRs of the δ branch of class A with a special focus on GPR55, which represented the most closely related receptor of the test series.

Table 3.35: Potency and selectivity of GPR35 agonists determined at class A δ-branch *orphan* GPCRs.

Target	210	197	196	277
				
human GPR35 K_i ± SEM (μM)[a]	**0.000426** ± 0.000037	**0.00271** ± 0.00032	**0.00897** ± 0.0015	**0.0062** ± 0.00065
human GPR35 EC_{50} ± SEM (μM)[b]	**0.00108** ± 0.00011	**0.00847** ± 0.00072	**0.0318** ± 0.0053	**0.0214** ± 0.0021
rat GPR35 EC_{50} ± SEM (μM)[c]	**0.157** ± 0.013	**0.0209** ± 0.0023	**0.0297** ± 0.0040	**0.0263** ± 0.0023
mouse GPR35 EC_{50} ± SEM (μM)[d]	**0.293** ± 0.042	**0.174** ± 0.019	**0.399** ± 0.083	**0.105** ± 0.015
human GPR55 EC_{50} ± SEM (μM)[e]	> 10	> 10	> 10	> 10

human GPR55 IC_{50} ± SEM (μM)[f]	> 10	> 10	> 10	> 10
human GPR84 IC_{50} ± SEM (μM)[g]	**6.37** ± 0.98	> 3	> 3	> 3
human GPR17 IC_{50} ± SEM (μM)[h]	> 10	> 10	> 10	> 10
human GPR18 IC_{50} ± SEM (μM)[i]	> 10	> 10	> 10	> 10

[a]radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35, compounds were tested versus the radioligand [^{3}H]PSB13253; [b]β-arrestin recruitment assay using human GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [c]β-arrestin recruitment assay using rat GPR35 CHO-cells (% activation in comparison to 10 μM zaprinast induced luminescence signal); [d]β-arrestin recruitment assay using mouse GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [e]β-arrestin recruitment assay using human GPR55 CHO-cells (% activation in comparison to 1 μM LPI induced luminescence signal) [f]β-arrestin recruitment assay using human GPR55 CHO-cells (% inhibition of 1 μM LPI induced luminescence signal); [g]cAMP accumulation assay using CHO-β-arrestin-GPR84 cells (% inhibition of Yazh-365 (30 nM) response); [h]calcium ion mobilization assay using 1321N1 astrocytoma cells (% inhibition of calcium-dependend fluorimetric signal upon activation by GPR17 agonist MDL29,951); [i]β-arrestin recruitment assay using human GPR18 CHO-cells (% inhibition of 10 μM THC induced luminescence signal)

All GPR35 agonists were highly selective for GPR35. They were not able to activate or inhibit GPR17, GPR84, GPR18 and GPR55 in our pharmacological test systems (Table 3.35). This result was expected as the SARs, that were determined for GPR35, were unique compared to the other receptors of the test series.

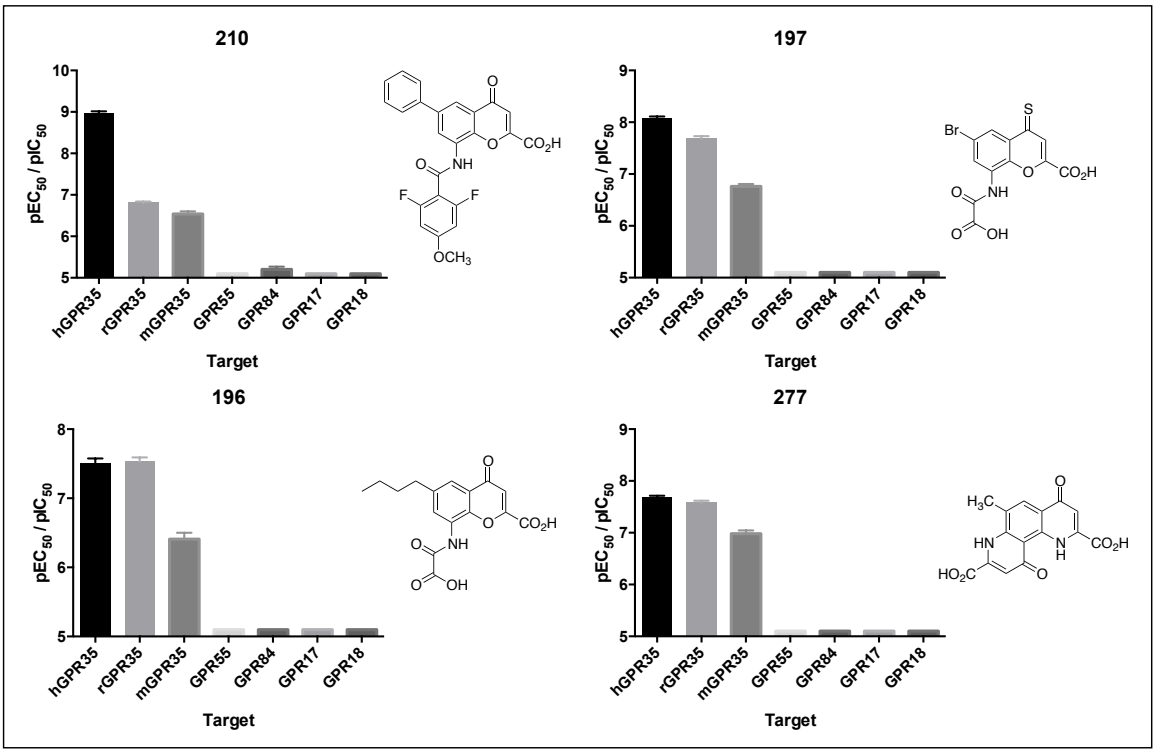
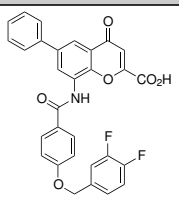
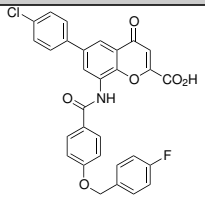
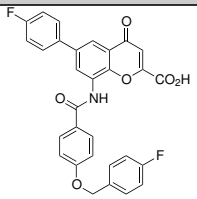
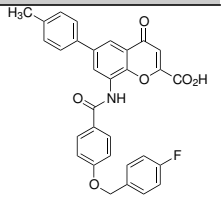


Figure 3.34: Potency and selectivity of GPR35 agonists at class A δ-branch *orphan* GPCRs. Potency of the compounds at hGPR35, mGPR35, rGPR35 and hGPR55 was determined in β-arrestin recruitment assays, respectively. Potency of the compounds at GPR84 was determined by cAMP accumulation assay. Potency of the compounds at hGPR17 was determined in the calcium ion mobilization assay.

3.6.2 Selectivity of the GPR84 antagonists

The most potent GPR84 antagonists **217**, **218**, **220**, and **222** were tested for their selectivity against other *orphan* GPCRs of the δ branch of class A and additionally against the free fatty acid receptors FFAR1 and FFAR4. The free fatty acid receptors FFAR1 (also known as GPR40) and FFAR4 (also known as GPR120) are highly relevant regarding the selectivity of GPR84 ligands because they are known to bind medium-chain fatty acids as GPR84. They also belong to the class A GPCRs.[220,221]

Table 3.36: Potency and selectivity of GPR84 antagonists determined at class A *δ*-branch *orphan* GPCRs and free fatty acid receptors (FFARs).

Target	217	218	220	222
				
human GPR35 K_i ± SEM (μM)[a]	**0.354** ± 0.049	>10	>10	>10
human GPR35 EC_{50} ± SEM (μM)[b]	> 10	> 10	> 10	> 10
rat GPR35 EC_{50} ± SEM (μM)[c]	**0.527** ± 0.055	> 10	> 10	> 10
mouse GPR35 EC_{50} ± SEM (μM)[d]	**1.61** ± 0.29	**2.77** ± 0.26	**1.52** ± 0.28	**3.22** ± 0.44
human GPR55 EC_{50} ± SEM (μM)[e]	> 10	> 10	> 10	> 10
human GPR55 IC_{50} ± SEM (μM)[f]	> 10	> 10	> 10	> 10
human GPR84 IC_{50} ± SEM (μM)[g]	**0.088** ± 0.018	**0.00734** ± 0.000168	**0.0592** ± 0.0039	**0.0404** ± 0.0072
human GPR84 IC_{50} ± SEM (μM)[j]	**0.112** ± 0.048	**0.00302** ± 0.00095	**0.00708** ± 0.00060	**0.0137** ± 0.0025
human GPR17 IC_{50} ± SEM (μM)[h]	> 10	> 10	> 10	> 10
human GPR18 IC_{50} ± SEM (μM)[i]	> 10	> 10	> 10	> 10

FFAR1 IC_{50} ± SEM (μM)[k]	> 10	> 10	> 10	> 10
FFAR1 EC_{50} ± SEM (μM)[l]	> 10	> 10	> 10	> 10
FFAR4 IC_{50} ± SEM (μM)[m]	> 10	> 10	> 10	> 10
FFAR4 EC_{50} ± SEM (μM)[n]	> 10	> 10	> 10	> 10

[a]radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35, compounds were tested versus the radioligand [^{3}H]PSB13253; [b]β-arrestin recruitment assay using human GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [c]β-arrestin recruitment assay using rat GPR35 CHO-cells (% activation in comparison to 10 μM zaprinast induced luminescence signal); [d]β-arrestin recruitment assay using mouse GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [e]β-arrestin recruitment assay using human GPR55 CHO-cells (% activation in comparison to 1 μM LPI induced luminescence signal) [f]β-arrestin recruitment assay using human GPR55 CHO-cells (% inhibition of 1 μM LPI induced luminescence signal); [g]cAMP accumulation assay using CHO-β-arrestin-GPR84 cells (% inhibition of Yazh-365 (30 nM) response); [h]calcium ion mobilization assay using 1321N1 astrocytoma cells (% inhibition of calcium-dependend fluorimetric signal upon activation by GPR17 agonist MDL29,951); [i]β-arrestin recruitment assay using human GPR18 CHO-cells (% inhibition of 10 μM THC induced luminescence signal); [j] β-arrestin recruitment assay using human GPR84 CHO-cells (% inhibition of the effect of 10 μM Yazh-365); [k], [l]compounds were screened at a concentration of 10 μM in calcium mobilization assays using 1321N1 astrocytoma cells recombinantly expressing human FFAR1 and in [m], [n]β-arrestin recruitment assays using CHO β-arrestin cells recombinantly expressing the prolink-tagged human FFAR4s. Effects were normalized to the signal induced by [k]10 μM of TUG-424 and [m]30 μM of TUG-891 at the hFFAR1 and hFFAR4s, respectively, corresponding to a maximal response. Antagonist screenings were performed versus [l]2 μM TUG-424 (hFFAR1) and [n]4μM TUG-891 (hFFAR4s)

All GPR84 antagonists that were evaluated at the different receptors were highly selective for GPR84. No potency was observed at the related FFAR receptors. Also at the class A receptors GPR18, GPR17 and GPR55 no inhibition or activation could be observed in our test assays. The most potent compounds **218**, **220**, and **222** were completely inactive at the human and the rat GPR35. Only compound **217**, which is

substituted with a second fluorine atom at the side chain showed agonistic activity at the human GPR35 and the rat GPR35 in the high nanomolar range. All four GPR84 antagonists showed agonistic activity in the micromolar range at the mouse GPR35 (Table 3.36).

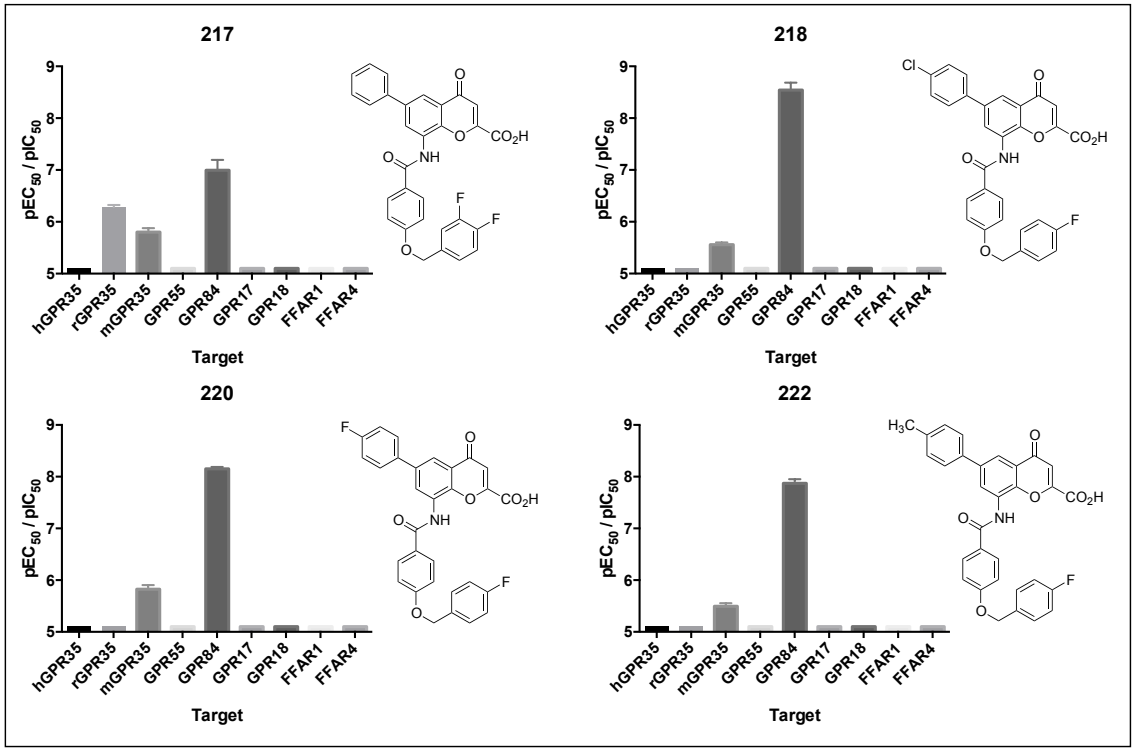
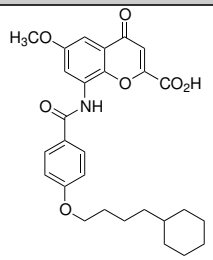
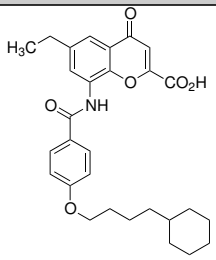


Figure 3.35: Potency and selectivity of GPR35 agonists at class A δ-branch *orphan* GPCRs. Potency of the compounds at hGPR35, mGPR35, rGPR35, hGPR84, FFAR4 and hGPR55 was determined in β-arrestin recruitment assays, respectively. Potency of the compounds at hGPR17 and FFAR1 was determined in the calcium ion mobilization assay.

3.6.3 Selectivity of the GPR17 antagonists

The most potent GPR17 antagonists of our series **255** and compoundANM217 were tested for their selectivity against the *orphan* class A GPCRs GPR35, GPR84, GPR18 and GPR55.

Table 3.37: Potency and selectivity of GPR17 antagonists determined at class A δ-branch *orphan* GPCRs.

Target	255	259
		
human GPR35 K_i ± SEM (μM)[a]	**0.012** ± 0.005	> 10
human GPR35 EC_{50} ± SEM (μM)[b]	> 10	**0.791** ± 0.228
rat GPR35 EC_{50} ± SEM (μM)[c]	> 10	> 10
mouse GPR35 EC_{50} ± SEM (μM)[d]	**2.78** ± 0.27	> 10
human GPR55 EC_{50} ± SEM (μM)[e] (efficacy)	> 10	> 10
human GPR55 IC_{50} ± SEM (μM)[f]	> 10	> 10
human GPR84 IC_{50} ± SEM (μM)[g]	> 3	> 10
human GPR17 IC_{50} ± SEM (μM)[h]	**0.445** ± 0.108	**0.666** ± 0.144
human GPR18 IC_{50} ± SEM (μM)[i]	> 10	n.d.

[a]radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35, compounds were tested versus the radioligand [^{3}H]PSB13253; [b]β-arrestin recruitment assay using human GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [c]β-arrestin recruitment assay using rat GPR35 CHO-cells (% activation in comparison to 10 μM zaprinast induced luminescence signal); [d]β-arrestin recruitment assay using mouse GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [e]β-arrestin recruitment assay using human GPR55 CHO-cells (% activation in comparison to 1 μM LPI induced luminescence signal) [f]β-arrestin recruitment assay using human GPR55 CHO-cells (% inhibition of 1 μM LPI induced luminescence signal); [g]cAMP accumulation assay using CHO-β-arrestin-GPR84 cells (% inhibition of Yazh-365 (30 nM) response); [h]calcium ion mobilization assay using 1321N1 astrocytoma cells (% inhibition of calcium-dependend fluorimetric signal upon activation by GPR17 agonist MDL29,951); [i]β-arrestin recruitment assay using human GPR18 CHO-cells (% inhibition of 10 μM THC induced luminescence signal)

The GPR17 antagonist are not selective. Compound **255** binds with high affinity to

the human GPR35 whereas compound **259** is able to activate the human GPR35 in the β-arrestin recruitment assay. For the development of drugs the selectivity is important. However GPR35 is only poorly investigated yet and its pharmacological functions remains unclear. It has to be investigated if an activation of GPR35 could be tolerated and whether this activation would have a negative side-effect during the therapy.

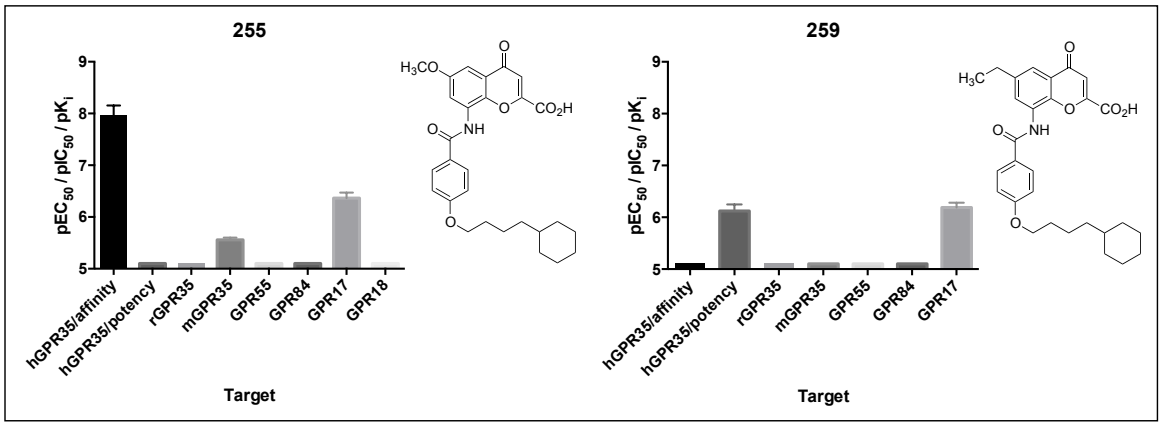
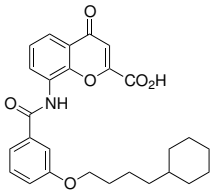
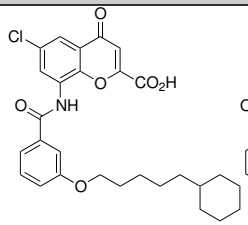
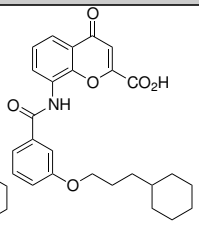
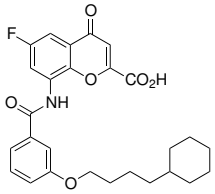


Figure 3.36: Potency and selectivity of GPR17 antagonists at class A δ-branch *orphan* GPCRs. Potency of the compounds at hGPR35, mGPR35, rGPR35, GPR18 and hGPR55 was determined in β-arrestin recruitment assays, respectively. Potency of the compounds at GPR84 was determined by cAMP accumulation assay. Potency of the compounds at hGPR17 was determined in the calcium ion mobilization assay. Affinity of the compounds at hGPR35 was determined by radioligand binding experiments.

3.6.4 Selectivity of the GPR55 agonists

To determine the selectivity of the GPR55 agonists, the most potent compounds of our series were tested at the G protein-coupled receptors GPR35, GPR18, GPR84 and GPR17. GPR35 and GPR84 are phylogenetically related to GPR55. Furthermore the GPR55 agonists were also tested for their selectivity against the cannabinoid receptors CB1 and CB2. This was highly interesting as GPR55 is discussed to be a third cannabinoid receptor.

Table 3.38: Potency and selectivity of GPR55 agonists determined at class A *δ*-branch *orphan* GPCRs.

Target	240	244	237	242
				
human GPR35 K_i ± SEM (*μ*M)[a]	**0.474** ± 0.043	**2.67** ± 0.590	**0.585** ± 0.070	**1.32** ± 0.090
human GPR35 EC_{50} ± SEM (*μ*M)[b]	> 10	> 10	> 10	> 10
rat GPR35 EC_{50} ± SEM (*μ*M)[c]	> 10	> 10	> 10	> 10
mouse GPR35 EC_{50} ± SEM (*μ*M)[d]	**2.58** ± 0.31	> 10	**2.43** ± 0.73	**2.90** ± 0.52
human GPR55 EC_{50} ± SEM (*μ*M)[e] (efficacy)	**0.474** ± 0.099 (72%)	**0.196** ± 0.041 (86%)	**0.111** ± 0.042 (29%)	**0.373** ± 0.038 (71%)
human GPR55 IC_{50} ± SEM (*μ*M)[f]	> 10	> 10	> 10	> 10
human GPR84 IC_{50} ± SEM (*μ*M)[g]	**6.36** ± 1.94	> 3	> 3	**1.03** ± 0.04
human GPR17 IC_{50} ± SEM (*μ*M)[h]	**1.71** ± 0.04	**1.58** ± 0.49	**4.28** ± 0.49	**2.67** ± 0.49
human GPR18 IC_{50} ± SEM (*μ*M)[i]	> 10	> 10	> 10	> 10
human CB1 K_i ± SEM (*μ*M)[j]	> 10	> 10	> 10	> 10

human CB2 $K_i \pm$ SEM (μM)[k]	> 10	> 10	> 10	> 10

[a]radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human GPR35, compounds were tested versus the radioligand [^{3}H]PSB13253; [b]β-arrestin recruitment assay using human GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [c]β-arrestin recruitment assay using rat GPR35 CHO-cells (% activation in comparison to 10 μM zaprinast induced luminescence signal); [d]β-arrestin recruitment assay using mouse GPR35 CHO-cells (% activation in comparison to 30 μM zaprinast induced luminescence signal); [e]β-arrestin recruitment assay using human GPR55 CHO-cells (% activation in comparison to 1 μM LPI induced luminescence signal) [f]β-arrestin recruitment assay using human GPR55 CHO-cells (% inhibition of 1 μM LPI induced luminescence signal); [g]cAMP accumulation assay using CHO-β-arrestin-GPR84 cells (% inhibition of Yazh-365 (30 nM) response); [h]calcium ion mobilization assay using 1321N1 astrocytoma cells (% inhibition of calcium-dependend fluorimetric signal upon activation by GPR17 agonist MDL29,951); [i]β-arrestin recruitment assay using human GPR18 CHO-cells (% inhibition of 10 μM THC induced luminescence signal); [j] radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human CB1 receptor, compounds were tested versus the radioligand [^{3}H]CP55,940; [k] radioligand binding experiments using membrane preparations of CHO cells recombinantly expressing the human CB2 receptor, compounds were tested versus the radioligand [^{3}H]CP55,940

The results showed that the selectivity of the GPR55 agonists was diverse. First of all none of the compounds was able to bind to the cannabinoid receptors and none of the compounds was able to activate or inhibit GPR18. But compound **282** and compound **242** did not only show effects at GPR55 but also at other GPCRs. Both compounds bound with high affinity to the human GPR35 and were able to activate the mouse orthologue of GPR35 in the micromolar range. They were also weak antagonists at GPR17 in the micromolar range. Compound **242** showed antagonistic activity at the human GPR84 around 1 μM. It showed the lowest selectivity for the human GPR55. However the activity of **282** and **242** still was at least 2-4 times higher compared to the other receptors.

The selectivity of **244** and **237** for GPR55 was higher. Both showed low affinity at the human GPR35 and a very low potency at the human GPR17. In contrast to that they showed a high agonistic activity at the human GPR55 in the nanomolar range.

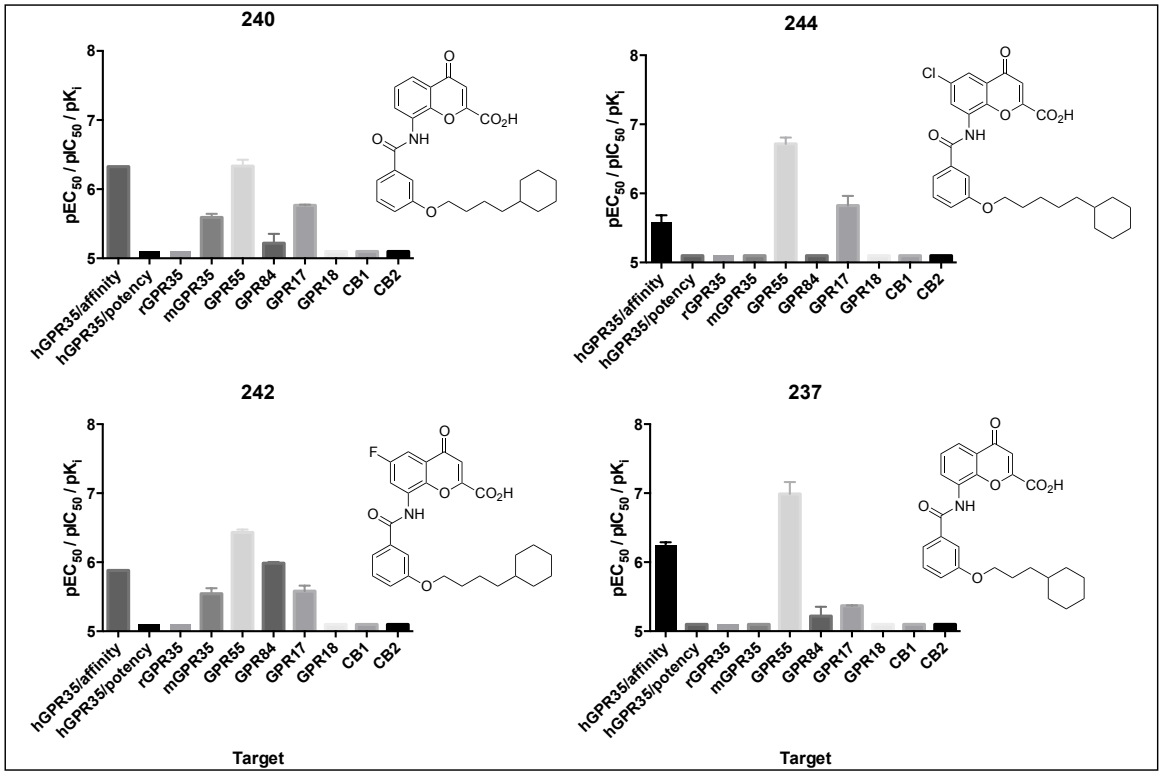


Figure 3.37: Potency and selectivity of GPR35 agonists at class A δ-branch *orphan* GPCRs. Potency of the compounds at hGPR35, mGPR35, rGPR35 and hGPR55 was determined in β-arrestin recruitment assays, respectively. Potency of the compounds at hGPR17 was determined in the calcium ion mobilization assay. Affinity of the compounds at hGPR35, CB1 and CB2 was determined by radioligand binding experiments.

The compound with the bestover all properties as a GPR55 agonist of our series was **244**. It is able to activate GPR55 in the nanomolar range with a high efficacy of 86% and it is highly selective to the other GPCRs we tested.

3.7 Biological evaluation of ecto-5'-nucleotidase inhibitors

3.7.1 Competition studies - Radiometric TLC *e*N assay

In this study 6 compounds, which were previously identified as potent and selective inhibitors at rat ecto-5'-nucleotidase (CD73), were further investigated at the human enzyme. We performed a radiometric thin-layer chromatography (TLC) assay using

[^{3}H]AMP as a substrate.[222] During the enzyme reaction adenosine 5'-monophosphate (AMP) is converted by CD73 to adenosine and further to inosine by adenosine deaminase. This conversion can be monitored by the TLC-assay. To this end the substrate AMP was mixted with the enzyme source (recombinant enzyme or human serum), β-glycerophosphate, the inhibitor and a small amount of the radioactive substrate [^{3}H]AMP in RPMI-1640 medium. The mixture was incubated and the reaction was stopped by pipetting aliquots of the reaction mixture on to a TLC plate together with a reference solution. Subsequent separation with a mixture of 1-butanol 1.5 vol. eq.; isoamylalcohol 1 vol. eq.; diethyleneglycol monoethylether 3 vol. eq.; ammonia solution 1.5 vol. eq.; pure water vol. 2.5 eq.. Then the spots were visualized by UV light, scraped off and quantified by scintillation β-counting.

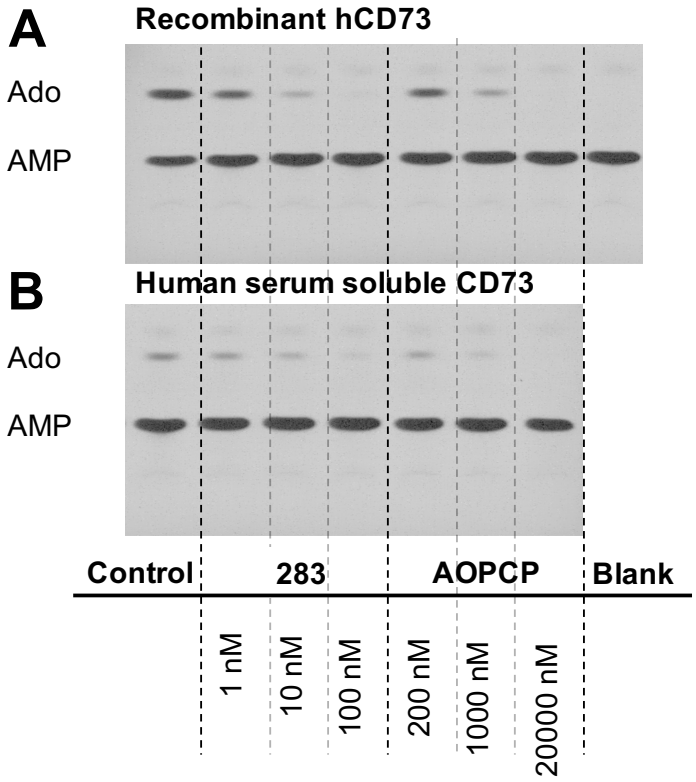


Figure 3.38: Autoradiographic analysis of the *e*N reaction using purified recombinant human enzyme (A) and human serum (B) without inhibitor (control), with inhibitor **283** and **AOPCP**, and without inhibitor and enzyme (Blank).

The TLC assay has got many advantages regarding the analysis of purine-converting

enzymes since it allows to measure a broad range of enzyme reactions without the need of modifications in the procedure except for reaction time and eluent. There is also no limitation to different enzyme sources, so that reactions using pure enzyme, serum or even cells can be measured. Furthermore it does not require expensive instruments such as HPLC so that an implementation of the assay is very easy and cheap. A big advantage of radiometric enzyme assays in general is their high sensitivity and stability.

All of the 6 compounds and the standard inhibitor α,β-methylene-ADP (AOPCP) were tested on purified recombinant human enzyme and human blood serum samples as enzyme source. To visualize the inhibition we prepared an autoradiographic analysis with AOPCP and inhibitor **283** in different concentrations compared to the control without inhibitor and blank (without enzyme and inhibitor) (Figure 3.38).

It was clearly observed that the conversion of AMP to adenosine was reduced in the presence of inhibitors.

Table 3.39: K_i values of inhibitors at recombinant human *e*N and at soluble *e*N in human serum and IC_{50} values of inhibitors at MDA-MB231 and HUVECs determined by radiometric TLC assay. For structures see Figure 3.39

compound	recombinant human *e*N[d] $K_i \pm$ SEM[a] (nM)	human serum *e*N $K_i \pm$ SEM[a](nM)	MDA-MB-231 $IC_{50} \pm$ SEM[b](nM)	HUVEC $IC_{50} \pm$ SEM[c](nM)
AOPCP	**88.4** ± 3.96	**487** ± 186	**5724** ± 1000	**1864** ± 467
284	**2.21** ± 0.40	**18.8** ± 12.5	-	-
285	**4.58** ± 0.23	**68.8** ± 23.2	-	-
286	**2.53** ± 0.735	**8.61** ± 2.79	-	-
287	**0.757** ± 0.061	**13.9** ± 5.74	-	-
288	**1.89** ± 0.061	**11.0** ± 1.04	-	-
283	**0.318** ± 0.02	**2.51** ± 1.07	**103.5** ± 16.9	**73.5** ± 11.6

[a] [^{3}H]AMP (40 μM) was used as substrate (Km value 40 μM and 65 μM respectively for recombinant human *e*N and *e*N in human serum[223])

[b] [^{3}H]AMP (400 μM) was used as substrate

[c] [^{3}H]AMP (200 μM) was used as substrate

[d] recombinant human CD73 was purified from transfected CHO cells[224]

Compound **283**, which was the most potent *e*N-inhibitor, was also tested in MDA-B-231 breast cancer cells and human umbilical vein endothelial cells (HUVECs), which are known to express *e*N. The results of the enzyme competition assays are shown in Table 4.1.

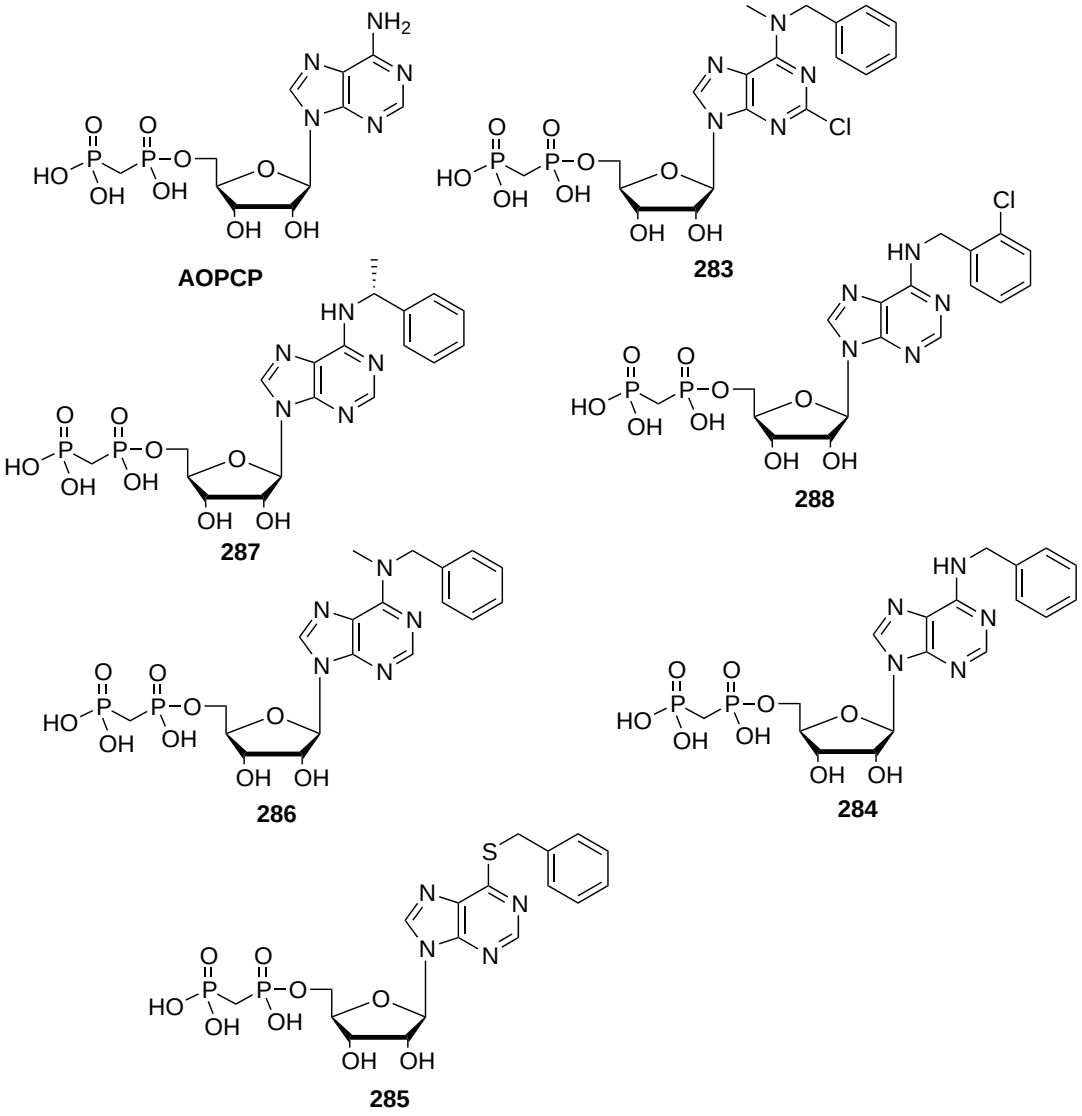


Figure 3.39: Structures of *e*N-inhibitors.

All compounds we tested are much more potent inhibitors of CD73 than the standard inhibitor AOPCP. They block the recombinant enzyme in the low nanomolar or even subnanomolar range. The most potent compound is **283** with a K_i of **0.318** ± 0.02 nM which is almost 300 times more potent than AOPCP. The *e*N inhibitors inhibit

the recombinant enzyme in the following range order of potency: **283** (about 300 folds more potent than AOPCP) > **287** (about 120 folds more potent than AOPCP) > **288** ≈ **284** ≈ **286** (40-50 folds more potent than AOPCP) > **285** (about 20 folds more potent than AOPCP). The K_i values are in the same range as the K_i values determined for recombinant rat CD73 using a different method.[171]

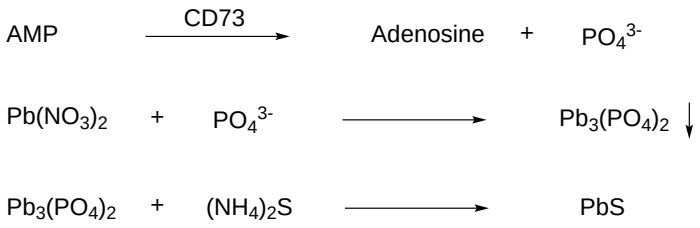
Interestingly the K_i values determined on human serum CD73 are about 10-fold higher than the ones measured on the recombinant enzyme. We suspect that this could be due to plasma protein binding of the compounds, which reduces the free fraction of the drugs. Further explanations could be different conformations of membrane- anchored, soluble and purified enzyme, or different viscosities of the systems. However all compounds were still much more potent than AOPCP.

The most potent inhibitor **283** was analysed in more detail using more complex biological systems. We started with an evaluation of the inhibitor in cancer (MDA-MB-231) and in non-cancer (HUVEC) cells. The IC_{50} values in both cell lines were comparable and in the range of 100 nM. It must be kept in mind that IC_{50} values cannot be compared to K_i values. We had to use high and different substrate concentrations for both cell lines because the quantity of the expression of CD73 is different. The substrate concentration in the assay used for both cell lines was about 10 times higher than that for recombinant and soluble serum CD73. Compared to the standard inhibitor AOPCP our new inhibitor **283** was about 55- and 25-fold more potent than AOPCP in MDA-MB-231 cells and HUVECs, respectively (Table 4.1).

In conclusion all tested inhibitors were confirmed to inhibit CD73 with K_i values in the low nanomolar or subnanomolar range. Inhibitor **283** is significantly more potent in cancer and also in non-cancer cell lines compared to the standard inhibitor AOPCP. We also observed that the potency is lower the more complex the biological system is that we used as an enzyme source. This has to be kept in mind with regard to animal studies. Inhibitors which show a decent potency in vitro are not automatically suitable for in vivo studies because their potency could be too low. With our inhibitor **283** we identified a pharmacological tool which shows an extremely high potency and which can therefore be used for in vivo studies.

3.7.2 Enzyme histochemistry

For the evaluation of the potent inhibitor **283** in tissues we performed a lead nitrate staining using human tonsil and mouse spleen sections.[225] Both tissues are known to express CD73. The lead nitrate staining is based on the chemical detection of the phosphate which is formed when AMP is cleaved by CD73. Phosphate is then reacted with $Pb(NO_3)_2$ to $Pb_3(PO_4)_2$ precipitates in the tissue. The excess of $Pb(NO_3)_2$ is washed out and the formed $Pb_3(PO_4)_2$ is reacted with $(NH_4)_2S$ to PbS which appears as brown staining in the tissue sections (Scheme 3.22).



Scheme 3.22: Lead-nitrate staining reaction of CD73

Human tonsils and mouse spleen tissue sections were stained with 1 mM AMP in the absence or the presence of 20 μM AOPCP or 600 nM of **283** and also with hematoxylin/eosin to distinguish between different tissue structures. Especially in the regions of the central arteries and the capsule intensive brown staining, which means high CD73 activity, is observed in both tissues. This activity was clearly reduced in the inhibitor treated samples. The inhibitor **283** at a concentration of 600 nM blocked CD73 more efficiently than AOPCP at a high concentration of 20 μM as shown by reduced brown staining (Figure 3.40).

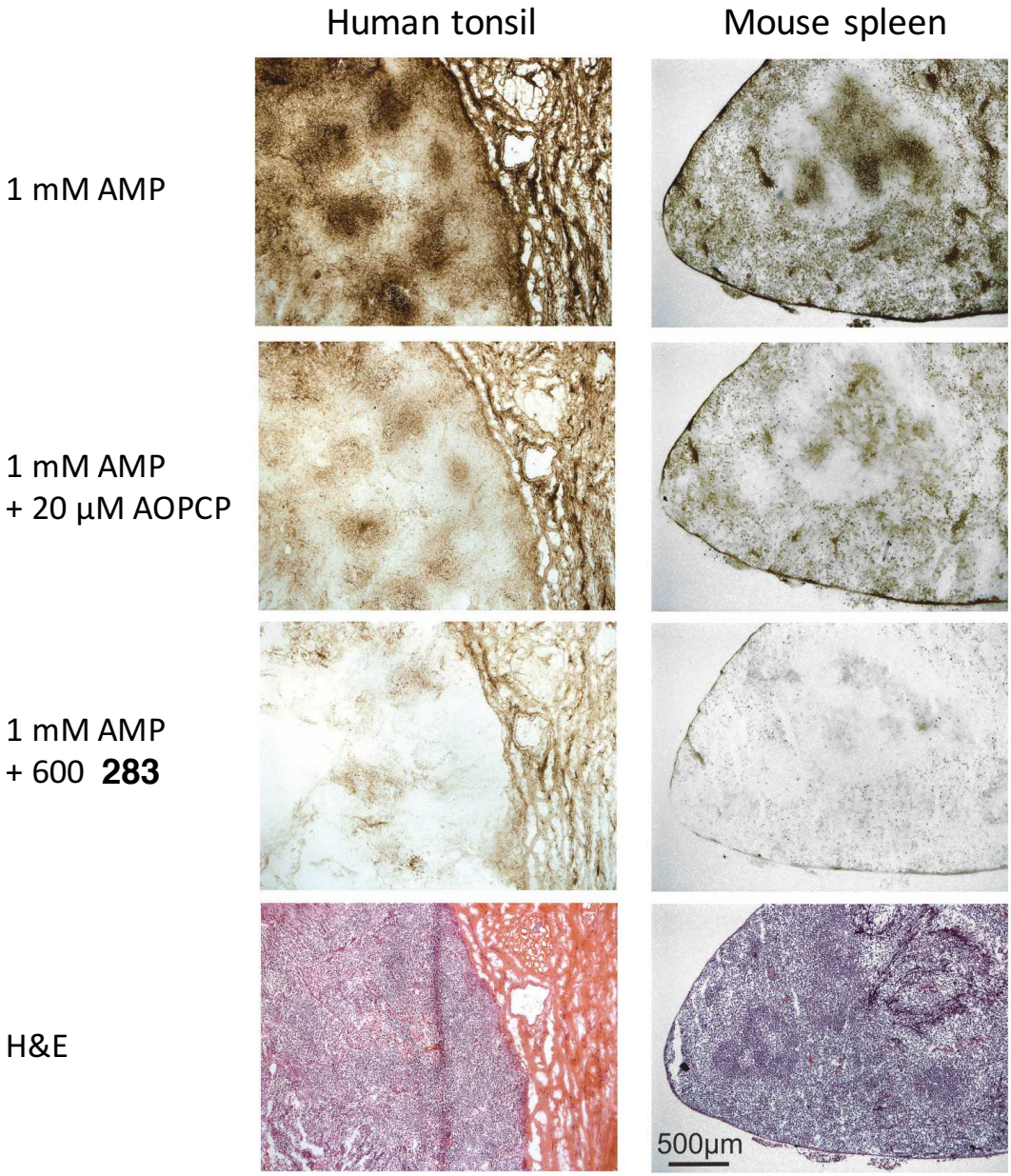


Figure 3.40: Enzyme histochemical staining of human tonsil and mouse spleen tissue section with 1 mM AMP in absence and presence of inhibitors **283** (600 nM) and **AOPCP** (20 μM)

3.7.3 Summary

We can conclude from our results that the new CD73 inhibitor **283** is not only superior to AOPCP at isolated enzyme and human soluble serum enzyme but also blocks CD73 much more efficiently in human and in mouse tissue sections. It represents the most potent inhibitor for CD73 known to date and is therefore an important pharmacological tool to further characterize the enzyme in vivo and also in functional studies.

4 Summary and outlook

4.1 Development of potent and selective ligands for *orphan* G protein-coupled receptors

Human **G protein-coupled receptors** (GPCRs) are a superfamily of about 800 proteins. Nearly 60% of all marketed drugs are targeting GPCRs. However the endogenous ligands of more than 100 GPCRs are still unknown or have not been confirmed yet. As these so-called *orphan* receptors might represent future drug targets these poorly studied receptors bear an enormous potential. Based on sequence homology and common structural elements G protein-coupled receptors can be grouped into different branches. In the present study we focused on purine- and cannabinoid-like receptors of the **δ-branch of class A** (rhodopsin-like) GPCRs. Our aim was to develop and optimize synthetic ligands for several promising *orphan* GPCRs of this receptor family. The newly developed ligands can be used as **pharmacological tools to further study the structures, functions or effects** initiated by those receptors. Although GPR17, GPR35, GPR55 and GPR84, on which we focussed, are poorly studied so far, there are indications that these receptors may be involved in pathological processes. Therefore potent agonists and antagonists represent potential drugs. The results of the study are summarized in the following paragraphs.

4.1.1 Synthesis

For the development of new potent and selective ligands for *orphan* GPCRs we synthesized a large library of **chromen-4-one derivatives** that were based on the structure of the cysteinyl-leukotriene receptor 1 antagonist pranlukast. The chromen-4-one core structure was modified extensively as shown in Figure 4.1.

The **preparation of the potential ligands** as shown in Scheme 4.1 began with the nitration of commercially available 6-substituted 2-hydroxyacetophenones (Scheme 4.1, **a**). The nitrophenyl derivatives were reacted in a Claisen condensation to obtain ethyl chromen-4-one-2-carboxylates (Scheme 4.1, **b**). Reduction of the nitro group at the 6-position yielded the corresponding amines (Scheme 4.1, **c**). The 6-bromino derivative **17** was further modified by several reactions (Scheme 4.1, **d-g**).

It was treated with Lawesson's reagent (Scheme 4.1, **f**) and subjected to Suzuki coupling with various phenylboronic acids (Scheme 4.1, **d**) or with butylboronic acid (Scheme 4.1, **e**) to give the 4-thio derivative, the 6-phenyl derivatives and the 6-butyl derivative, respectively. No convenient method for Suzuki coupling reactions at the 6-position of chromen-4-ones has been described in the literature so far.

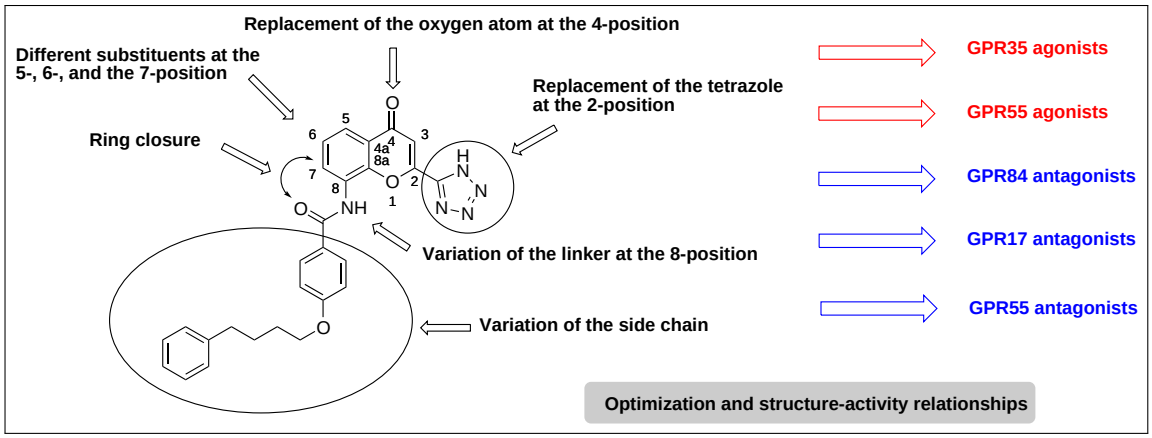


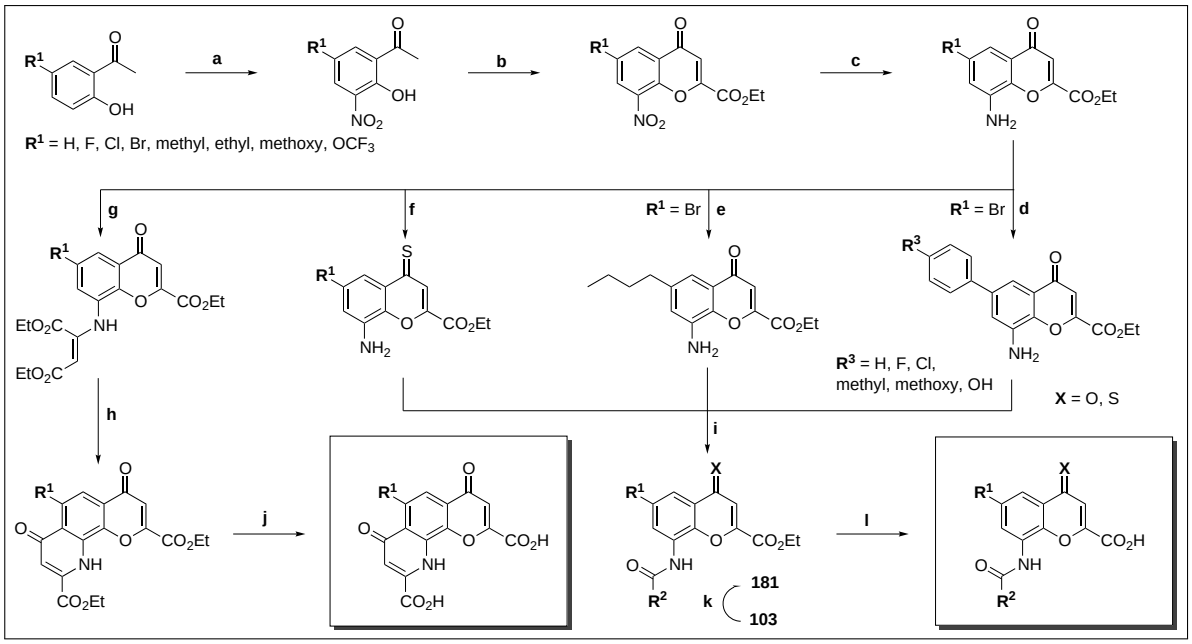
Figure 4.1: The lead structure pranlukast was structurally optimized to obtain ligands for the *orphan* receptors GPR17, GPR35, GPR55, and GPR84. Structure-activity relationships of the chromen-4-one derivatives for the individual receptors were analyzed.

Usually the substituent at the 6-position is introduced before the Claisen condensation is performed. We developed a **more elegant strategy** by synthesizing large amounts of **17** (ethyl 8-amino-6-bromo-4-oxo-4*H*-chromene-2-carboxylate) followed by variation of the 6-position in a single step. To this end the reaction conditions for the Suzuki coupling reaction of the hydrolytically unstable chromen-4-ones had to be optimized. We developed a Suzuki coupling method which worked **under dry conditions** to prevent the cleavage of the chromen-4-one ring. The reaction was performed in a pressure tube under argon which resulted in **short reaction times**. The new method worked also with **alkyl-boronic acids** with slight modifications. Suzuki coupling with **aromatic boronic acids** gave higher yields than coupling with

aliphatic boronic acids.

Two amines were also reacted in a modified Conrad-Limpach reaction with diethyl acetylendicarboxylate followed by ring closure reaction in boiling Dowtherm A®, a heat transfer fluid consisting of diphenyl ($C_{12}H_{10}$) and diphenyl oxide ($C_{12}H_{10}O$) (Scheme 4.1, **g-h**).

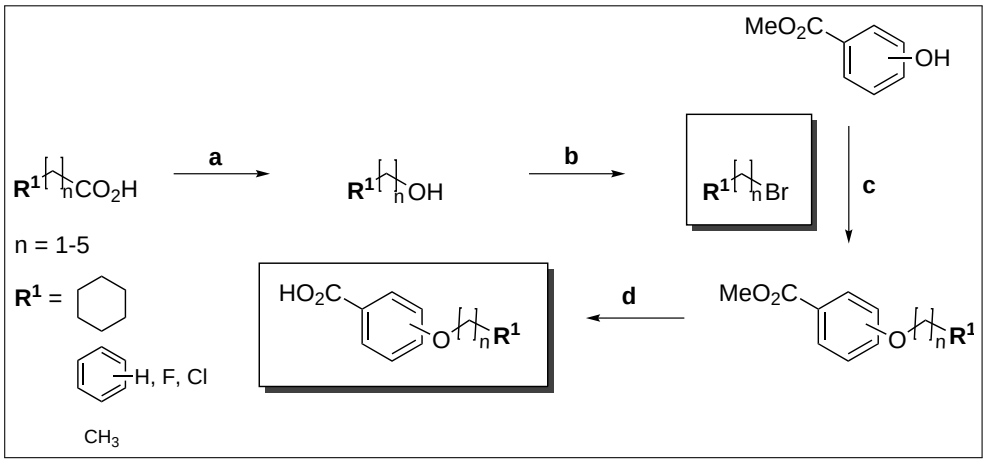
The amide coupling with the corresponding acid chlorides/carboxylic acids gave the desired amides (Scheme 4.1, **i**). Several linker modifications were performed, including the introduction of an urea and a thiourea linker. One compound was also methylated with methyl iodide to obtain a methylated amide as a linker (Scheme 4.1, **k**).



Scheme 4.1: Synthesis and modification of the chromen-4-one core structure. **Reagents and conditions:** (**a**) HNO_3, CH_3COOH, rt, 2 h, 42-95% yield; (**b**) $(COOEt)_2$, KO^tBu, DMF, argon, 0-5 °C, 2-3 h, concd. aq. HCl, ice-water, concd. aq. HCl, EtOH, reflux, overnight, 53-98% yield; (**c**) $SnCl_2x2\ H_2O$, aq. HCl (2N), EtOH, 65 °C, 45 min, 57-95% yield; (*d*) phenylboronic acid, toluene, DMF, K_2CO_3, tetrakis(triphenylphosphine)palladium(0), argon, pressure flask, 120 °C, overnight, 46-89% yield; (**e**) butylbronic acid, toluene, DMF, K_2CO_3, bis(triphenylphosphine)palladium(II) dichloride, triphenylphosphine, pressure flask, 120 °C, overnight, 17% yield; (**f**) Lawesson's reagent, toluene, 100 °C, 16 h, 40-68% yield; (**g**) diethyl acetylendicarboxylate, MeOH, rt, overnight, 33-77% yield; (**h**) Dowtherm A®, 250 °C, 2-30 min, 20-28% yield; (**i**) carboxylic acid chloride, DIPEA, DCM, THF, rt, 1-3 days, 9-99% yield, (**j**) NaOH (1N), THF, EtOH, rt, 5 min, 52-71% yield; (**k**) CH_3I, K_2CO_3, DMF, rt, 2 days, 76% yield; (**l**) K_2CO_3, H_2O, THF, EtOH, rt, overnight, 30-99% yield. For exact structures see chapter 3.

The final step was the hydrolysis of the ethyl ester at the 2-position to yield the chromen-4-one-2-carboxylic acid derivatives (Scheme 4.1, **j, l**).

For the synthesis of ligands for GPR17, GPR55, and GPR84 long side-chains in the 8-position were needed that were not commercially available. We synthesized these intermediates starting from the aliphatic carboxylic acid which was reduced to give the corresponding alcohol (Scheme 4.2, **a**). The alcohol was then brominated (Scheme 4.2, **b**) and coupled to several methyl hydroxybenzoates via a Williamsson ether synthesis (Scheme 4.2, **c**). Finally the methyl ester was cleaved (Scheme 4.2, **d**) and the carboxylic acid chlorides were prepared which could be coupled to the amines as previously described.



Scheme 4.2: Synthesis of the side-chain. **Reagents and conditions**: (**a**) $LiAlH_4$, THF, rt, 4 h, 85-83% yield; (**b**) PBr_3, toluene, 0-5 °C, 30 min, reflux, 120 min, 86% yield (crude product); (**c**) K_2CO_3, DMF, 115 °C, 4 h, 37-100% yield; **d** method 1: microwave, MeOH, KOH, 100 °C, 30 min; method 2: pressure tube, MeOH, KOH, 120 °C, 4 h, 23-97% yield. For exact structures see Table 3.5 and Table 3.6

Altogether we **successfully synthesized 82 chromen-4-one-2-carboxylic acid derivatives** of which only one was previously described in the literature. These compounds were evaluated in our group for their potency to activate or block the *orphan* GPCRs GPR17, GPR35, GPR55 and GPR84.

4.1.2 GPR35

One receptor we mainly focused on was **GPR35** which has been observed to be expressed in the gastrointestinal tract, in liver, in the central nervous system, the

cardiovascular system and the immune system. GPR35 agonists were proposed as potential drugs for **inflammation and pain** and for **cardiovascular diseases**, such as hypertension.

The potencies of chromen-4-one-2-carboxylic acids at the human GPR35 were determined by β-arrestin recruitment assays and the affinities were measured by radioligand binding studies. Structure-activity relationships are summarized in Figure 4.2. Besides the introduction of substituents at the 6-position, like a phenyl ring or a bromine atom, the replacement of the oxygen atom at the 4-position by a sulfur atom resulted in an increase in potency. Furthermore, fluorine atoms in the *ortho*- and *meta*-position and a methoxy group in the *para*-position of the benzamide at position 8 were beneficial. The introduction of a second carboxylic acid function as well as ring closure at position 7 and 8 did not result in an increase in potency.

The most potent human GPR35 agonist of this series of compounds was compound **210** with an EC_{50} value of **0.00108** $\pm$ 0.00011 μM. It is the **most potent GPR35 agonist known to date** and it is **highly selective** versus GPR55, which is phylogenetically the closest relative of GPR35, and it is also selective versus other related δ-branch GPCRs including GPR17 and GPR84.

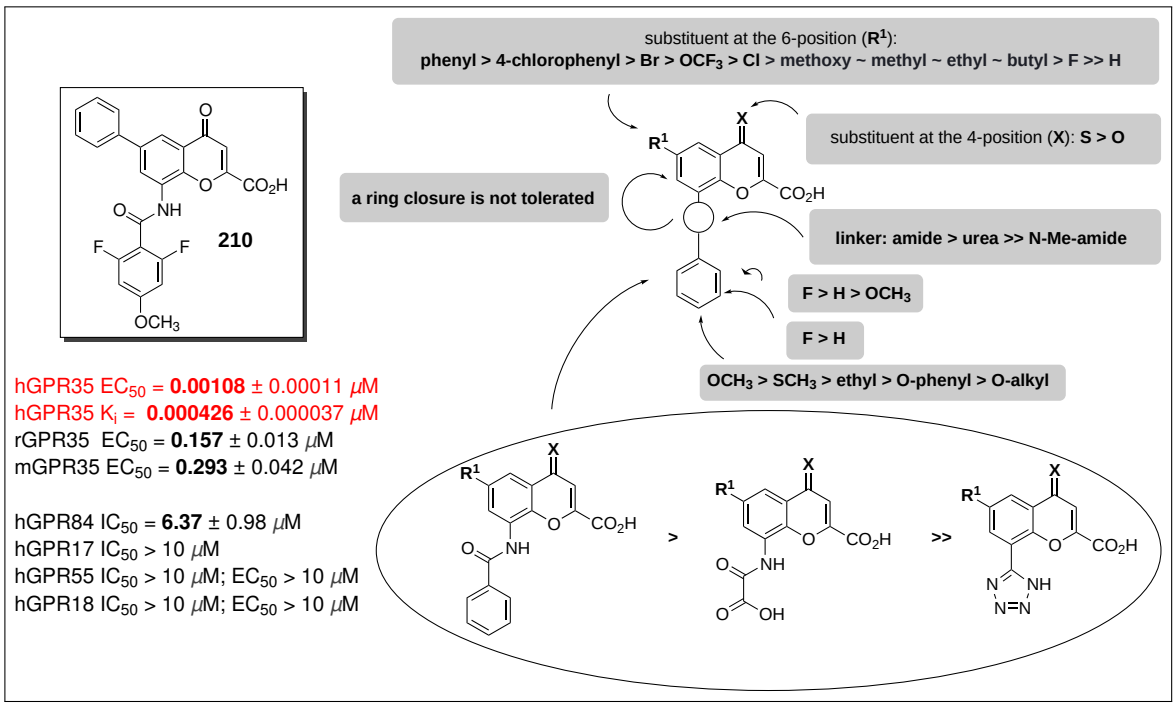


Figure 4.2: Structure-activity relationships for chromenones at the human GPR35. The most potent compound **210** was further evaluated at various related targets.

The GPR35 agonist **210** showed high **orthologue selectivity** for the human receptor and only moderate potency at the rodent orthologues. However, for preclinical in vivo studies potent ligands at rodent receptors are required. Therefore we optimized the chromen-4-one derivatives with regard to their potency at the mouse and the rat GPR35.

Structure-activity relationships are summarized in Figure 4.3. In contrast to the human GPR35 (hGPR35) the introduction of a second carboxylic acid function at position 8 increased potency at the rodent receptors. At the 6-position large substituents like a bromine atom or a phenyl ring were well tolerated, but in contrast to the human receptor a butyl substituent was preferred. The introduction of a thiourea linker instead of an amide linker further increased potency. We observed differences not only between the hGPR35 and the rodent orthologues but also between the mouse GPR35 (mGPR35) and the rat GPR35 (rGPR35). Whereas the ring closure between position 7 and 8 was well tolerated by the mGPR35 it resulted in decreased potency at the rGPR35.

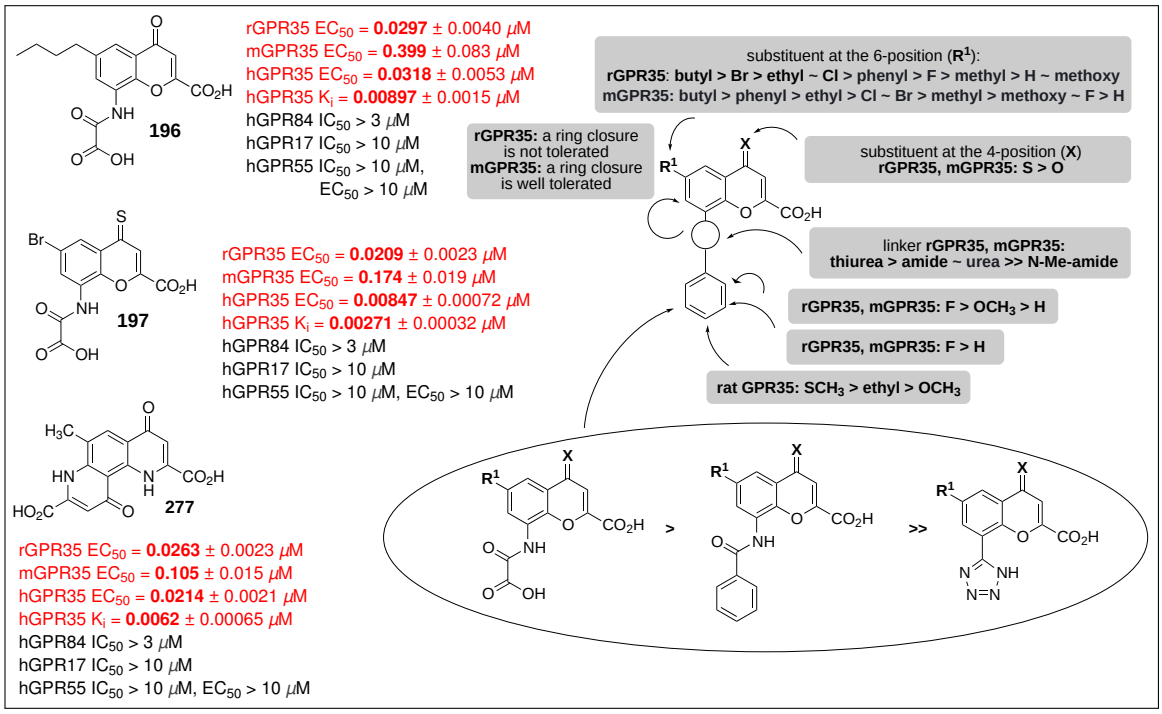


Figure 4.3: Structure-activity relationships of chromenones as agonists at the rodent GPR35. The most potent compounds **196**, **197**, and **277** were further evaluated at various related targets.

Comparable to the hGPR35, the replacement of the oxygen atom at position 4 by a sulfur atom increased the potency at both rodent orthologues. The most potent chromen-4-one-based agonists at mouse and rat GPR35 were **196** (rGPR35: EC_{50} = **0.0297** ± 0.0040 μM; mGPR35 EC_{50} = **0.399** ± 0.083 μM) and **197** (rGPR35: EC_{50} = **0.0209** ± 0.0023 μM; mGPR35 EC_{50} = **0.174** ± 0.019 μM). To further increase the potency at the mGPR35 we synthesized a derivative of bufrolin (for structure see chapter 1), which was recently identified as a GPR35 agonist. Compound **277** showed **high potency at all tested GPR35 orthologues** (rGPR35: EC_{50} = **0.0263** ± 0.0023 μM; mGPR35 EC_{50} = **0.105** ± 0.015 μM; hGPR35 EC_{50} = **0.0214** ± 0.0021 μM).

For further optimization an introduction of various alkyl side-chains and bromoalkyl side-chains at the 6-position would be interesting. Furthermore additional bufrolin derivatives should be synthesized to develop further GPR35 agonists that are equally potent at the human and the rodent GPR35.

Besides full agonists we also identified potential biased agonists which do not activate or block the β-arrestin pathway but which showed high affinities in the low nanomolar range at the human GPR35 (**255**, $EC_{50} > 10$ μM; K_i = **0.012** ± 0.005 μM). These compounds should be further investigated regarding their functional activity.

4.1.3 GPR84

A further receptor that was investigated in this study was the *orphan* receptor **GPR84**. It is known to be expressed in immune cells. Therefore GPR84 antagonist are potential drugs for the treatment of inflammatory diseases. The structure-activity relationships are summarized in Figure 4.4. The potencies of chromen-4-one-2-carboxylic acids at GPR84 were determined by cAMP accumulation assays in CHO cells recombinantly expressing the human GPR84. The most potent compounds were further evaluated in β-arrestin recruitment assays and radioligand binding experiments.

As GPR84 is known to be activated by medium-chain free fatty acids we synthesized a broad range of chromen-4-one derivatives that contained a long lipophilic side-chain at the 8-position. The introduction of additional halogen atoms, preferably fluorine atoms, was well tolerated at the second phenyl ring but not at the first

phenyl ring. The most remarkable increase in potency could be achieved by the modification of the 6-position. The introduction of a larger, *para*-substituted phenyl ring strongly increased the inhibitory potency of the GPR84 antagonists. A chlorine atom at the *para*-position of the phenyl ring was preferred. The modification of the linker as well as the replacement of the oxygen atom at the 4-position by a sulfur atom had no effect on potency.

The most potent compound of our series was **218** (IC_{50} = **0.00734** ± 0.000168 μM), which is the **most potent GPR84 antagonist known to date and displays high selectivity** versus GPR17, GPR35 and GPR55 and the free fatty acid receptors FFAR1 and FFAR2.

To improve the potency of GPR84 antagonists a further enlargement of the substituent at the 6-position would be interesting. However this would also result in higher lipophilicity.

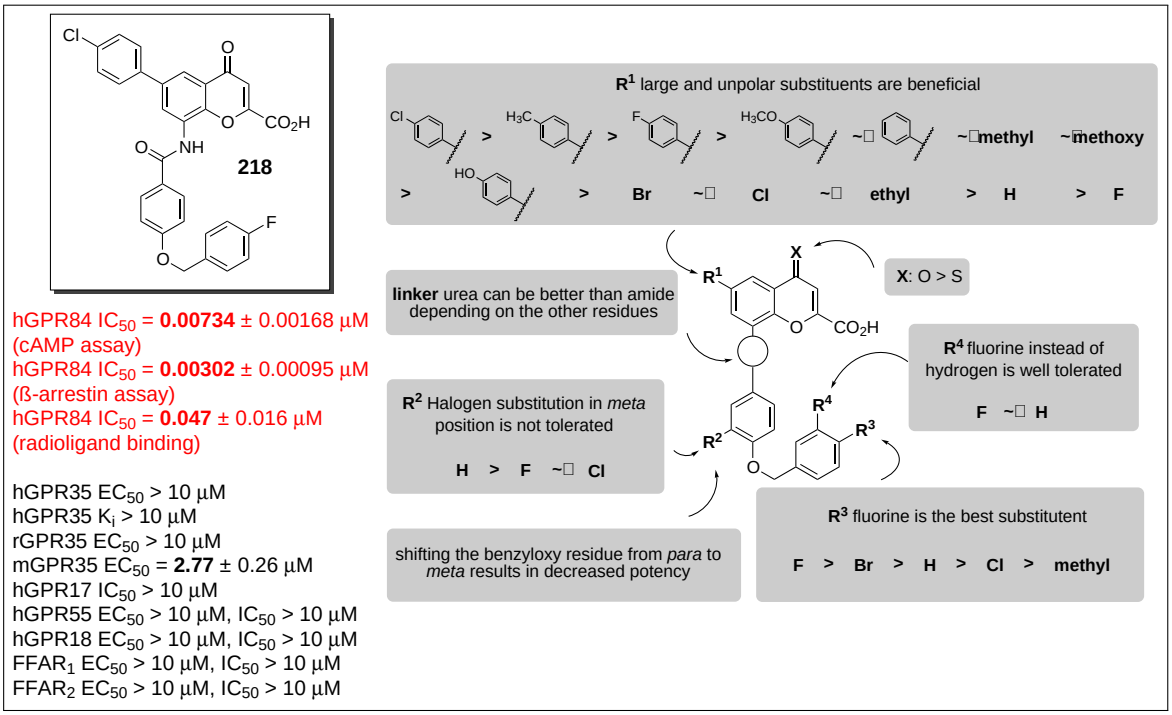


Figure 4.4: Structure-activity relationships for chromenones at the human GPR84. The most potent GPR84 antagonists **218** was evaluated in more detail.

4.1.4 GPR17

The *orphan* receptor **GPR17** is discussed as a novel drug target for **multiple sclerosis** as there are indications that GPR17 antagonists could **promote the myelination** . We optimized chromen-4-one derivatives regarding their inhibitory potency at GPR17. Calcium ion mobilization assays in 1321N1 astrocytoma cells that recombinantly expressed the human GPR17 were performed in our group. The most potent compounds were further evaluated in radioligand-binding experiments. Structure-activity relationships are summarized in Figure 4.5.

To increase the inhibitory potency of the compounds at GPR17 the introduction of long alkyl side-chains in the *para*-position of the phenylamide at position 8 was beneficial. The optimal chain-length was 4 C-atoms that were connected to a cyclohexyl ring. At the 6-position halogen atoms were not tolerated. A methoxy group mostly increased the potency at GPR17. The replacement of the linker at the 8-position as well as the replacement of the oxygen atom at the 4-position by a sulfur atom only showed minor effects on the potency.

The most potent compound of the series was **255**, which has an IC_{50} value of **0.445** ± 0.108 μM. It is **highly selective** versus GPR55 and GPR84 but it shows also a high affinity for GPR35.

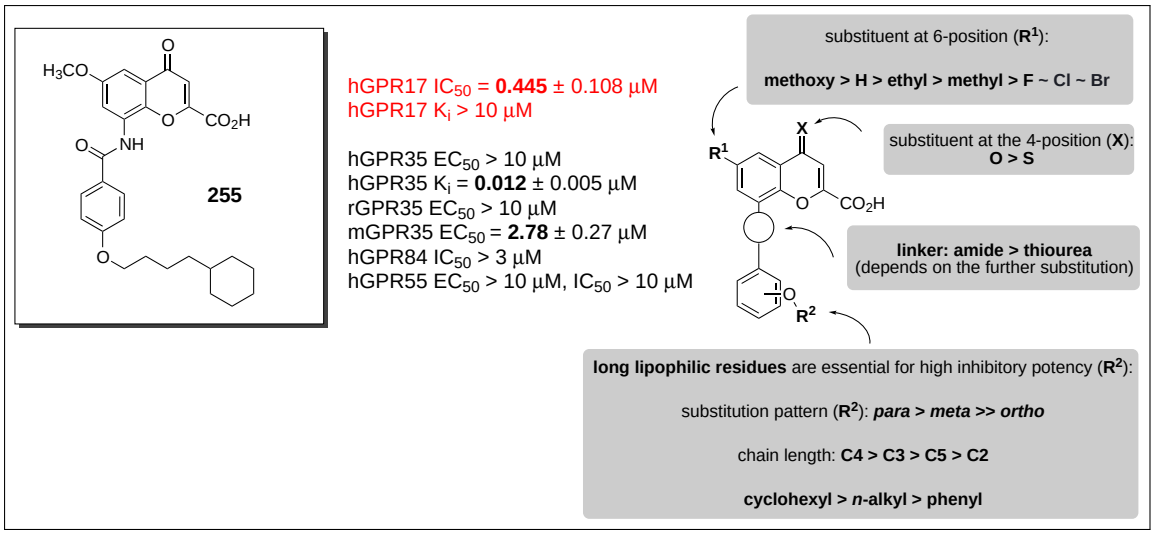


Figure 4.5: Structure-activity relationships for chromenones at the human GPR17. The most potent GPR17 antagonists **255** was evaluated in more detail.

4.1.5 GPR55

GPR55 is the phylogenetically closest relative of GPR35 and it interacts with certain cannabinoids. It is widely expressed in bone, intestine and brain. GPR55 activation was shown to be **pro-carcinogenic**. GPR55 antagonists my be developed as anticancer drugs. We synthesized a broad range of potent ligands for GPR55, including **agonists** and **partial agonists** with potencies in the nanomolar range and **antagonists** with low micromolar potencies. Structure-activity relationships are displayed in Figure 4.6. Pharmacological evaluation was performed by the β-arrestin recruitment assay in our group.

The modification of the 6-position, the linker, or of position 4 did not result in an increase in potency, in contrast to modifications that were performed at the side-chain in position 8. Long and lipophilic residues were essential for high potency at GPR55. Shifting the side-chain from the *meta*-position to the *para*-position resulted in a switch from agonistic to antagonistic activity.

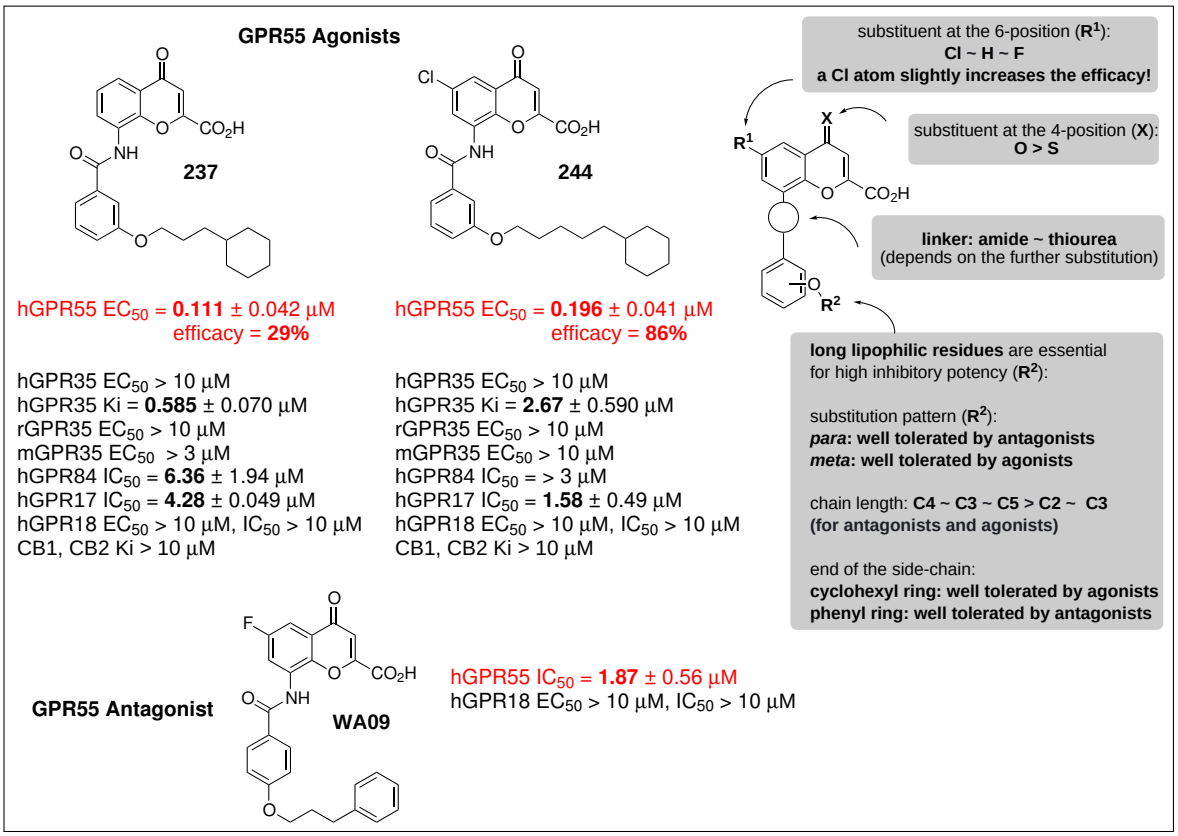


Figure 4.6: Structure-activity relationships for chromenones at the human GPR55. The most potent GPR55 agonists **237** and **244** were evaluated in more detail.

The same switch could be observed when the cyclohexyl ring at the end of the side-chain was replaced by a phenyl ring. The prolongation of the side-chain resulted in an increase in efficacy, but in a slight decrease of potency.

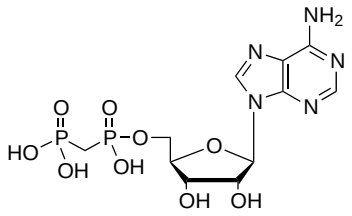
6-Chloro-8-(3-((5-cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid, **244** (EC_{50} ± SEM = **196** ± 41.0 *n*M, Emax = 86%), is one of the most potent agonists for human GPR55 based on the chromen-4-one scaffold and it is **highly selective** versus the closely related GPR35 as well as versus GPR17, GPR84 and the cannabinoid receptors CB_1 and CB_2.

In conclusion we successfully developed **potent and selective agonists and antagonists respectively, for the *orphan* receptors** GPR17, GPR35, GPR55 and GPR84. Some of the compounds represent the most potent ligands for their receptors known to date. The new chromen-4-one derivatives are important **tools for further investigation of the receptors in vitro and in vivo**, and can be used as **lead compounds for the development of new drugs**. The chromen-4-one was confirmed to be a **privileged structure for the development of ligands for class A *δ*-branch GPCRs**. In the future our comprehensive and structurally diverse library of chromenones can be pharmacologically evaluated at further receptors of this class of GPCRs and subsequently be individually optimized.

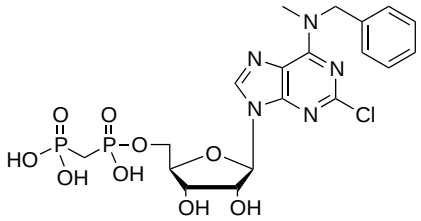
4.2 Biological evaluation of potent and selective ecto-5'-nucleotidase inhibitors

Ectonucleotidases hydrolyze extracellular nucleotides and are thereby involved in **purinergic signalling**. **Ecto-5'-nucleotidase (*e*NT/CD73) mediates hydrolysis of adenosine-5'-monophosphate (AMP) to adenosine** and subsequently activates adenosine receptors. Is is therefore involved in several physiological and pathological processes such as **immunity, inflammation, neurotransmission and tumorigenesis**. The inhibitory potencies of **new small molecule inhibitors**, which were developed in our group, where measured by using a thin layer chromatographic (TLC) assay with [^{3}H]AMP as a substrate, and compared with the standard CD73 inhibitor *α*,*β*-methylene-ADP (AOPCP).

Table 4.1: K_i values of inhibitors at recombinant human *e*N and at soluble *e*N in human serum and IC_{50} values of inhibitors at MDA-MB231 and HUVECs determined by radiometric TLC assay. For structures see Figure 3.39



AOPCP



283

compound	recombinant human *e*N[d] $K_i \pm SEM$[a]	human serum *e*N $K_i \pm SEM$[a]	MDA-MB-231 $IC_{50} \pm SEM$[b]	HUVEC $IC_{50} \pm SEM$[c]
AOPCP	**88.4** ± 3.96	**487** ± 186	**5724** ± 1000	**1864** ± 467
284	**2.21** ± 0.40	**18.8** ± 12.5	-	-
285	**4.58** ± 0.23	**68.8** ± 23.2	-	-
286	**2.53** ± 0.735	**8.61** ± 2.79	-	-
287	**0.757** ± 0.061	**13.9** ± 5.74	-	-
288	**1.89** ± 0.061	**11.0** ± 1.04	-	-
283	**0.318** ± 0.02	**2.51** ± 1.07	**103.5** ± 16.9	**73.5** ± 11.6

[a] [^{3}H]AMP (40 μM) was used as substrate (Km value 40 μM and 65 μM respectively for recombinant human *e*N and *e*N in human serum[223])

[b] [^{3}H]AMP (400 μM) was used as substrate

[c] [^{3}H]AMP (200 μM) was used as substrate

[d] recombinant human CD73 was purified from transfected CHO cells[224]

The ability of the most potent CD73 inhibitor, **283**, to block AMP hydrolysis in different cells and tissues was further confirmed in **TLC assays with cultured human MDA-MB-231 breast cancer cell lines and human umbilical vein endothelial cells (HUVEC)**, as well as in lead nitrate-based **histochemical analysis of ecto-5'-nucleotidase distribution in human tonsil and mouse spleen sections**. The studies showed the **superiority of our new inhibitors compared to AOPCP** in all biological test systems and represent a basis for future in vivo studies.

5 Experimental part

5.1 General remarks

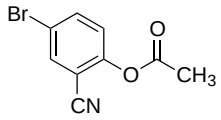
All commercially available reagents were used as purchased (Acros, Alfa Aesar, Sigma-Aldrich, or Fluorochem). Solvents were employed without additional purification or drying except for dichloromethane, which was distilled over calcium hydride. The reactions were monitored by thin-layer chromatography (TLC) using aluminum sheets with silica gel 60 F254 (Merck). Column chromatography was performed with silica gel, 0.060-0.200 mm, pore diameter ca. 6 nm. All synthesized compounds were finally dried in vacuum at 8-12 Pa (0.08-0.12 mbar) using a sliding vane rotary vacuum pump (Vacuubrand GmbH). ^{1}H and ^{13}C NMR data were collected either on a Bruker Avance 500 MHz NMR spectrometer at 500 MHz (^{1}H) or 126 MHz (^{13}C) or on a Bruker Ascend 600 MHz NMR spectrometer at 600 MHz (^{1}H) or 151 MHz (^{13}C). DMSO-d_6 was employed as a solvent at 303 K, unless otherwise noted. Chemical shifts are reported in parts per million relative to the deuterated solvent, that is, dimethylsulfoxide (DMSO), δ (^{1}H) 2.49 ppm, δ (^{13}C) 39.7 ppm. Coupling constants J are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). melting points (mps) were determined on a Büchi 530 melting point apparatus and are uncorrected. The purities of isolated products were determined by electrospray ionization (ESI)-mass spectra obtained on an liquid chromatography-mass spectrometry (LC-MS) instrument (Applied Biosystems API 2000 LC-MS/MS, high-performance liquid chromatography (HPLC) Agilent 1100) using the following procedure: the compounds that contained a chromen-4-one ring system were dissolved at a concentration of 0.5 mg/mL in acetonitrile/water containing 2 mM ammonium acetate. Then 10 μL of the sample was injected into an HPLC column (Macherey-Nagel Nucleodur 3 μm C18, 50 x 2.00 mm). Elution was performed with a gradient of water/acetonitrile (containing 2 mM ammonium acetate) from 90:10 to 0:100 for 20 min at a flow rate of 300 μL/min, starting the

gradient after 10 min. ultraviolet (UV) absorption was detected from 200 to 950 nm using a diode array detector. For all other compounds the same method was used, but acetonitrile was replaced by methanol. Purity of all compounds was determined at 254 nm.

5.2 Synthesis and characterization

5.2.1 4-Bromo-2-cyanophenyl acetate

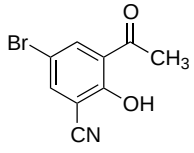
5.2.1.1 4-Bromo-2-cyanophenyl acetate 269 (*ANM380*)[226]



5-Bromo-2-hydroxybenzonitrile (9.1 g, 46 mmol) was stirred with acetic anhydride (20 mL) and three drops of concd. H_2SO_4 were added. After 1 hour the reaction mixture was poured on ice and the obtained solid was filtered, washed with water, and dried in vacuum at rt. The product was isolated as white crystals (9.3 g, 84% yield). ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 7.42 (d, J = 8.8 Hz, 1H, 6-H), 7.98 (ddd, J = 8.8, 2.4, 0.6 Hz, 1H, 5-H), 8.24 (d, J = 2.4 Hz, 1H, 3-H). ^{13}C NMR: δ 20.6 ($OCO\underline{C}H_3$), 108.4 (C-2), 114.0 (CN), 118.7 (C-4), 125.7 (C-6), 135.8 (C-3), 138.0 (C-5), 151.5 (C-1), 168.3 (C=O). LC-MS (m/z): positive mode 198 $[M\text{-}COCH_3]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 90.0%. lit. mp 60-61 °C.

5.2.2 3-Acetyl-5-bromo-2-hydroxybenzonitrile

5.2.2.1 3-Acetyl-5-bromo-2-hydroxybenzonitrile 265 (*ANM381*)[226]

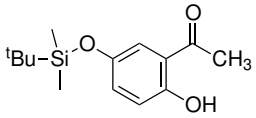


Compound **269** (9.12 g, 38 mmol) and $AlCl_3$ (16 g, 120 mmol) were heated to 160 °C under stirring. After 3 hours the reaction mixture was cooled down to rt and poured on 200 g of ice containing 20 mL of concd. HCl. The slurry was extracted three times with 50 mL of EtOAc, acidified with 1 N HCl, washed with brine and dried over $MgSO_4$. After removing the solvent under reduced pressure the crude product was recrystallized in DCM. The product was isolated as a pale brown solid (2.48 g, 27% yield). ^{1}H NMR: δ 2.69 (s, 3H, $COCH_3$), 8.22 (d, J = 2.5 Hz, 1H, 4-H), 8.35 (d, J = 2.5 Hz, 1H, 6-H), 12.74 (s, 1H, OH). ^{13}C NMR: δ 27.5 ($CO\underline{C}H_3$), 103.4 (C-1), 110.1 (CN), 114.2 (C-6), 122.2 (C-3), 138.9 (C-4), 141.5 (C-6), 161.6 (C-2), 204.3 (C=O). LC-MS (m/z): positive mode 239

$[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 92.8%. lit. mp 151-152 °C.

5.2.3 1-(5-((*Tert*-butyldimethylsilyl)oxy)-2-hydroxyphenyl)ethanone

5.2.3.1 1-(5-((*Tert*-butyldimethylsilyl)oxy)-2-hydroxyphenyl)ethanone 289 (*ANM208*)[227]



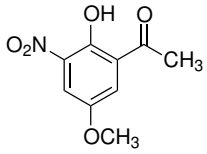
Dihydroxyacetophenon (5.00 g, 33 mmol), TBDMSCl (5.90 g, 39 mmol), and imidazole (1.97 g, 29 mmol) were dissolved in 80 mL of dry DMF under an argon atmosphere and stirred for 12 h at rt. After addition of 60 mL of H_2O the product was extracted with 3x15 mL EtOAc, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified on a column of silica gel (10% EtOAc in cyclohexane) and was isolated as a yellow oil (4.21 g, 48% yield). ^{1}H NMR: δ 0.17 (s, 6H, Si($C\underline{H}_3$)$_2$), 0.94 (s, 9H, SiC($C\underline{H}_3$)$_3$), 2.59 (s, 3H, $COCH_3$), 6.85 (d, 1H, J = 8.9 Hz, 5-H), 7.05 (dd, 1H, J = 3.0, 8.9 Hz, 6-H), 7.21 (d, 1H, J = 3.0 Hz, 3-H), 11.40 (s, 1H, OH). ^{13}C NMR: δ 4.5 (Si($\underline{C}H_3$)$_2$), 18.0 (Si$\underline{C}$), 25.7 (SiC($\underline{C}H_3$)$_3$), 28.1 (CO$\underline{C}H_3$), 118.6 (C-6), 120.5 (C-3), 120.7 (C-6), 128.5 (C-5), 147.0 (C-4), 155.3 (C-1), 203.6 ($\underline{C}OCH_3$). LC-MS (m/z): positive mode 267 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.0%.

5.2.4 1-(2-Hydroxy-3-nitrophenyl)ethanones

5.2.4.1 General procedure for the synthesis of 1-(2-hydroxy-3-nitrophenyl)ethanones (GP-1)

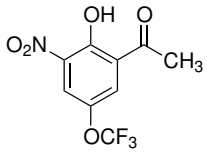
The appropriate 2-hydroxyacetophenone (10 mmol) was dissolved in 6.74 mL of glacial acetic acid. Then a mixture of fumic nitric acid (0.46 mL, 11.1 mmol) and glacial acetic acid (0.85 mL, 13.5 mmol) was added dropwise and the reaction mixture was stirred for 120 min at rt. The product was precipitated by pouring it on ice and the solid was subsequently filtered off, washed with water (30 mL), and dried in vacuum at 50 °C.

5.2.4.2 1-(2-Hydroxy-5-methoxy-3-nitrophenyl)ethanone 1 (*ANM29*)[175]



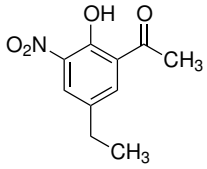
The compound was synthesized according to GP-1 using 2-hydroxy-5-methoxyacetophenone (1.66 g, 10 mmol). The product was isolated as an orange solid (1.31 g, 62%). ^{1}H NMR: δ 2.70 (s, 3H, $COCH_3$), 3.84 (s, 3H, O-CH_3), 7.74 (d, J = 3.2 Hz, 1H, 3-H), 7.79 (d, J = 3.2 Hz, 1H, 5-H), 12.20 (s, 1H, OH). ^{13}C NMR: δ 28.5 (CO$\underline{C}H_3$), 56.6 (O-CH_3), 115.8 (C-5), 122.5 (C-3), 124.1 (C-2), 138.3 (C-6), 147.3 (C-1), 150.5 (C-4), 204 ($\underline{C}OCH_3$). LC-MS (m/z): positive mode 209 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. lit. mp 112-114 °C.

5.2.4.3 1-(2-Hydroxy-3-nitro-5-(trifluoromethoxy)phenyl)ethanone 2 (*ANM50*)



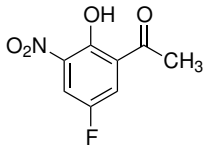
The compound was synthesized according to GP-1 using 2-hydroxy-5-(trifluoromethoxy)acetophenone (1.76 g, 8 mmol). The product was isolated as a yellow solid (881 mg, 42%). ^{1}H NMR: δ 2.63 (s, 3H, $COCH_3$), 7.89-7.93 (m, 1H, 3-H), 8.05 (s, 1H, 5-H). ^{13}C NMR: δ 40.1 (CO$\underline{C}H_3$), 117.3 (C-2), 119.4 (OCF_3), 123.9 (C-5), 128.1 (C-6), 129.0 (C-3), 134.1 (C-1), 158.3 (C-4), 201.0 ($\underline{C}OCH_3$). LC-MS (m/z): positive mode 264 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 87-88 °C.

5.2.4.4 1-(5-Ethyl-2-hydroxy-3-nitrophenyl)ethanone 3 (*ANM83*)[176]



The compound was synthesized according to GP-1 using 5-ethyl-2-hydroxyacetophenone (3.3 g, 20 mmol). The product was isolated as a yellow solid (3.83 g, 92%). ^{1}H NMR: δ 1.20 (t, J = 7.6, 3H, $CH_2\underline{CH_3}$), 2.64-2.68 (m, 2H, $\underline{CH_2}CH_3$), 2.71 (s, 3H, $COCH_3$), 8.04-8.09 (m, 1H, 3-H), 8.09-8.11 (m, 1H, 5-H), 12.77 (s, 1H, OH). ^{13}C NMR: δ 15.4 ($CH_2\underline{C}H_3$), 26.8 ($\underline{C}H_2CH_3$), 28.2 (CO$\underline{C}H_3$), 122.9 (C-2), 130.1 (C-5), 134.7 (C-4), 136.2 (C-3), 138.1 (C-6), 152.4 (C-1), 204.9 ($\underline{C}OCH_3$). LC-MS (m/z): positive mode 208 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 92.7%. lit. mp 125-126 °C.

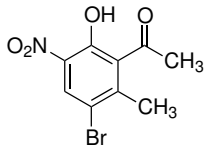
5.2.4.5 1-(5-Fluoro-2-hydroxy-3-nitrophenyl)ethanone 4 (*THB30*)[176]



The compound was synthesized according to GP-1 using 5-fluoro-2-hydroxyacetophenone (2.1 g, 13.4 mmol). The product was isolated as a white solid (1.34 g, 50%). ^{1}H NMR: δ 2.69 (s, 3H, $COCH_3$), 8.16

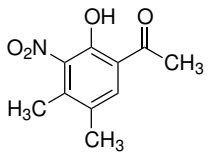
(dd, J = 3.2, 8.6 Hz, 1H, 2-H), 8.21 (dd, J = 3.2, 8.0 Hz, 1H, 6-H). ^{13}C NMR: δ 28.5 ($CO\underline{C}H_3$), 118.1-118.3 (C-2), 123.1-123.3 (C-6), 124.1-124.2 (C-5), 150.5-151.7 (C-1), 153.6 (C-3, C-4), 203.5 ($\underline{C}OCH_3$). LC-MS (m/z): negative mode 198 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. lit. mp 93-94 °C.

5.2.4.6 1-(3-Bromo-6-hydroxy-2-methyl-5-nitrophenyl)ethanone 5 (*ANM85*)



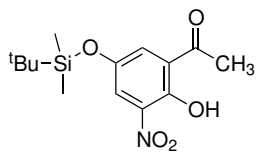
The compound was synthesized according to GP-1 using 1-(3-bromo-6-hydroxy-2-methylphenyl)ethanone (2.18 g, 9.5 mmol). The product was isolated as a pale yellow solid (2.5 g, 95%). ^{1}H NMR: δ 2.40 (d, J = 0.7 Hz, 3H, CH_3), 2.57 (s, 3H, $COCH_3$), 8.15 (d, J = 0.7 Hz, 1H, 3-H). ^{13}C NMR: δ 21.6 (CH_3), 27.6 ($CO\underline{C}H_3$), 121.1 (C-4), 121.3 (C-2), 129.5 (C-6), 133.8 (C-3), 150.1 (C-1), 204.3 ($\underline{C}OCH_3$). LC-MS (m/z): negative mode 271 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 80.0%. mp 163-164 °C.

5.2.4.7 1-(3-Bromo-6-hydroxy-2-methyl-5-nitrophenyl)ethanone 6 (*ANM207*)[177]



The compound was synthesized according to GP-X using 1-(2-hydroxy-4,5-dimethylphenyl)ethanone (1.97 g, 12 mmol). The product was isolated as a yellow solid (2.12 g, 84%). ^{1}H NMR: δ 2.17 (s, 3H, C5-C$\underline{H}_3$), 2.28 (s, 3H, C4-C$\underline{H}_3$), 2.66 (s, 3H, $COCH_3$), 7.95 (d, J = 0.9 Hz, 1H, 3-H), 12.42 (s, 1H, OH). ^{13}C NMR: δ 14.7 (C4-$\underline{C}H_3$), 18.7 (C5-$\underline{C}H_3$), 27.2 ($CO\underline{C}H_3$), 118.7 (C-2), 128.3 (C-4), 133.2 (C-3), 136.4 (C-5), 141.4 (C-6), 150.1 (C-1), 204.8 ($\underline{C}OCH_3$). LC-MS (m/z): positive mode 210 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 87.0%. lit. mp 143-144 °C.

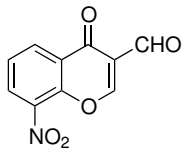
5.2.4.8 1-(5-((*Tert*-butyldimethylsilyl)oxy)-2-hydroxy-3-nitrophenyl)ethanone 7 (*ANM213*)



The compound was synthesized according to GP-1 using compound **289** (8.8 g, 33 mmol). The product was isolated as a brown solid (6.3 g, 62%). ^{1}H NMR: δ 0.22 (s, 6H, $Si(CH_3)_2$), 0.96 (s, 9H, $SiC(CH_3)_3$), 2.68 (s, 3H, $COCH_3$), 7.61 (d, J = 3.1 Hz, 1H, 3-H), 7.66 (d, J = 3.0 Hz, 1H, 5-H), 12.26 (s, 1H, OH). ^{13}C NMR: δ 4.7 ($Si(\underline{C}H_3)_2$), 18.0 (Si-C), 25.6 ($SiC(\underline{C}H_3)_3$), 28.5 ($CO\underline{C}H_3$), 121.7 (C-5), 124.2 (C-2), 127.6 (C-3), 138.3 (C-4), 146.3 (C-6), 148.5 (C-1), 203.7 ($\underline{C}OCH_3$). LC-MS (m/z): positive mode 312 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 74.0%. mp 87-89 °C.

5.2.5 8-Nitro-4-oxo-4*H*-chromene-3-carbaldehyde

5.2.5.1 8-Nitro-4-oxo-4*H*-chromene-3-carbaldehyde 40 (*ANM185*)[228]



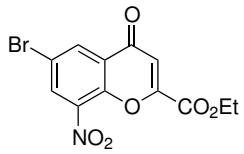
1-(2-Hydroxy-3-nitrophenyl)ethanone (1.36 g, 7.5 mmol) was dissolved in 6 mL of DMF cooled down to 0-5 °C. Then $POCl_3$ (2.80 mL, 30 mmol) was added dropwise and the reaction mixture stirred at rt overnight. After the reaction mixture was poured on water the obtained solid was filtered, washed with 2x10 mL of water and dried in vaccum at 50 °C The product was isolated as a white solid (1.12 g, 68%). ^{1}H NMR: δ 7.74 (t, J = 8.0 Hz, 1H, 6-H), 8.45 (dd, J = 1.7, 8.0 Hz, 1H, 5-H), 8.54 (dd, J = 1.7, 8.0 Hz, 1H, 7-H), 10.09 (s, 1H, CHO). ^{13}C NMR: δ 120.8 (C-3), 126.3 (C-6), 126.6 (C-4a), 130.8 (C-7), 131.1 (C-5), 139.1 (C-8), 147.8 (C-8a), 163.4 (C-2), 173.5 (C-4), 187.9 (CHO). LC-MS (m/z): positive mode 220 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: not detectable. lit. mp 133-134 °C.

5.2.6 Ethyl 4-oxo-4*H*-chromene-2-carboxylates

5.2.6.1 General procedure for the synthesis of the ethyl 4-oxo-4*H*-chromene-2-carboxylates (GP-2)

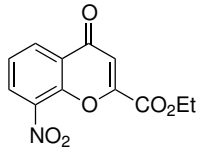
The appropriate 5'-substituted 1-(2-hydroxyphenyl)ethanone (4.0 mmol) and diethyl oxalate (1.3 mL, 9.8 mmol) were dissolved in 27 mL of anhydrous DMF under an argon atmosphere and were cooled to 0-5 °C. Potassium tert-butoxide (1.79 g, 15.6 mmol) was added, and the reaction mixture was stirred under an argon atmosphere for 2-3 h at 0-5 °C. Then a cooled solution of concd. (12 M) aq HCl solution (2.6 mL, 31.2 mmol) in 27 mL of water was added, and the mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in 20 mL of EtOH, and concd. (12 M) aq HCl (1.3 mL, 15.6 mmol) was subsequently added. The reaction mixture was refluxed overnight under an argon atmosphere. The mixture was concentrated under reduced pressure until the product started to crystallize and was subsequently cooled to 0-5 °C for completion of crystallization. The product was filtered off, washed with ice-cold EtOH in small portions, and dried in vacuum at 50 °C.

5.2.6.2 Ethyl 6-bromo-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 8 (*ANM1)*[179]



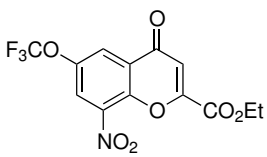
The compound was synthesized according to GP-2 using 1-(5-bromo-2-hydroxy-3-nitrophenyl)-ethanone (3.12 g, 12 mmol). The product was isolated as a pale-white solid (3.8 g, 93%). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 8.42 (d, J = 2.5 Hz, 1H, 5-H), 8.77 (d, J = 2.5 Hz, 1H, 7-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.1 ($\underline{C}H_2CH_3$), 114.62 (C-3), 117.3 (C-6), 126.8 (C-4a), 132.58 (C-7), 133.1 (C-5), 140.0 (C-8), 146.7 (C-8a), 152.4 (C-2), 159.3 ($\underline{C}O_2Et$), 175.0 (C-4). LC-MS (m/z): positive mode 358 $[M+NH_4]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.3%. lit. mp 141-142 °C.

5.2.6.3 Ethyl 8-nitro-4-oxo-4*H*-chromene-2-carboxylate 10 (*ANM44)*[32]



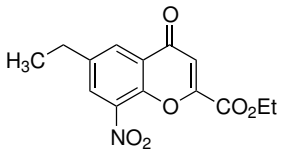
The compound was synthesized acording to GP-2 using 1-(2-hydroxy-3-nitrophenyl)ethanone (2.17 g, 12 mmol). The product was isolated as a white solid (2.8 g, 89%). ^{1}H NMR: δ 1.34 (t, J = 7.1, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.07 (s, 1H, 3-H), 7.70 (t, J = 8.0 Hz, 1H, 6-H), 8.36 (dd, J = 1.7, 8.0 Hz, 1H, 5-H), 8.54 (dd, J = 1.7, 7.9 Hz, 1H, 7-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.0 ($\underline{C}H_2CH_3$), 114.6 (C-3), 125.5 (C-8), 125.8 (C-6), 130.8 (C-7), 131.0 (C-5), 139.2 (C-8a), 147.6 (C-4a), 152.3 (C-2), 159.5 ($\underline{C}O_2Et$), 176.10 (C-4). LC-MS (m/z): positive mode 264 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. lit. mp 122-123 °C.

5.2.6.4 Ethyl 8-nitro-4-oxo-6-(trifluoromethoxy)-4*H*-chromene-2-carboxylate 9 (*ANM52)*



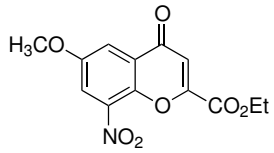
The compound was synthesized according to GP-2 using **2** (795 mg, 3 mmol). The product was isolated as a white solid (550 mg, 53%). ^{1}H NMR: δ 1.34 (t, J = 7.1, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.12 (s, 1H, 3-H), 8.23 (d, J = 3.1 Hz, 1H, 5-H), 8.70 (d, J = 3.0 Hz, 1H, 7-H). ^{13}C NMR: δ 13.0 ($CH_2\underline{C}H_3$), 34.4 (O-CF_3), 63.2 ($\underline{C}H_2CH_3$), 114.3 (C-3), 122.1 (C-7), 124.6 (C-5), 126.7 (C-4a), 140.2 (C-8), 143.9 (C-8a), 146.3 (C-2), 152.6 (C-6), 159.3 (CO_2Et), 175.4 (C-4). LC-MS (m/z): positive mode 365 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 91.3%. mp 120-121 °C.

5.2.6.5 Ethyl 6-ethyl-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 11 (*ANM84)*



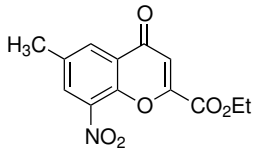
The compound was synthesized according to GP-2 using **3** (2.51 g, 12 mmol). The product was isolated as a white solid (2.27 g, 65%). ^{1}H NMR: δ 1.24 (t, 3H, J = 7.6 Hz, C6-$CH_2\underline{C}H_3$), 1.34 (t, J = 7.1 Hz, 3H, $CH_2\underline{C}H_3$), 2.83 (q, 2H, J = 7.6 Hz, C6-$\underline{C}H_2CH_3$), 4.39 (q, J = 7.1, 2H, $\underline{C}H_2CH_3$), 7.04 (s, 1H, 3-H), 8.18 (dd, J = 0.8, 2.3 Hz, 1H, 5-H), 8.43 (dd, J = 0.6, 2.3 Hz, 1H, 7-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 15.1 (C6-$CH_2\underline{C}H_3$), 27.2 (C6-$\underline{C}H_2CH_3$), 63.0 ($\underline{C}H_2CH_3$), 114.4 (C-3), 125.3 (C-8), 128.9 (C-7), 130.5 (C-5), 139.0 (C-8a), 142.1 (C-6), 145.9 (C-4a), 152.2 (C-2), 159.5 (CO_2Et), 176.1 (C-4). LC-MS (m/z): positive mode 292 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 90.1%. mp 116-117 °C.

5.2.6.6 Ethyl 6-methoxy-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 15 (*ANM36)*[65]



The compound was synthesized according to GP-2 using **1** (844 mg, 4 mmol). The product was isolated as a white solid (917 mg, 78%). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2\underline{C}H_3$), 3.95 (s, 3H, O-CH_3), 4.39 (q, J = 7.1 Hz, 2H, $\underline{C}H_2CH_3$), 7.02 (s, 1H, 3-H), 7.72 (d, J = 3.2 Hz, 1H, 5-H), 8.16 (d, J = 3.2 Hz, 1H, 7-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 56.8 (O-CH_3), 63.0 ($\underline{C}H_2CH_3$), 111.5 (C-7), 114.5 (C-3), 119.2 (C-5), 126.3 (C4a), 140.0 (C-8), 141.9 (C-8a), 152.2 (C-2), 155.8 (C-6), 159.5 (CO_2Et), 175.7 (C-4). LC-MS (m/z): positive mode 294 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.4%. lit. mp 175-176 °C.

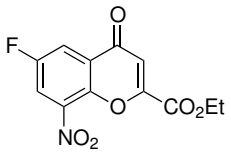
5.2.6.7 Ethyl-6-methyl-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 14 (*ANM31)*[183]



The compound was synthesized according to GP-2 using 2-hydroxy-5-methyl-3-nitroacetophenone (781 mg, 4 mmol). The product was isolated as a white solid (763 mg, 69%). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2\underline{C}H_3$), 2.50 (s, 3H, C6-CH_3), 4.39 (q, J = 7.1 Hz, 2H, $\underline{C}H_2CH_3$), 7.03 (s, 1H, 3-H), 8.16 (dd, J = 0.9, 2.2 Hz, 1H, 5-H), 8.40 (dd, J = 0.6, 2.2 Hz, 1H, 7-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 20.2 (C6-$\underline{C}H_3$) 63.0 ($\underline{C}H_2CH_3$), 114.4 (C-3), 125.2 (C-8), 130.2 (C-7), 131.5 (C-5), 136.2 (C-6), 138.8 (C-8), 145.8 (C-4a), 152.2 (C-2), 159.5 (CO_2Et), 176.0 (C-4). LC-MS (m/z): positive mode

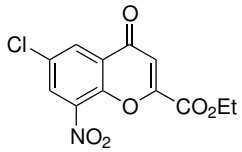
278 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.4%. lit. mp 148-151 °C.

5.2.6.8 Ethyl 6-fluoro-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 12 (*THB37*)[95]



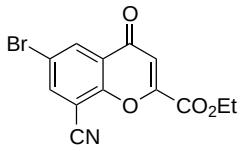
The compound was synthesized according to GP-2 using **4** (1.3 g, 6.5 mmol). The product was isolated as a white solid (1.25 g, 68%). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.07 (s, 1H, 3-H), 8.14 (dd, J = 3.2, 7.6 Hz, 1H, 5-H), 8.62 (dd, J = 3.2, 7.8 Hz, 1H, 7-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.1 ($\underline{C}H_2CH_3$), 113.8 (C-3), 116.0-116.1 (C-7), 119.5-119.7 (C-5), 126.7-126.8 (C-4a), 139.9 (C-8), 144.4 (C-8a), 152.5 (C-2), 156.4 (C-6), 159.4 (CO_2Et), 175.46 (C-4). LC-MS (m/z): positive mode 282 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 90.2%. lit. mp 112-113 °C.

5.2.6.9 Ethyl 6-chloro-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 13 (*THB38*)[182]



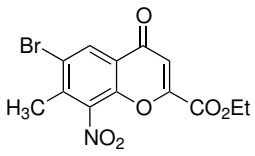
The compound was synthesized according to GP-2 using 1-(5-chloro-2-hydroxy-3-nitrophenyl)ethanone (2.6 g, 12 mmol). The product was isolated as a white solid (3.5 g, 98%). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.10 (s, 1H, 3-H), 8.30 (d, J = 2.6 Hz, 1H, 5-H), 8.69 (d, J = 2.6 Hz, C-7). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.1 ($\underline{C}H_2CH_3$), 114.5 (C-3), 126.6 (C-4a), 129.6 (C-7), 129.9 (C-8), 130.6 (C-5), 140.0 (C-8a), 146.4 (C-2), 152.4 (C-6), 159.3 (CO_2Et), 175.1 (C-4). LC-MS (m/z): positive mode 298 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 85.8%. lit. mp 126-127 °C.

5.2.6.10 Ethyl 6-bromo-8-cyano-4-oxo-4*H*-chromene-2-carboxylate 266 (*ANM382*)



The compound was synthesized according to GP-2 using **265** (2.16 g, 9 mmol). The product was isolated as a pale-white solid (2.29 g, 79%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.41 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.08 (s, 1H, 3-H), 8.37 (d, J = 2.4 Hz, 1H, 5-H), 8.72 (d, J = 2.5 Hz, 1H, 7-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.1 ($\underline{C}H_2CH_3$), 105.2 (C-8), 112.9 (CN), 114.7 (C-3), 118.0 (C-6), 125.7 (C-4a), 132.6 (C-5), 141.7 (C-7), 152.4 (C-8a), 154.9 (C-2), 159.3 (CO_2H), 175.4 (C-4). LC-MS (m/z): positive mode 324 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.3%. mp 172-173 °C.

5.2.6.11 Ethyl 6-bromo-7-methyl-8-nitro-4-oxo-4*H*-chromene-2-carboxylate 16 (*ANM89*)



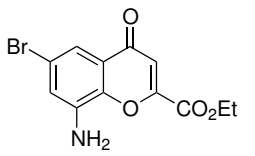
The compound was synthesized according to GP-2 using **5** (2.40 g, 9 mmol). The product was isolated as a white solid (2.03 g, 63%). ^{1}H NMR: δ 1.31 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.55 (d, J = 0.8 Hz, 3H, CH_3), 4.41 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.03 (s, 1H, 3-H), 8.20 (d, J = 1 Hz, 1H, 5-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 22.3 (CH_3), 63.1 ($\underline{C}H_2CH_3$), 114.8 (C-3), 121.9 (C-6), 123.7 (C-4a), 127.5 (C-5), 137.2 (C-8, C-7), 144.9 (C-8a), 151.8 (C-2), 159.2 (CO_2Et), 175.7 (C-4). LC-MS (m/z): positive mode 356 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 91.5%. mp 204-205 °C.

5.2.7 Ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylates

5.2.7.1 General procedure for the synthesis of the ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylates (GP-3)

The appropriate nitro derivative (2.5 mmol) was suspended in a mixture of EtOH (7 mL) and diluted aq HCl (2 N, 7 mL). Tin(II)chloride dihydrate (2.26 g, 10.0 mmol) was subsequently added, and the reaction mixture was heated at 65 °C until the solid was completely dissolved. The mixture was stirred for an additional 20 min at the same temperature. The EtOH was removed under reduced pressure, and the resulting suspension was added to 50 mL of EtOAc containing sodium carbonate (7 g). The precipitated tin complex was filtered off and washed with 30 mL of EtOAc. The filtrate was washed with brine (30 mL), dried over anhydrous MgSO4, and concentrated under reduced pressure. The resulting solid was dried in vacuum at 50 °C.

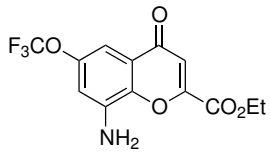
5.2.7.2 Ethyl 8-amino-6-bromo-4-oxo-4*H*-chromene-2-carboxylate 17 (*ANM2*)[180]



The compound was synthesized according to GP-3 using **8** (3.36 g, 9.8 mmol). The product was isolated as a yellow solid (2.89 g, 95%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.86 (s, 2H, NH), 6.90 (s, 1H, 3-H), 7.19 (d, J = 2.4 Hz, 1H, 7-H), 7.22 (d, J = 2.4 Hz, 1H, 5-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.8

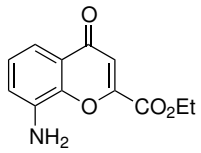
($\underline{C}H_2CH_3$), 112.0 (C-3), 113.4 (C-5), 119.2 (C-7), 119.2 (C-6), 125.5 (C-4a), 140.9 (C-8), 143.0 (C-8a), 151.9 (C-2), 160.0 (CO_2Et), 176.7 (C-4). LC-MS (m/z): negative mode 310 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. lit. mp 161-162 °C.

5.2.7.3 Ethyl 8-amino-4-oxo-6-(trifluoromethoxy)-4*H*-chromene-2-carboxylate 18 (*ANM61)*



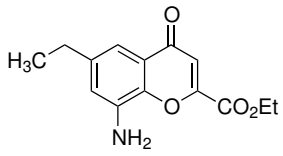
The compound was synthesized according to GP-3 using **9** (486 mg, 1.4 mmol). The product was isolated as a yellow solid (253 mg, 57%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.01 (s, 2H, NH), 6.91 (s, 1H, 3-H), 6.94 (d, J = 2.5 Hz, 1H, 7-H), 7.01 (d, J = 2.8 Hz, 1H, 5-H). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 62.9 ($\underline{C}H_2CH_3$), 100.4 (C-7), 109.1 (C-5), 113.1 (C-3), 119.2 (OCF_3), 125.0 (C-4a), 141.3 (C-8), 142.4 (C-8a), 146.4 (C-6), 152.0 (C-2), 160.0 (CO_2Et), 177.1 (C-4). LC-MS (m/z): positive mode 318 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 90.0%. mp 100-102 °C.

5.2.7.4 Ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylate 19 (*ANM75)*[181]



The compound was synthesized according to GP-3 using **10** (2.63 g, 10 mmol). The product was isolated as a yellow solid (2.12 g, 91%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.50 (s, 2H, NH), 6.86 (s, 1H, 3-H), 7.11 (d, J = 1.9, 7.4 Hz, 1H, 7-H), 7.18-7.20 (m, 2H, 5-H, 6-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 110.6 (C-3), 113.2 (C-5), 117.9 (C-7), 124.4 (C-4a), 126.3 (C-6), 138.8 (C-8), 143.8 (C-8a), 151.7 (C-2), 160.2 (CO_2Et), 177.9 (C-4). LC-MS (m/z): positive mode 233 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 153-154 °C.

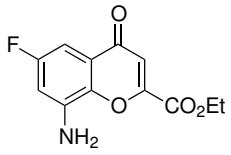
5.2.7.5 Ethyl 8-amino-6-ethyl-4-oxo-4*H*-chromene-2-carboxylate 20 (*ANM87)*



The compound was synthesized according to GP-3 using **11** (2.11 g, 7.3 mmol). The product was isolated as a yellow solid (1.45 g, 76%). ^{1}H NMR: δ 1.17 (t, 3H, J = 7.6 Hz, Ar-$CH_2C\underline{H}_3$), 1.35 (t, J = 7.1 Hz, 3H, $C\underline{H}_2CH_3$), 2.59 (q, 2H, J = 7.6 Hz, Ar-$C\underline{H}_2CH_3$), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.43 (s, 2H, NH), 6.84 (s, 1H, 3-H), 6.98 (d, J = 2.1 Hz, 1H, 7-H), 7.0 (d, J = 2.0 Hz, 1H, 5-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$); 15.4 (Ar-$CH_2\underline{C}H_3$), 28.1 (Ar-$\underline{C}H_2CH_3$), 62.7 ($\underline{C}H_2CH_3$), 109.2 (C-3), 113.1 (C-5), 117.8

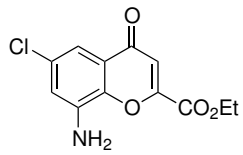
(C-7), 124.2 (C-4a), 138.6 (C-8), 142.1 (C-6), 142.5 (C-8a), 151.5 (C-2), 160.3 (CO_2Et), 177.8 (C-4). LC-MS (m/z): positive mode 262 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.2%. mp 132-133 °C.

5.2.7.6 Ethyl 8-amino-4-oxo-4*H*-chromene-2-carboxylate 21 (*THB39*)[95]



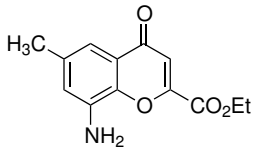
The compound was synthesized according to GP-3 using **12** (1.2 g, 4.2 mmol). The product was isolated as a yellow solid (644 mg, 60%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.91 (s, 2H, NH), 6.78 (dd, J= 3.0, 8.5 Hz, 1H, 3-H), 6.81?6.93 (m, 2H, 5-H, 7-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 94.4-94.6 (C-5), 104.3-104.6 (C-7), 112.6 (C-3), 125.2-125.3 (C-4a), 140.8 (C-8a), 141.4-141.5 (C-8), 151.8 (C-2), 159.2- 161.2 (C-6), 160.0 (CO_2Et), 177.2 (C-4). LC-MS (m/z): positive mode 252 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.8%. lit. mp 164-165 °C.

5.2.7.7 Ethyl 8-amino-6-chloro-4-oxo-4*H*-chromene-2-carboxylate 22 (*THB40*)[95]



The compound was synthesized according to GP-3 using **13** (2.7 g, 9.1 mmol). The product was isolated as a yellow solid (1.7 g, 62%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.87 (s, 2H, NH), 6.89 (s, 1H, 3-H), 7.01?7.10 (m, 2H, 5-H, 7-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 108.8 (C-3), 113.3 (C-7), 116.3 (C-5), 125.2 (C-4a), 131.0 (C-8), 140.8 (C-8a), 142.7 (C-6), 151.9 (C-2), 160.0 (CO_2Et), 176.8 (C-4). LC-MS (m/z): positive mode 268 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%. lit. mp 150-151 °C.

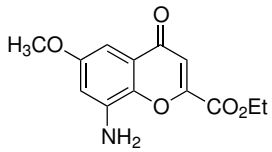
5.2.7.8 Ethyl-8-amino-6-methyl-4-oxo-4*H*-chromene-2-carboxylate 23 (*ANM32*)[183]



The compound was synthesized according to GP-3 using **14** (693 mg, 2.5 mmol). The product was isolated as a yellow solid (561 mg, 91%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.28 (s, 3H, Ar-CH_3), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.45 (s, 2H, NH), 6.83 (s, 1H, 3-H), 6.94 (dd, J = 0.5, 2.1 Hz, 1H, 7-H), 6.97 (dd, J = 0.9, 2.2 Hz, 0.8 Hz, 1H, 5-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 21.1 (Ar-CH_3), 62.7 ($\underline{C}H_2CH_3$), 110.4 (C-5),

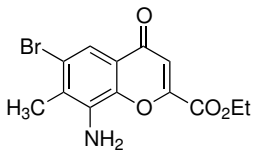
113.1 (C-3), 118.8 (C-7), 124.5 (C-4a), 135.9 (C-6), 138.5 (C-8), 142.3 (C-8a), 151.5 (C-2), 160.3 (CO_2Et), 177.8 (C-4). LC-MS (m/z): positive mode 248 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.0%. lit. mp 127-132 °C.

5.2.7.9 Ethyl-8-amino-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate 24 (*ANM37)*[184]



The compound was synthesized according to GP-3 using **15** (733 mg, 2.5 mmol). The product was isolated as a yellow solid (623 mg, 95%). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.76 (s, 3H, O-CH_3), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.56 (s, 2H, NH), 6.61 (d, J = 2.9 Hz, 1H, 5-H), 6.70 (d, J = 2.9 Hz, 1H, 7-H), 6.84 (s, 1H, 3-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 55.5 (O-CH_3), 63.0 ($\underline{C}H_2CH_3$), 91.6 (C-7), 105.8 (C-5), 112.5 (C-3), 125.1 (C4a), 139.8 (C-8), 140.2 (C-8a), 151.3 (C-2), 157.7 (C-6), 160.2 (CO_2Et), 177.5 (C-4). LC-MS (m/z): positive mode 264 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. lit. mp 144-145 °C.

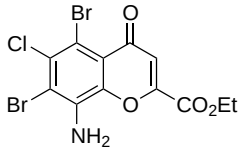
5.2.7.10 Ethyl 8-amino-6-bromo-7-methyl-4-oxo-4*H*-chromene-2-carboxylate 25 (*ANM99)*



The compound was synthesized according to GP-3 using **16** (1.90 g, 5.3 mmol). The product was isolated as a yellow solid (1.6 g, 92%). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.39 (d, J = 0.8 Hz, 3H, CH_3), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.46 (s, 2H, NH), 6.88 (s, 1H, 3-H), 7.19 (d, J = 0.9 Hz, 1H, 5-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 23.2 (CH, CH_3), 62.8 ($\underline{C}H_2CH_3$), 111.3 (C-3), 113.52 (C-5), 114.2 (C-6), 122.6 (C-4a), 135.5 (C-8), 136.4 (C-7), 142.0 (C-8a), 151.7 (C-2), 160.00 (CO_2Et), 177.3 (C-4). LC-MS (m/z): positive mode 328 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.5%. mp 164-166 °C.

5.2.8 Ethyl 8-amino-5,7-dibromo-6-chloro-4-oxo-4*H*-chromene-2-carboxylate

5.2.8.1 Ethyl 8-amino-5,7-dibromo-6-chloro-4-oxo-4*H*-chromene-2-carboxylate 38 (*ANM310)*



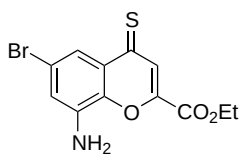
Compound **22** (267 mg, 1.0 mmol), NBS (214 mg, 1.2 mmol), and three drops of AIBN were dissolved in 10 mL of benzene and three drops of DMF and heated to reflux for 6 h. Then the reaction mixture was poured into ice containing water and the precipitated solid filtered, washed three times with 5 mL water and dried in the oven at 60 °C. The product was isolated as a yellow solid (86 mg, 20%). ^{1}H NMR: δ 1.39 (t, J = 7.7 Hz, 3H, $CH_2C\underline{H}_3$), 4.44 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.74 (s, 2H, NH), 6.91 (s, 1H, 3-H). ^{13}C NMR: δ 13.6 ($CH_2\underline{C}H_3$), 62.6 ($\underline{C}H_2CH_3$), 102.5 (C-5), 110.4 (C-7), 114.4 (C-3), 121.4 (C-4a), 133.0 (C-6), 137.7 (C-8), 142.9 (C-8a), 150.0 (C-2), 159.3 (CO_2Et), 175.2 (C-4). LC-MS (m/z): positive mode 412 $[M+H]^+$ measured in MeOH –> ester at 2-position changed to methylester. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 251-253 °C.

5.2.9 Ethyl 4-thioxo-4*H*-chromene-2-carboxylates

5.2.9.1 General procedure for the synthesis of the ethyl 4-thioxo-4*H*-chromene-2-carboxylates (GP-4)

To a solution of the appropriate ethyl 8-amino-4-oxo-4H-chromene-2-carboxylate (3 mmol) in toluene (20 mL) was added Lawesson's reagent (606 mg, 1.5 mmol) and the reaction mixture heated to 100 °C for 16 hours. After addition of aq. $NaHCO_3$ solution (80 mL) the product was extracted with DCM (3 x 20 mL) and separated by column chromatography on a column of silica gel (50% cyclohexane in EtOAc).

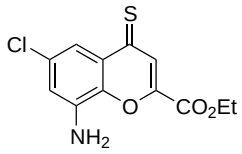
5.2.9.2 Ethyl 8-amino-6-bromo-4-thioxo-4*H*-chromene-2-carboxylate 34 (*ANM274)*



The compound was synthesized according to GP-4 using **17** (936 mg, 3 mmol) and was isolated as a red-browne solid (619 mg, 63% yield). ^{1}H NMR: δ 1.36 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 4.39 (q, 2H, J = 7.1 Hz, $C\underline{H}_2CH_3$), 5.95 (s, 2H, NH), 7.25 (d, 1H, J = 2.3

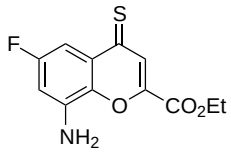
Hz, 5-H), 7.51 (d, 1H, J = 2.3 Hz, 7-H), 7.60 (s, 1H, 3-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.9 ($\underline{C}H_2CH_3$), 114.5 (C-7), 119.3 (C-5), 121.0 (C-6), 124.6 (C-3), 132.0 (C-4a), 138.5 (C-8a), 141.4 (C-8), 142.4 (C-2), 160.4 (CO_2Et), 201.6 (C-4). LC-MS (m/z): positive mode 329 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 84.9%. mp 176-177 °C.

5.2.9.3 Ethyl 8-amino-6-chloro-4-thioxo-4*H*-chromene-2-carboxylate 35 (*ANM272*)



The compound was synthesized according to GP-4 using **22** (267 mg, 1 mmol) and was isolated as a red-browne solid (112 mg, 40% yield). ^{1}H NMR: δ 1.36 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 4.30 (q, 2H, J = 7.1 Hz, $C\underline{H}_2CH_3$), 5.96 (s, 2H, NH), 7.11 (d, 1H, J = 2.5 Hz, 5-H), 7.38 (d, 1H, J = 2.5 Hz, 7-H), 7.60 (s, 1H, 3-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.9 ($\underline{C}H_2CH_3$), 111.4 (C-7), 116.4 (C-5), 124.5 (C-3), 131.7 (C-6), 132.7 (C-4a), 138.2 (C-8a), 141.3 (C-8), 142.4 (C-2), 160.4 (CO_2Et), 201.8 (C-4). LC-MS (m/z): positive mode 284 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.1%. mp 190-192 °C.

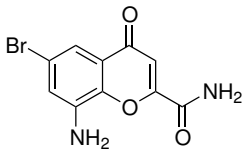
5.2.9.4 Ethyl 8-amino-6-fluoro-4-thioxo-4*H*-chromene-2-carboxylate 36 (*ANM314*)



The compound was synthesized according to GP-4 using **21** (689 mg, 2.6 mmol) and was isolated as a green solid (224 mg, 68% yield). ^{1}H NMR: δ 1.36 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.02 (s, 2H, NH), 6.93 (d, J = 3.0 Hz, 1H, 5-H), 7.11 (d, J = 3.0 Hz, 1H, 7-H), 7.61 (s, 1H, 3-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.9 ($\underline{C}H_2CH_3$), 97.0-97.1 (C-7), 104.6-104.8 (C-5), 124.0 (C-3), 131.9 (C-4a), 136.3 (C-8a), 142.0 (C-2), 142.4 (C-8), 160.5 (CO_2Et), 162.2 (C-6), 202.0-202.1 (C-4). LC-MS (m/z): positive mode 268 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 84.0%. mp 175-177 °C.

5.2.10 8-Amino-6-bromo-4-oxo-4*H*-chromene-2-carboxamide

5.2.10.1 8-Amino-6-bromo-4-oxo-4*H*-chromene-2-carboxamide 39 (*ANM46*)



Compound **17** (255 mg, 0.9 mmol) was dissolved in 1 mL of MeOH. After addition of 2 mL of a solution of NH_3 in MeOH (7N) the reaction mixture was stirred over night at rt under an argon atmosphere. The product was obtained by filtration and washed

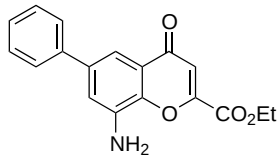
twice with 3 mL of MeOH and was dried in vacuum at 50 °C. The product was isolated as a pale white solid (243 mg, 95% yield). ^{1}H NMR: δ 6.43 (s, 2H, C8-NH_2), 6.81 (s, 1H, 3-H), 7.13 (d, J = 2.4 Hz, 1H, 7-H), 7.15 (d, J = 2.4 Hz, 1H, 5-H), 8.18-8.68 (s, 2H, $CONH_2$). ^{13}C NMR: δ 110.7 (C-3), 111.4 (C-5), 118.6 (C-7), 119.1 (C-6), 125.6 (C-4a), 141.0 (C-8), 142.6 (C-8a), 155.6 (C-2), 160.4 (CONH), 176.7 (C-4). LC-MS (m/z): positive mode 284 $[M+H]^+$, negative mode 281 $[M-2H]^{2-}$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.4%. mp > 350 °C.

5.2.11 Ethyl 4-oxo-6-phenyl-4*H*-chromene-2-carboxylates

5.2.11.1 General procedure for the synthesis of the ethyl 4-oxo-6-phenyl-4*H*-chromene-2-carboxylates (GP-5)

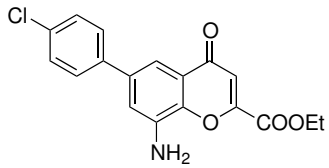
Compound **17** (1.0 mmol) and the appropriate phenylboronic acid (1.2 mmol) were suspended in 16 mL of toluene and 1 mL DMF in a 30 mL pressure flask (ACE®-glass). After addition of K_2CO_3 (2 mmol) the suspension was degased in an ultrasonic bath for 5 min. Then tetrakis(triphenylphosphine)palladium(0) (0.025 mmol) was added under an argon atmosphere and the mixture was heated to 120 °C overnight. The reaction mixture was dissolved in 30 mL of EtOAc, filtrated, washed twice with saturated NaCl in water solution and separated by column chromatography on a column of silica gel (1:4 cyclohexane/EtOAc).

5.2.11.2 Ethyl-8-amino-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 26 (*ANM38*)



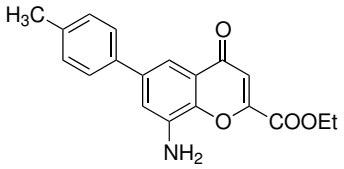
The compound was synthesized according to GP-5 using **17** (312 mg, 1 mmol) and phenylboronic acid (146 mg, 1.2 mmol). The product was isolated as a yellow solid (275 mg, 89% yield). ^{1}H NMR: δ 1.37 (t, J = 7.1 Hz, 3H, $CH_2\underline{CH_3}$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H_2}CH_3$), 5.64 (s, 2H, NH), 6.90 (s, 1H, 3-H), 7.36-7.40 (m, 2H, 4'-H, 7-H), 7.41 (d, J = 2.2 Hz, 1H, 5-H), 7.45-7.51 (m, 2H, 3'-H, 5'-H), 7.60-7.65 (m, 2H, 2'-H, 6'-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 108.3 (C-5), 113.3 (C-3), 116.1 (C-7), 124.6 (C-4a), 126.8 (C-2', C-6'), 128.0 (C-4'), 129.2 (C-3', C-5'), 138.3 (C-6), 139.3 (C-8), 139.2 (C-1'), 143.5 (C-8a), 151.7 (C-2), 160.2 (CO_2Et), 177.8 (C-4). LC-MS (m/z): negative mode 308 $[M-H]^-$, positive mode 310 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.1%. mp 138-139 °C.

5.2.11.3 Ethyl-8-amino-6-(4-chlorophenyl)-4-oxo-4*H*-chromene-2-carboxylate 27 (*ANM118)*



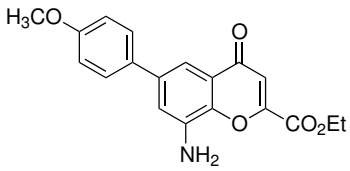
The compound was synthesized according to GP-5 using **17** (312 mg, 1 mmol) and 4-chlorophenylboronic acid (164 mg, 1.05 mmol). The product was isolated as a yellow solid (158 mg, 46% yield). ^{1}H NMR: δ 1.36 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.66 (s, 2H, NH), 6.90 (s, 1H, 3-H), 7.37 (d, J = 2.2 Hz, 3'-H, 5'-H), 7.39 (d, J = 2.2 Hz, 1H, 5-H), 7.49-7.54 (m, 2H, 3'-H, 5'-H), 7.62-7.67 (m, 2H, 2'-H, 6'-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 108.3 (C-5), 113.3 (C-3), 115.8 (C-7), 124.7 (C-4a), 128.6 (C-2', C-6'), 129.1 (C-3', C-5'), 132.8 (C-6), 137.0 (C-4'), 138.3 (C-1'), 139.4 (C-8), 143.7 (C-8a), 151.7 (C-2), 160.2 (CO_2Et), 177.8 (C-4). LC-MS (m/z): positive mode 344 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.8%. mp 183-184 °C.

5.2.11.4 Ethyl 8-amino-4-oxo-6-(p-tolyl)-4*H*-chromene-2-carboxylate 28 (*ANM119)*



The compound was synthesized according to GP-5 using **17** (312 mg, 1 mmol) and 4-tolylboronic acid (143 mg, 1.05 mmol). The product was isolated as a yellow solid (191 mg, 59% yield). ^{1}H NMR: δ 1.36 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.34 (s, 3H, Ar-CH_3), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.61 (s, 2H, NH), 6.89 (s, 1H, 3-H), 7.28 (m, 2H, 3'-H, 5'-H), 7.36 (d, J = 2.2 Hz, 1H, 7-H), 7.40 (d, J = 2.2 Hz, 1H, 5-H), 7.52 (m, 2H, 2'-H, 6'-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 20.8 (Ar-CH_3), 62.8 ($\underline{C}H_2CH_3$), 108.0 (C-5), 113.2 (C-3), 115.9 (C-7), 124.6 (C-4a), 126.6 (C-2', C-6'), 129.8 (C-3', C-5'), 136.6 (C-4'), 137.3 (C-6), 138.2 (C-1'), 139.3 (C-8), 143.4 (C-8a), 151.6 (C-2), 160.2 (CO_2Et), 177.9 (C-4). LC-MS (m/z): positive mode 324 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.1%. mp 127-128 °C.

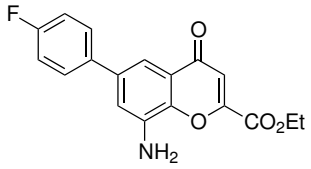
5.2.11.5 ethyl 8-amino-6-(4-methoxyphenyl)-4-oxo-4*H*-chromene-2-carboxylate 29 (*ANM127)*



The compound was synthesized according to GP-5 using **17** (312 mg, 1 mmol) and 4-methoxyphenylboronic acid (160 mg, 1.05 mmol). The product was isolated as a yellow solid (255 mg, 75% yield). ^{1}H NMR: δ 1.36 (t, J =

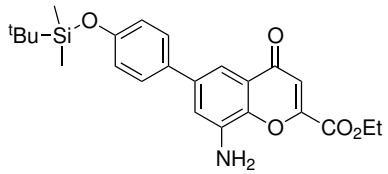
7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.79 (s, 3H, O-CH_3), 4.40 (q, *J* = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.59 (s, 2H, NH), 6.89 (s, 1H, 3-H), 7.03 (m, 2H, 3'-H, 5'-H), 7.33 (d, *J* = 2.2 Hz, 1H, 7-H), 7.37 (d, *J* = 2.2 Hz, 1H, 5-H), 7.56 (m, 2H, 2'-H, 6'-H). ^{13}C NMR: *δ* 14.0 ($CH_2\underline{C}H_3$), 55.3 (O-CH_3), 62.8 ($\underline{C}H_2CH_3$), 107.66 (C-5), 113.2 (C-3), 114.6 (C-3', C-5'), 114.8 (C-7), 124.6 (C-4a), 127.9 (C-2', C-6'), 131.8 (C-6), 138.0 (C-1'), 139.2 (C-8), 143.2 (C-8a), 151.6 (C-2), 159.3 (C-4'), 160.2 (CO_2Et), 177.9 (C-4). LC-MS (m/z): positive mode 340 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.3%. mp 162-163 °C.

5.2.11.6 Ethyl 8-amino-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylate 30 (*ANM128)*



The compound was synthesized according to GP-5 using **17** (312 mg, 1 mmol) and 4-fluorophenylboronic acid (147 mg, 1.05 mmol). The product was isolated as a yellow solid (243 mg, 74% yield). ^{1}H NMR: *δ* 1.36 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, *J* = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.64 (s, 2H, NH), 6.90 (s, 1H, 3-H), 7.30 (m, 2H, 3'-H, 5'-H), 7.35 (d, *(*J) = 2.2 Hz, 1H, 7-H), 7.37 (d, *J* = 2.2 Hz, 1H, 5-H), 7.66 (m, 2H, 2'-H, 6'-H). ^{13}C NMR: *δ* 14.0 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 108.2 (C-5), 113.3 (C-3), 116.1 (C-3', C-5', C-7), 124.6 (C-4a), 128.9 (C-2', C-6'), 136.0 (C-6), 137.3 (C-1'), 139.3 (C-8), 143.5 (C-8a), 151.7 (C-2), 160.2 (CO_2Et), 161.2-163.1 (C-4'), 177.8 (C-4). LC-MS (m/z): positive mode 328 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.3%. mp 176-177 °C.

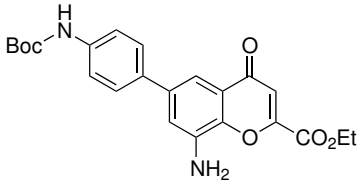
5.2.11.7 Ethyl 8-amino-6-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-4-oxo-4*H*-chromene-2-carboxylate 31 (*ANM191)*



The compound was synthesized according to GP-5 using **17** (312 mg, 1 mmol) and (4-(tert-butyldimethylsilyl)phenyl)boronic acid (248 mg, 1.05 mmol). The product was isolated as a yellow solid (281 mg, 64% yield). ^{1}H NMR: *δ* 0.21 (s, 6H, Si-$(CH_3)_2$), 0.96 (s, 9H, $SiC(CH_3)_3$), 1.36 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.40 (q, *J* = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.59 (s, 2H, NH), 6.89 (s, 1H, 3-H), 6.90-6.98 (m, 2H, 3'-H, 5'-H), 7.33 (d, *J* = 2.2 Hz, 1H, 7-H), 7.36 (d, *J* = 2.2 Hz, 1H, 5-H), 7.50-7.54 (m, 2H, 2'-H, 6'-H). ^{13}C NMR: *δ* 4.4 ($Si(\underline{C}H_3)_2$), 14.0 ($CH_2\underline{C}H_3$), 18.1 ($Si\underline{C}$), 25.7 ($SiC(\underline{C}H_3)_3$), 62.8 ($\underline{C}H_2CH_3$), 107.7 (C-5), 113.2 (C-3), 115.8 (C-7), 120.5 (C-3', C-5'), 124.6 (C-4a), 128.0 (C-2', C-6'), 132.6 (C-6), 127.9 (C-1'), 139.2 (C-8), 143.2

(C-8a), 151.6 (C-2), 155.2 (C-4'), 160.2 (CO_2Et), 177.8 (C-4). LC-MS (m/z): positive mode 440 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.7%. mp 155-157 °C.

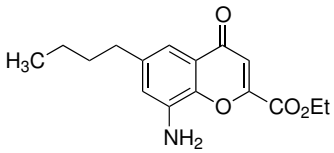
5.2.11.8 Ethyl 8-amino-6-(4-((*tert*-butoxycarbonyl)amino)phenyl)-4-oxo-4*H*-chromene-2-carboxylate 32 (*ANM192*)



The compound was synthesized according to GP-5 using **17** (614 mg, 2 mmol) and 4-(N-Boc-amino)phenylboronic acid (498 mg, 2.1 mmol). The product was isolated as a yellow solid (506 mg, 60% yield). ^{1}H NMR: δ 1.36 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 4.40 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.60 (s, 2H, NH), 6.89 (s, 1H, 3-H), 7.34 (d, J = 2.2 Hz, 1H, 5-H), 7.52-7.56 (m, 4H, 2'-H, 3'-H, 5'-H, 6'-H), 9.45 (s, 1H, CONH). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 28.3 ($C(\underline{C}H_3)_3$), 62.8 ($\underline{C}H_2CH_3$), 79.3 ($\underline{C}(CH_3)_3$), 107.7 (C-5), 113.2 (C-3), 115.7 (C-7), 118.6 (C-3', C-5'), 124.6 (C-4a), 127.0 (C-2', C-6'), 133.0 (C-6), 138.0 (C-1'). 139.2 (C-8), 139.5 (CONH), 143.3 (C-8a), 151.6 (C-2), 152.9 (C-4'), 160.2 (CO_2Et), 177.9 (C-4). LC-MS (m/z): positive mode 425 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 222-223 °C.

5.2.12 Ethyl 8-amino-6-butyl-4-oxo-4*H*-chromene-2-carboxylate

5.2.12.1 Ethyl 8-amino-6-butyl-4-oxo-4*H*-chromene-2-carboxylate 33 (*ANM80*)

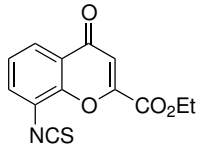


Compound **17** (156 mg, 0.5 mmol) and butylboronic acid (56 mg, 0.55 mmol) were suspended in 8 mL of toluene and 0.5 mL DMF in a 30 mL pressure flask (ACE®-glass). After addition of K_2CO_3 (1 mmol) the suspension was degased in an ultrasonic bath for 5 min. Then bis(triphenylphosphine)palladium(II)dichloride (0.025 mmol) and triphenylphosphine (0.5 mmol) were added under an argon atmosphere and the mixture was heated to 120 °C overnight. The reaction mixture was dissolved in 30 mL of EtOAc, filtrated, washed twice with saturated NaCl in water solution and separated by column chromatography on a column of silica gel (1:4 cyclohexane/EtOAc). The product was isolated as a yellow solid (25 mg, 17% yield). ^{1}H NMR: δ 0.89 (t, J = 7.2 Hz, 3H, 4'-H), 1.26-1.28 (m, 2H, 3'-H), 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.53-1.55 (m, 2H, 2'-H), 2.55-2.57 (m, 2H, 1'-H), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.43 (s, 2H, NH), 6.84 (s, 1H, 3-H), 6.96 (d, J = 2.1 Hz, 1H, 7-H), 6.99 (d, J = 1.9 Hz, 1H, 5-H). ^{13}C NMR: δ 13.3 (C-4'), 14.0 ($CH_2\underline{C}H_3$), 21.8 (C-3'), 33.0 (C-2'), 34.7 (C-1'), 62.7 ($\underline{C}H_2CH_3$), 109.8 (C-3), 113.1 (C-5), 118.2 (C-7), 124.2 (C-4a),

138.5 (C-6), 140.7 (C-8), 142.5 (C-8a), 151.5 (C-2), 160.3 (CO_2Et), 177.8 (C-4). LC-MS (m/z): positive mode 290 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.4%. mp 119-121 °C.

5.2.13 Ethyl 8-isothiocyanato-4-oxo-4*H*-chromene-2-carboxylate

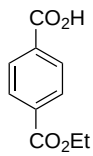
5.2.13.1 Ethyl 8-isothiocyanato-4-oxo-4*H*-chromene-2-carboxylate 184 (*ANM162)*



Thiophosgene (150 mg, 1.3 mmol) was dissolved in EtOAc (5.5 mL) cooled down to -78 °C (acetone/dry ice mixture) under an argon atmosphere. Then triethylamine (0.37 mL, 2.7 mmol) in 4 mL EtOAc was added dropwise over a period of 30 min and the reaction mixture stirred for 15 min. After that **19** (280 mg, 1.2 mmol) in THF (6.6 mL) was added dropwise over a period of 30 min and the reaction mixture was stirred overnight at rt. After addition of 26 mL EtOAc the organic layer was washed two times with 10 mL H_2O each and two times with 20 mL brine each and dried over $MgSO_4$. After removing the solvent under reduced pressure the product was isolated as a pink-white solid (310 mg, 94% yield). ^{1}H NMR: δ 1.38 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.41 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.51 (t, J = 7.9 Hz, 1H, 6-H), 7.85 (dd, J = 1.6, 7.8 Hz, 1H, 7-H), 7.94 (dd, J = 1.6, 8.0 Hz, 1H, 5-H). ^{13}C NMR: δ 14.1 ($CH_2\underline{C}H_3$), 63.0 ($\underline{C}H_2CH_3$), 114.3 (C-3), 121.3 (C-8), 123.9 (C-5), 124.9 (C-4a), 126.2 (C-7), 130.6 (C-6), 140.7 (NCS), 151.5 (C-8a), 152.1 (C-2), 159.7 (CO_2Et), 176.9 (C-4). LC-MS (m/z): positive mode 276 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 108-109 °C.

5.2.14 4-(Ethoxycarbonyl)benzoic acid

5.2.14.1 4-(Ethoxycarbonyl)benzoic acid 290 (*ANM219)*[229]



Diethyl terephthalate (2.22 g, 10 mmol) was dissolved in 30 mL EtOH and stirred for 15 min at 40-50 °C. Then KOH (560 mg, 10 mmol) was added and the reaction mixture refluxed for 4 h. After removing the solvent under reduced pressure the product was suspended in 60 mL of water and extracted with 3 x 15 mL of DCM. The water-phase was then acidified with 10 % HCl and extracted with 3 x 10 mL diethyl ether. The diethyl ether phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The product was isolated as a white solid (950 mg, 49% yield). ^{1}H NMR: δ 1.32 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$),

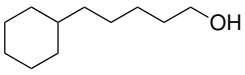
4.33 (q, J = 7.1 Hz, 2H, C$\underline{H_2}$CH$_3$), 8.04 (s, 4H, 2-H, 3-H, 5-H, 6-H), 13.29 (s, 1H, OH). ^{13}C NMR: δ 14.2 (CH$_2\underline{C}$H$_3$), 61.3 ($\underline{C}$H$_2$CH$_3$), 129.4 (C-3, C-5), 129.7 (C-2, C-6), 133.6 (C-1), 134.9 (C-4), 165.2 (CO_2Et), 166.7 (O_2H). LC-MS (m/z): positive mode 195 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 167-171 °C.

5.2.15 Alkylalcohols

5.2.15.1 General procedure for the synthesis of alkylalcohols (GP-6)

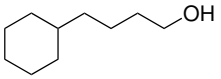
To a solution of 16 mL of $LiAlH_4$ in THF (1 M), 10 mmol of the appropriate alkylcarboxylic acid dissolved in 8 mL of dry THF was dropped slowly over 30 min. An increase of temperature was observed. After that the reaction mixture was stirred for 4 h at rt and then quenched with water at 0-5 °C. Carefully H_2SO_4 (10 M) was added until the precipitated solid was dissolved. The product was extracted with 3 x 30 mL EtOAc and the combined organic layers were washed with Brine, dried over $MgSO_4$ and filtered. After removing the solvent under reduced pressure the product was used without further characterization.

5.2.15.2 5-Cyclohexylpentan-1-ol 41 (*ANM86*)[193]



The product was synthesized according to GP-6 using 5-cyclohexylpentanoic acid (921 mg, 5 mmol) and was isolated as a colourless oil (793 mg, 93% yield). The product was used without further purification and characterization.

5.2.15.3 4-Cyclohexylbutan-1-ol 42 (*ANM159*)[193]



The product was synthesized according to GP-6 using 5-cyclohexylbutanoic acid (1.70 g, 10 mmol) and was isolated as a colourless oil (1.32 g, 85% yield). The product was used without further purification and characterization.

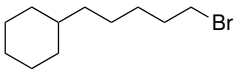
5.2.16 Alkylbromides

5.2.16.1 General procedure for the synthesis of alkylbromides (GP-7)

10.9 mmol of the aliphatic alcohol were dissolved in 2 mL of toluene under argon atmosphere and cooled down to 0-5 °C. Then very slowly 12.1 mmol of PBr_3 were

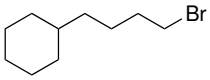
added and the reaction mixture was stirred for 30 min at rt and then heated for 2 h at 100 °C. After cooling the reaction mixture to 0-5 °C it was poured on ice, washed with Brine and the water phase extracted with 2 x 10 mL of diethyl ether. The combined organic layers were dried over $MgSO_4$, the solvent removed under reduced pressure and the product was finally dried in vacuum at 45 °C.

5.2.16.2 (5-Bromopentyl)cyclohexane 43 (*ANM144)*[193]



The product was synthesized according to GP-7 using **41** (766 mg, 4.5 mmol) and was isolated as a colourless oil (899 mg, 86% yield). The product was used without further purification and characterization.

5.2.16.3 (5-Bromobutyl)cyclohexane 44 (*ANM160)*[193]



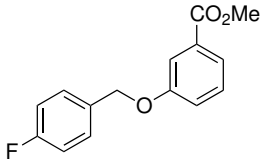
The product was synthesized according to GP-7 using **42** (1.25 g, 8.0 mmol) and was isolated as a colourless oil (1.50 g, 86% yield). The product was used without further purification and characterization.

5.2.17 Methyl alkyloxybenzoates

5.2.17.1 General procedure for the synthesis of methyl alkyloxybenzoates (GP-8)

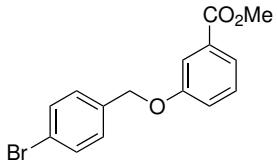
The appropriate alkyl bromide (7.0 mmol), methyl 4-hydroxybenzoate (1.07 g, 7.0 mmol), and potassium carbonate (1.10 g, 8.0 mmol) were suspended in 15 mL of anhydrous DMF. The reaction mixture was heated at 110-115 °C under an argon atmosphere overnight. DMF was removed under reduced pressure. After addition of 60 mL water, the resulting suspension was extracted with ethyl acetate (3 x 25 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. If purification by column chromatography was necessary, see individual compounds. The product was dried in vacuum at 50 °C.

5.2.17.2 Methyl-3-(4-fluorobenzyloxy)benzoate 60 (*ANM8)*[202]



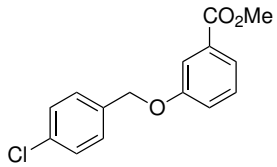
The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (0.87 mL, 7.0 mmol) and methyl 3-hydroxybenzoate (1.06 g, 7.0 mmol), and was isolated as white solid (1.56 g, 86% yield). LC-MS (m/z): positive mode 261 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%.

5.2.17.3 Methyl-3-(4-bromobenzyloxy)benzoate 62 (*ANM9)*[203]



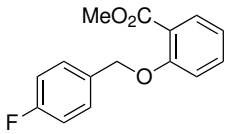
The compound was synthesized according to GP-8 using 4-bromobenzyl bromide (1.75 mL, 7.0 mmol) and methyl 3-hydroxybenzoate (1.06 g, 7.0 mmol), and was isolated as white solid (1.95 g, 87% yield). LC-MS (m/z): positive mode 323 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%.

5.2.17.4 Methyl-3-(4-chlorobenzyloxy)benzoate 64 (*ANM10)*[204]



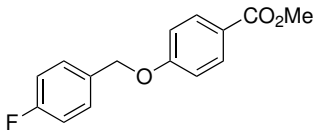
The compound was synthesized according to GP-8 using 4-chlorobenzyl bromide (1.44 g, 7.0 mmol) and methyl 3-hydroxybenzoate (1.06 g, 7.0 mmol), and was isolated as white solid (1.59 g, 82% yield). LC-MS (m/z): positive mode 277 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.7%.

5.2.17.5 Methyl-2-(fluorobenzyloxy)benzoate 72 (*ANM11)*[207]



The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (0.87 mL, 7.0 mmol) and methyl 2-hydroxybenzoate (1.06 g, 7.0 mmol), and was isolated as yellow oil (1.54 g, 85% yield). LC-MS (m/z): positive mode 278 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 89.6%.

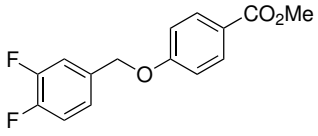
5.2.17.6 Methyl-4-(fluorobenzyloxy)benzoate 46 (*ANM54)*[195]



The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (0.87 mL, 7.0 mmol) and methyl 4-hydroxybenzoate (1.06 g, 7.0 mmol), and was isolated as white solid (1.60 g, 88% yield). LC-MS (m/z): positive mode 261 $[M+H]^+$. Purity by

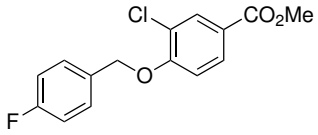
HPLC-UV (254 nm)-ESI-MS: 99.2%.

5.2.17.7 Methyl-4-(3,4-difluorobenzyloxy)benzoate 48 (*ANM114)*[197]



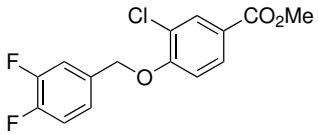
The compound was synthesized according to GP-8 using 3,4-difluorobenzyl bromide (0.90 mL, 7.0 mmol) and methyl 4-hydroxybenzoate (1.06 g, 7.0 mmol), and was isolated as white solid (1.73 g, 89% yield). LC-MS (m/z): positive mode 279 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.0%.

5.2.17.8 Methyl-3-chloro-4-(4-fluorobenzyloxy)benzoate 50 (*ANM115)*



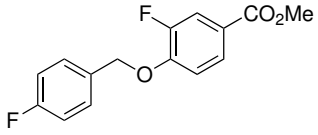
The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (0.87 mL, 7.0 mmol) and methyl 3-chloro-4-hydroxybenzoate (1.3 g, 7.0 mmol), and was isolated as white solid (1.77 g, 86% yield). LC-MS (m/z): positive mode 295 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.0%.

5.2.17.9 Methyl-3-chloro-4-(3,4-difluorobenzyloxy)benzoate 52 (*ANM146)*[198]



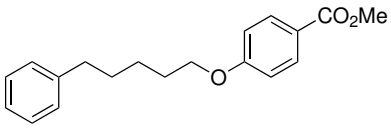
The compound was synthesized according to GP-8 using 3,4-difluorobenzyl bromide (1.31 g, 7.0 mmol) and methyl 3-chloro-4-hydroxybenzoate (1.3 g, 7.0 mmol), and was isolated as white solid (1.92 g, 88% yield). LC-MS (m/z): positive mode 313 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 92.4%.

5.2.17.10 Methyl-3-fluoro-4-(4-fluorobenzyloxy)benzoate 54 (*THB7)*



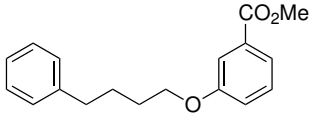
The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (1.00 g, 5.88 mmol) and methyl 3-fluoro-4-hydroxybenzoate (1.00 g, 5.88 mmol), and was isolated as white solid (1.51 g, 92% yield).). ^{1}H NMR: δ 3.82 (s, 3H, $CO_2C\underline{H}_3$), 5.25 (s, 2H, CH_2), 7.17-7.29 (m, 2H, 3'-H, 5'-H), 7.38 (t, J = 8.5 Hz, 1H, 5-H), 7.46-7.57 (m, 2H, 2'-H, 6'-H), 7.70 (dd, J = 2.1 Hz, 11.8 Hz, 1H, 6-H), 7.77 (ddd, J = 1.1, 2.1, 8.6 Hz, 1H, 2-H). ^{13}C NMR: δ 52.3 ($CO_2\underline{C}H_3$), 69.8 (CH_2), 115.0 (C-5), 115.4-115.6 (C-3', C-5'), 116.6-116.7 (C-3), 122.6 (C-1), 126.7 (C-2', C-6'), 130.4 (C-6), 130.5 (C-2), 132.3 (C-1'), 150.3-152.2 (C-4), 161.2-163.1 (C-4'), 165.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 279 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.0%. mp 89-90 °C.

5.2.17.11 Methyl 4-((5-phenylpentyl)oxy)benzoate 74 (*THB18)*[208]



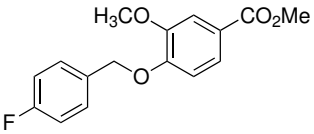
The compound was synthesized according to GP-8 using (5-bromopentyl)benzene (1.00 g, 4.4 mmol) and methyl 4-hydroxybenzoate (748 mg, 4.4 mmol), and was isolated as white solid (1.51 g, 92% yield).). LC-MS (m/z): positive mode 299 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.7%.

5.2.17.12 Methyl 3-(4-phenylbutoxy)benzoate 84 (*THB32)*



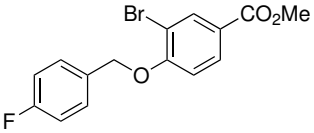
The compound was synthesized according to GP-8 using (5-bromobutyl)benzene (1.00 g, 4.69 mmol) and methyl 3-hydroxybenzoate (797 mg, 4.69 mmol), and was isolated as a colourless oil (1.14 g, 90% yield). ^{1}H NMR: δ 1.71 (tt, J = 3.4, 7.0 Hz, 4H, 2'-H, 3'-H), 2.63 (t, J = 7.1 Hz, 2H, 4'-H), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 3.98-4.05 (m, 2H, 1'-H), 7.13-7.31 (m, 5H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H), 7.36-7.44 (m, 2H, 2-H, 6-H), 7.52 (dt, J = 1.2, 7.6 Hz, 2H, 5-H, 6-H). ^{13}C NMR: δ 27.4 (C-3'), 28.3 (C-2'), 34.9 (C-4'), 52.3 ($CO_2\underline{C}H_3$), 67.7 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.4 (C-6), 125.8 (C-4'), 128.4 (C-3", C-5"), 128.4 (C-2", C-6"), 130.0 (C-5'), 131.1 (C-1), 142.1 (C-1"), 158.8 (C-3), 166.2 ($\underline{C}O_2Me$). LC-MS (m/z): positive mode 285 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 78.5%.

5.2.17.13 Methyl 4-(4-fluorobenzyloxy)-3-methoxybenzoate 56 (*ANM112)*[201]



The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (0.87 mL, 7.00 mmol) and methyl 4-hydroxy-3-methoxybenzoate (1.28 g, 7.00 mmol), and was isolated as a white solid (1.83 g, 90% yield). LC-MS (m/z): positive mode 291 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%.

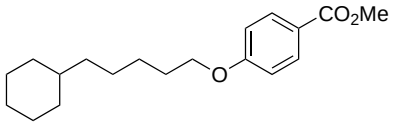
5.2.17.14 Methyl 3-bromo-4-((4-fluorobenzyl)oxy)benzoate 58 (*ANM113)*



The compound was synthesized according to GP-8 using 4-fluorobenzyl bromide (0.87 mL, 7.00 mmol) and methyl 3-bromo-4-hydroxybenzoate (1.62 g, 7.00 mmol), and was iso-

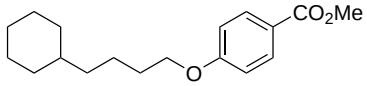
lated as a white solid (2.16 g, 91% yield). LC-MS (m/z): positive mode 337 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.2%.

5.2.17.15 Methyl 4-((5-cyclohexylpentyl)oxy)benzoate 78 (*ANM145)*



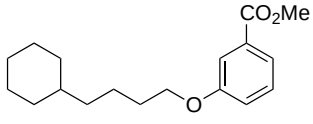
The compound was synthesized according to GP-8 using **43** (931 mg, 4.00 mmol) and methyl 4-hydroxybenzoate (609 mg, 4.00 mmol), and was isolated as a white solid (570 g, 47% yield). ^{1}H NMR: δ 0.81-0.86 (m, 2H, Cyclohexyl), 1.12-1.19 (m, 6H, 5'-H, Cyclohexyl), 1.30-1.38 (m, 4H, 3'-H, 4'-H), 1.61-1.70 (m, 7H, 2'-H, Cyclohexyl), 3.80 (s, 3H, $CO_2C\underline{H}_3$), 4.02 (t, J = 6.5 Hz, 2H, 1'-H), 7.01 (d, J = 8.9 Hz, 2H, 3-H, 5-H), 7.84-7.90 (m, 2H, 2-H, 6-H). ^{13}C NMR: δ 25.9-26.1 (C-4', C-5'), 26.0 (C-3", C-5"), 26.3 (C-4"), 28.7 (C-3'), 33.0 (C-2", C-6"), 37.0 (C-2'), 37.1 (C-1"), 51.9 ($CO_2\underline{C}H_3$), 68.0 (C-1'), 114.6 (C-3, C-5), 121.8 (C-1), 131.3 (C-2, C-6), 162.7 (C-4), 166.0 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 305 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 59-60 °C.

5.2.17.16 Methyl 4-(4-cyclohexylbutoxy)benzoate 76 (*ANM163)*



The compound was synthesized according to GP-8 using **44** (1.50 g, 7.00 mmol) and methyl 4-hydroxybenzoate (1.06 g, 7.00 mmol), and was isolated as a white solid (1.57 g, 77% yield). ^{1}H NMR: δ 0.81-0.86 (m, 2H, Cyclohexyl), 1.11-1.22 (m, 6H, 4'-H, Cyclohexyl), 1.37-1.45 (m, 2H, 3'-H), 1.60-1.69 (m, 7H, 2'-H, Cyclohexyl), 3.80 (s, 3H, $CO_2C\underline{H}_3$), 4.02 (t, J = 6.5 Hz, 2H, 1'-H), 7.01 (d, J = 8.9 Hz, 2H, 3-H, 5-H), 7.85-7.91 (m, 2H, 2-H, 6-H). ^{13}C NMR: δ 22.8 (C-4'), 26.0 (C-3", C-5"), 26.3 (C-4"), 28.9 (C-3'), 32.9 (C-2", C-6"), 36.7 (C-2'), 37.1 (C-1"), 51.9 ($CO_2\underline{C}H_3$), 68.0 (C-1'), 114.5 (C-3, C-5), 121.8 (C-1), 131.3 (C-2, C-6), 162.7 (C-4), 166.0 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 291 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.1%. mp < 50 °C.

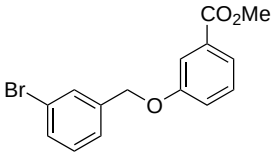
5.2.17.17 Methyl 3-(4-cyclohexylbutoxy)benzoate 100 (*ANM164)*



The compound was synthesized according to GP-8 using **44** (857 g, 4.00 mmol) and methyl 3-hydroxybenzoate (609 mg, 4.00 mmol), and was isolated as a white solid (673 g, 58% yield). ^{1}H NMR: δ 0.81-0.86 (m, 2H, Cyclohexyl), 1.11-1.22 (m, 6H, 4'-H, Cyclohexyl),

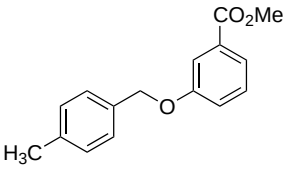
1.37-1.45 (m, 2H, 3'-H), 1.60-1.69 (m, 7H, 2'-H, Cyclohexyl), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 3.94-4.03 (m, 2H, 1'-H), 7.14-7.24 (m, 1H, 4-H), 7.39-7.48 (m, 2H, 2-H, 5-H), 7.51 (dq, J = 1.3, 7.5 Hz, 1H, 6-H). ^{13}C NMR: δ 22.8 (C-4'), 25.9 (C-3", C-5"), 26.3 (C-4"), 29.0 (C-3'), 32.9 (C-2", C-6"), 36.7 (C-2'), 37.1 (C-1"), 52.3 ($CO_2\underline{C}H_3$), 67.8 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.3 (C-6), 130.0 (C-5), 131.1 (C-1), 158.8 (C-4), 166.3 ($\underline{C}O_2Me$). LC-MS (m/z): positive mode 291 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.9%. mp < 50 °C.

5.2.17.18 Methyl 3-((3-bromobenzyl)oxy)benzoate 66 (*ANM225*)



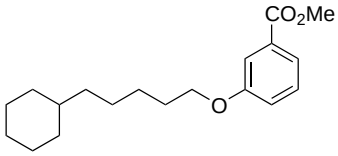
The compound was synthesized according to GP-8 using 4-bromobenzyl bromide (1.75 g, 7.00 mmol) and methyl 3-hydroxybenzoate (1.07 g, 7.00 mmol), and was isolated as a white solid (2.20 g, 97% yield). LC-MS (m/z): positive mode 321 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 78.8%.

5.2.17.19 Methyl 3-((4-methylbenzyl)oxy)benzoate 68 (*ANM227*)



The compound was synthesized according to GP-8 using 4-methylbenzyl bromide (1.30 g, 7.00 mmol) and methyl 3-hydroxybenzoate (1.07 g, 7.00 mmol), and was isolated as a white solid (1.56 g, 88% yield). LC-MS (m/z): positive mode 257 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 74.5%.

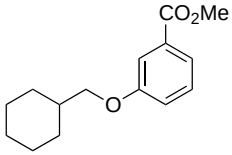
5.2.17.20 Methyl 3-((5-cyclohexylpentyl)oxy)benzoate 45 (*ANM239*)



The compound was synthesized according to GP-8 using **43** (1.94 g, 8.30 mmol) and methyl 3-hydroxybenzoate (1.26 g, 8.30 mmol), and was isolated as a colourless oil (940 mg, 37% yield). ^{1}H NMR: δ 0.74-0.91 (m, 2H, Cyclohexyl), 1.02-1.25 (m, 6H, 5'-H, Cyclohexyl), 1.25-1.46 (m, 4H, 3'-H, 4'-H), 1.52-1.76 (m, 7H, 2'-H, Cyclohexyl), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 4.00 (t, J = 6.5 Hz, 2H, 1'-H), 7.20 (ddd, J = 8.2, 2.6, 1H, 1.0 Hz, 4-H), 7.35-7.48 (m, 2H, 2-H, 5-H), 7.47-7.56 (m, 1H, 6-H). ^{13}C NMR: δ 25.9 (C-4', C-5'), 26.0 (C-3", C-5"), 26.3 (C-4"), 28.7 (C-3'), 33.0 (C-2", C-6"), 37.0 (C-2'), 37.1 (C-1"), 52.3 ($CO_2\underline{C}H_3$), 67.9 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.4 (C-6), 130.0 (C-5), 131.1 (C-1), 158.9 (C-4), 166.2 ($\underline{C}O_2Me$). LC-MS (m/z):

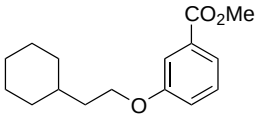
positive mode 305 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.4%.

5.2.17.21 Methyl 3-(cyclohexylmethoxy)benzoate 94 (*ANM244*)[211]



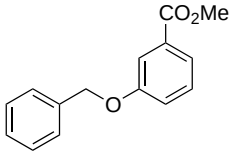
The compound was synthesized according to GP-8 using (bromomethyl)cyclohexane (1.42 g, 8.00 mmol) and methyl 3-hydroxybenzoate (1.22 g, 8.00 mmol), and was isolated as a colourless oil (911 mg, 46% yield). LC-MS (m/z): positive mode 249 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.8%.

5.2.17.22 Methyl 3-(2-cyclohexylethoxy)benzoate 96 (*ANM245*)



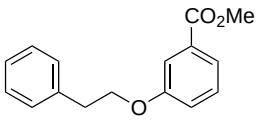
The compound was synthesized according to GP-8 using (bromoethyl)cyclohexane (1.53 g, 8.00 mmol) and methyl 3-hydroxybenzoate (1.22 g, 8.00 mmol), and was isolated as a colourless oil (1.89 g, 90% yield). ^{1}H NMR: δ 0.88-1.02 (m, 2H, Cyclohexyl), 1.07-1.27 (m, 2H, Cyclohexyl), 1.44-1.48 (m, 2H, Cyclohexyl), 1.55-1.78 (m, 7H, 2'-H, Cyclohexyl), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 4.04 (t, J = 6.6 Hz, 2H, 1'-H), 7.20 (ddd, J = 8.2, 2.7, 1.1 Hz, 1H, 4-H), 7.38-7.44 (m, 2H, 2-H, 5-H), 7.49-7.54 (m, 1H, 6-H). ^{13}C NMR: δ 25.9 (C-3", C-5"), 26.2 (C-4"), 34.2 (C-1"), 32.8 (C-2", C-6"), 36.1 (C-2'), 52.3 ($CO_2\underline{C}H_3$), 65.9 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.4 (C-6), 130.0 (C-5), 131.1 (C-1), 158.8 (C-4), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 263 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.9%.

5.2.17.23 Methyl 3-(benzyloxy)benzoate 70 (*ANM246*)[206]



The compound was synthesized according to GP-8 using (bromomethyl)benzene (1.73 g, 8.00 mmol) and methyl 3-hydroxybenzoate (1.22 g, 8.00 mmol), and was isolated as a white solid (1.77 g, 91% yield). LC-MS (m/z): positive mode 243 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.2%.

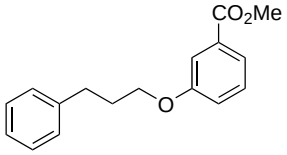
5.2.17.24 Methyl 3-phenethoxybenzoate 80 (*ANM247*)



The compound was synthesized according to GP-8 using (2-bromoethyl)benzene (1.48 g, 8.00 mmol) and methyl 3-hydroxybenzoate (1.22 g, 8.00 mmol), and was isolated as a colourless

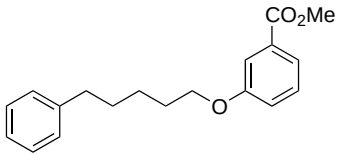
oil (1.32 g, 64% yield). ^{1}H NMR: δ 3.04 (t, J = 6.8 Hz, 2H, 2'-H), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 4.24 (t, J = 6.8 Hz, 2H, 1'-H), 7.19-7.24 (m, 2H, 4-H, 4"-H), 7.28-7.34 (m, 4H, 2"-H, 3"-H, 5"-H, 6"-H), 7.39-7.44 (m, 2H, 2-H, 5-H), 7.51-7.54 (m, 1H, 6-H). ^{13}C NMR: δ 35.0 (C-2'), 52.3 ($CO_2\underline{C}H_3$), 68.5 (C-1'), 114.6 (C-2), 119.8 (C-4), 121.5 (C-6), 126.4 (C-4"), 128.4 (C-2", C-6"), 129.1 (C-3", C-5"), 130.1 (C-5), 131.1 (C-1), 138.4 (C-1"), 158.6 (C-4), 166.1 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 257 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.0%.

5.2.17.25 Methyl 3-(3-phenylpropoxy)benzoate 82 (*ANM248*)



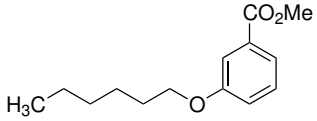
The compound was synthesized according to GP-8 using (3-bromopropyl)benzene (1.59 g, 8.00 mmol) and methyl 3-hydroxybenzoate (1.22 g, 8.00 mmol), and was isolated as a colourless oil (1.70 g, 79% yield). ^{1}H NMR: δ 1.96-2.07 (m, 2H, 2'-H), 2.69-2.76 (m, 2H, 3'-H), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 4.00 (t, J = 6.3 Hz, 2H, 1'-H), 7.14-7.30 (m, 6H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H, 4-H), 7.38-7.45 (m, 2H, 2-H, 5-H), 7.50-7.55 (m, 1H, 6-H). ^{13}C NMR: δ 30.4 (C-2'), 31.5 (C-3'), 52.3 ($CO_2\underline{C}H_3$), 67.1 (C-1'), 114.6 (C-2), 119.8 (C-4), 121.5 (C-6), 126.0 (C-4"), 128.4 (C-2", C-3", C-5", C-6"), 130.0 (C-5), 131.1 (C-1), 141.4 (C-1"), 158.8 (C-4), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 271 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 92.0%.

5.2.17.26 Methyl 3-((5-phenylpentyl)oxy)benzoate 86 (*ANM271*)



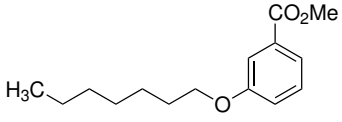
The compound was synthesized according to GP-8 using (5-bromopentyl)benzene (1.82 g, 8.00 mmol) and methyl 3-hydroxybenzoate (1.22 g, 8.00 mmol), and was isolated as a colourless oil (2.1 g, 88% yield). ^{1}H NMR: δ 1.39-1.48 (m, 2H, 3'-H), 1.57-1.67 (m, 2H, 4'-H), 1.70-1.78 (m, 2H, 2'-H), 2.56-2.60 (m, 2H, 5'-H), 3.83 (s, 2H, $CO_2C\underline{H}_3$), 3.99 (t, J = 6.45 Hz, 2H, 1'-H), 7.13-7.20 (m, 4H, 2"-H, 4"-H, 6"-H, 4-H), 7.25 (t, J = 7.58 Hz, 2H, 3"-H, 5"-H), 7.39-7.43 (m, 2H, 2-H, 5-H), 7.50-7.53 (m, 1H, 6-H). ^{13}C NMR: δ 25.2 (C-3'), 28.5 (C-2'), 30.8 (C-5'), 35.2 (C-4'), 52.3 ($CO_2\underline{C}H_3$), 67.8 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.4 (C-4), 125.7 (C-4"), 128.3-128.4 (C-2", C-3", C-5", C-6"), 130.0 (C-5), 131.1 (C-1), 142.3 (C-1"), 158.8 (C-4), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 299 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 90.5%.

5.2.17.27 Methyl 3-(hexyloxy)benzoate 88 (*ANM321*)[210]



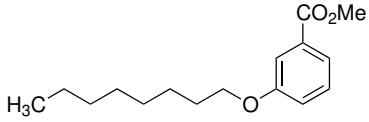
The compound was synthesized according to GP-8 using 1-bromohexane (0.986 mL, 7.00 mmol) and methyl 3-hydroxybenzoate (1.07 g, 7.00 mmol), and was isolated as a yellow oil (1.10 g, 67% yield). ^{1}H NMR: δ 0.82-0.91 (m, 3H, 6'-H), 1.16-1.34 (m, 4H, 4'-H, 5'-H), 1.36-1.46 (m, 2H, 3'-H), 1.64-1.76 (m, 2H, 2'-H), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 3.99 (t, J = 6.5 Hz, 2H, 1'-H), 7.13-7.18 (m, 1H, 4-H), 7.38-7.44 (m, 2H, 2-H, 6-H), 7.49-7.54 (m, 1H, 5-H). ^{13}C NMR: δ 14.0 (C-6'), 22.2 (C-5'), 25.2 (C-4'), 28.7 (C-3'), 31.1 (C-2'), 52.3 ($CO_2\underline{C}H_3$), 67.9 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.4 (C-6), 130.0 (C-5), 131.1 (C-1), 158.9 (C-3), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 237 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.2%.

5.2.17.28 Methyl 3-(heptyloxy)benzoate 90 (*ANM322*)



The compound was synthesized according to GP-8 using 1-bromoheptane (1.10 mL, 7.00 mmol) and methyl 3-hydroxybenzoate (1.07 g, 7.00 mmol), and was isolated as a yellow oil (1.12 g, 64% yield). ^{1}H NMR: δ 0.80-0.90 (m, 3H, 7'-H), 1.20-1.45 (m, 8H, 6'-H, 5'-H, 4'-H, 3'-H), 1.65-1.85 (m, 2H, 2'-H), 3.80-3.90 (m, 3H, $CO_2C\underline{H}_3$), 3.98-4.09 (m, 2H, 1'-H), 7.18-7.22 (m, 1H, 4-H), 7.39-7.44 (m, 2H, 2-H, 6-H), 7.50-7.53 (m, 1H, 5-H). ^{13}C NMR: δ 14.0 (C-7'), 22.1 (C-5'), 28.7 (C-4'), 32.3 (C-3'), 35.2 (C-2'), 52.3 ($CO_2\underline{C}H_3$), 67.8 (C-1'), 114.4 (C-2), 119.9 (C-4), 121.3 (C-6), 130.0 (C-5), 131.1 (C-1), 158.8 (C-3), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 251 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 63.4%.

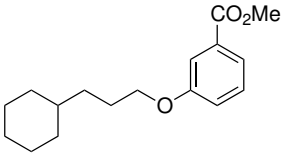
5.2.17.29 Methyl 3-(octyloxy)benzoate 92 (*ANM323*)



The compound was synthesized according to GP-8 using 1-bromooctane (1.22 mL, 7.00 mmol) and methyl 3-hydroxybenzoate (1.07 g, 7.00 mmol), and was isolated as a yellow oil (1.61 g, 87% yield). ^{1}H NMR: δ 0.80-0.88 (m, 3H, 8'-H), 1.20-1.45 (m, 10H, 7'-H, 6'-H, 5'-H, 4'-H, 3'-H), 1.60-1.80 (m, 2H, 2'-H), 3.80-3.85 (m, 3H, $CO_2C\underline{H}_3$), 3.98-4.05 (m, 2H, 1'-H), 7.18-7.21 (m, 1H, 4-H), 7.38- 7.44 (m, 2H, 2-H, 6-H), 7.50-7.54 (m, 1H, 5-H). ^{13}C NMR: δ 14.1 (C-8'), 22.2 (C-7'), 25.6 (C-6'), 28.7 (C-5'), 28.8 (C-4'), 28.8 (C-3'), 31.3 (C-2'), 52.3 ($CO_2\underline{C}H_3$), 67.9 (C-1'), 114.5 (C-2), 119.9 (C-4), 121.4 (C-6), 130.1 (C-5), 131.1 (C-1), 158.9 (C-3), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive

mode 265 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.3%.

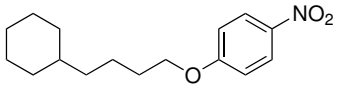
5.2.17.30 Methyl 3-(3-cyclohexylpropoxy)benzoate 98 (*THB58)*



The compound was synthesized according to GP-8 using (3-bromopropyl)cyclohexane (1.4 g, 7.00 mmol) and methyl 3-hydroxybenzoate (1.07 g, 7.00 mmol), and was isolated as a colourless oil (1.02 g, 53% yield). ^{1}H NMR: δ 0.81-0.92 (m, 2H, 3'-H), 1.05-1.33 (m, 8H, 2"-H, 3"-H, 5"-H, 6"-H), 1.57-1.76 (m, 5H, 2'-H, 1"-H, 4"-H), 3.28 (s, 3H, $CO_2C\underline{H}_3$), 3.98 (t, J = 6.5 Hz, 2H, 1'-H), 7.19 (ddd, J = 1.0, 2.7, 8.2 Hz, 1H, 4-H), 7.36-7.44 (m, 2H, 5-H, 6-H), 7.48-7.54 (m, 1H, 2-H). ^{13}C NMR: δ 25.9 (C-3", C-5"), 26.1 (C-4"), 26.3 (C-2'), 32.9 (C-2", C-6"), 33.3 (C-3'), 36.9 (C-1"), 52.3 ($CO_2\underline{C}H_3$), 68.2 (C-1'), 114.5 (C-5), 119.9 (C-6), 121.4 (C-4), 130.0 (C-2), 131.1 (C-1), 158.8 (C-3), 166.2 ($\underline{C}O_2$Me). LC-MS (m/z): positive mode 277 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.0%.

5.2.18 1-(4-Cyclohexylbutoxy)-4-nitrobenzene

5.2.18.1 1-(4-Cyclohexylbutoxy)-4-nitrobenzene 186 (*ANM161)*[230]



The product was synthesized according to GP-8 using **44** (877 mg, 4 mmol) and 4-nitrophenol (556 mg, 4 mmol) and was isolated as a white solid (720 mg, 65% yield). ^{1}H NMR: δ 0.83-0.87 (m, 2H, Cyclohexyl), 1.12-1.19 (m, 6H, 4'-H, Cyclohexyl), 1.38-1.42 (m, 2H, 3'-H), 1.61-1.70 (m, 7H, 2'-H, Cyclohexyl), .10 (t, J = 4.1 Hz, 2H, 1'-H), 7.09-7.15 (m, 2H, 3-H, 5-H), 8.16-8.20 (m, 2H, 2-H, 6-H). ^{13}C NMR: δ 22.7 (C-4'), 25.9 (C-3", C-5"), 26.3 (C-4'), 28.8 (C-3'), 32.9 (C-2", C-6"), 36.6 (C-2'), 37.1 (C-1"), 68.8 (C-1'), 115.1 (C-3, C-5), 126.0 (C-2, C-6), 140.8 (C-1), 164.2 (C-4). LC-MS (m/z): positive mode 278 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.1%. lit. mp 51-52 °C.

5.2.19 Benzoic acids

5.2.19.1 General procedure for the synthesis of the alkyloxybenzoic acids (GP-9)

5.2.19.2 Method 1 (GP-9a)

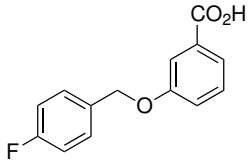
In a 10 mL microwave-reaction vessel equipped with a magnetic stirring bar, the appropriate ester (2 mmol) was suspended in MeOH (1 mL) and aq. KOH solution (1 N, 3 mL). The vessel was sealed, and the mixture was irradiated in a microwave

oven at 100 °C for 60 min (3-10 bar, 40-65 W). The reaction mixture was cooled to rt, and 20 mL of water were added. The product was precipitated by dropwise addition of diluted aq. HCl (2 N) until a pH $\leq$ 2 was reached. The solid was subsequently filtered off, washed with water (15 mL), and dried in vacuum at 50 °C.

5.2.19.3 Method 2 (GP-9b)

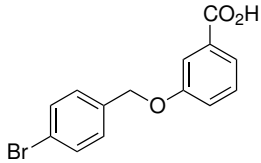
In a 30 mL pressure tube (ACE®-glass), the appropriate ester (3 mmol) was suspended in MeOH (5 mL) and KOH (1N, 7 mL) and heated to 120 °C for 4 h. After addition of 80 mL of water the product was precipitated by dropwise addition of diluted aq. HCl (2 N) until a pH $\leq$ 2 was reached. The solid was subsequently filtered off, washed with water (15 mL), and dried in vacuum at 50 °C.

5.2.19.4 3-(4-Fluorobenzyloxy)benzoic acid 61 (*ANM12*)[196]



The compound was synthesized according to GP-9a using **60** (521 mg, 2.0 mmol), and was isolated as a white solid (422 mg, 86% yield). ^{1}H NMR: δ 5.13 (s, 2H, CH_2), 7.18-7.23 (m, 2H, 3'-H, 5'-H), 7.25 (ddd, J = 8.2, 2.7 Hz, 1H, 4-H), 7.40 (t, J = 7.9 Hz, 1H, 5-H), 7.48-7.52 (m, 3H, 2-H, 2'-H, 6'-H), 7.52-7.55 (m, 1H, 6-H). ^{13}C NMR: δ 68.8 (CH_2), 115.1 (C-2), 115.4 (C-3', C-5'), 119.9 (C-6), 122.0 (C-4), 129.9 (C-2', C-6'), 130.1 (C-5), 132.4 (C-1'), 133.2 (C-1), 158.4 (C-3), 162.9 (C-4'), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 245 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 150-152 °C.

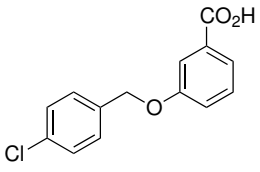
5.2.19.5 3-(4-Bromobenzyloxy)benzoic acid 63 (*ANM13*)[196]



The compound was synthesized according to GP-9a using **62** (642 mg, 2.0 mmol), and was isolated as a white solid (548 mg, 89% yield). ^{1}H NMR: δ 5.13 (s, 2H, CH_2), 7.29 (ddd, J = 8.2, 2.7 Hz, 1H, 4-H), 7.37 (t, J = 7.9 Hz, 1H, 5-H), 7.40-7.43 (m, 2H, 2'-H, 6'-H), 7.51 (dd, J = 2.6 Hz, 1H, 2-H), 7.51-7.54 (m, 2H, 3'-H, 5'-H). ^{13}C NMR: δ 68.7 (CH_2), 115.1 (C-2), 119.3 (C-6), 121.1 (C-4'), 122.0 (C-4), 129.7 (C-5), 129.9 (C-2', C-6'), 131.5 (C-3', C-5'), 133.9 (C-3), 136.6 (C-1'), 158.2 (C-3), 167.4 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 306 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit.

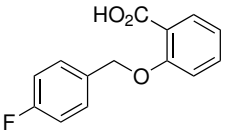
mp 180-183 °C.

5.2.19.6 3-(4-Chlorobenzyloxy)benzoic acid 65 (*ANM14*)[196]



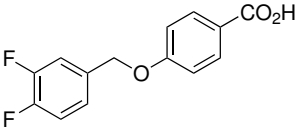
The compound was synthesized according to GP-9a using **64** (553 mg, 2.0 mmol), and was isolated as a white solid (494 mg, 94% yield). ^{1}H NMR: δ 5.16 (s, 2H, CH_2), 7.25 (ddd, J = 8.2, 2.7 Hz, 1H, 4-H), 7.41 (t, J = 7.9 Hz, 1H, 5-H), 7.43-7.50 (m, 4H, 2'-H, 3'-H, 5'-H, 6'-H), 7.51 (dd, J = 2.6 Hz, 1H, 2-H), 7.52-7.55 (m, 1H, 6-H). ^{13}C NMR: δ 68.7 (CH_2), 115.1 (C-2), 119.9 (C-6), 122.1 (C-4), 128.6 (C-3', C-5'), 129.6 (C-2', C-6'), 129.9 (C-5), 132.5 (C-4'), 132.6 (C-1), 136.0 (C-1'), 158.3 (C-3), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 261 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.6%. lit. mp 95-96 °C.

5.2.19.7 2-(4-Fluorobenzyloxy)benzoic acid 73 (*ANM15*)[196]



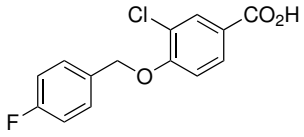
The compound was synthesized according to GP-9a using **72** (521 mg, 2.0 mmol), and was isolated as a white solid (479 mg, 97% yield). ^{1}H NMR: δ 5.17 (s, 2H, CH_2), 7.00 (td, J = 0.9, 7.5 Hz, 1H, 5-H), 7.16-7.23 (m, 3H, 3-H, 3'-H, 5'-H), 7.47 (ddd, J = 1.8, 7.4 Hz, 1H, 5-H), 7.51-7.56 (m, 2H, 2'-H, 6'-H), 7.64 (dd, J = 1.8, 7.6 Hz, 1H, 6-H), 12.59 (s, 1H, OH). ^{13}C NMR: δ 69.1 (CH_2), 114.1 (C-3), 115.3 (C-3', C-5'), 120.2 (C-5), 122.2 (C-1), 129.4 (C-2', C-6'), 130.8 (C-6), 133.0 (C-4), 133.4 (C-1'), 157.0 (C-2), 162.5 (C-4'), 167.5 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 245 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.8%. lit. mp 82-83 °C.

5.2.19.8 4-(3,4-Difluorobenzyloxy)benzoic acid 49 (*ANM122*)



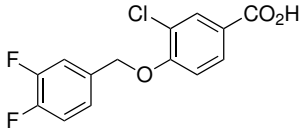
The compound was synthesized according to GP-9a using **48** (557 mg, 2.0 mmol), and was isolated as a white solid (507 mg, 96% yield). ^{1}H NMR: δ 5.16 (s, 2H, CH_2), 7.07-7.11 (m, 2H, 3-H, 5-H), 7.30-7.34 (m, 1H, 6'-H), 7.45 (dt, J = 8.4 Hz, 1H, 5'-H), 7.53 (ddd, J = 7.8, 2.1 Hz, 1H, 2'-H), 7.87-7.91 (m, 2H, 2-H, 6-H), 12.6 (s, 1H, OH). ^{13}C NMR: δ 68.3 (CH_2), 114.8 (C-3, C-5), 117.0 (C-2'), 117.8 (C-5'), 123.6 (C-1), 123.9 (C-6'), 131.5 (C-2, C-6), 134.5 (C-1'), 148.2-148.6 (C-4'), 150.3-150.5 (C-3'), 161.7 (C-4), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 263 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 212-213 °C.

5.2.19.9 3-Chloro-4-(4-fluorobenzyloxy)benzoic acid 51 (*ANM126)*



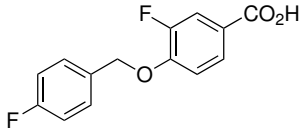
The compound was synthesized according to GP-9a using **50** (589 mg, 2.0 mmol), and was isolated as a white solid (538 mg, 96% yield). ^{1}H NMR: δ 5.27 (s, 2H, CH_2), 7.21-7.27 (m, 2H, 3'-H, 5'-H), 7.33 (d, J = 8.7 Hz, 1H, 5-H), 7.50-7.55 (m, 2H, 2'-H, 6'-H), 7.88 (dd, J = 8.6, 2.1 Hz, 1H, 6-H), 7.91 (d, J = 2.1 Hz, 1H, 2-H), 12.93 (s, 1H, OH). ^{13}C NMR: δ 69.8 (CH_2), 114.0 (C-5), 115.6 (C-3', C-5'), 121.6 (C-3), 124.4 (C-1), 130.0 (C-6), 130.1 (C-2', C-6'), 130.9 (C-2), 132.4 (C-1'), 157.1 (C-4), 161.1-163.0 (C-4'), 166.1 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 279 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 209-210 °C.

5.2.19.10 3-Chloro-4-(3,4-difluorobenzyloxy)benzoic acid 53 (*ANM148)*[199]



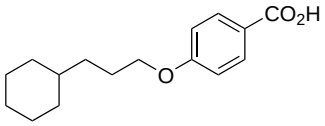
The compound was synthesized according to GP-9a using **52** (625 mg, 2.0 mmol), and was isolated as a white solid (549 mg, 92% yield). ^{1}H NMR: δ 5.27 (s, 2H, CH_2), 7.32 (d, J = 8.7 Hz, 1H, 6'-H), 7.34 (dt, J = 1.9, 4.8 Hz, 1H, 5-H), 7.47 (dt, J = 8.4 Hz, 1H, 5'-H), 7.54 (ddd, J = 7.8, 2.1 Hz, 1H, 2'-H), 7.88 (dd, J = 2.1, 8.6 Hz, 1H, 6-H), 7.92 (d, J = 2.1 Hz, 1H, 2-H), 13.0 (s, 1H, OH). ^{13}C NMR: δ 69.2 (CH_2), 113.9 (C-5), 116.8-116.9 (C-2'), 117.7-117.9 (C-5'), 121.6 (C-3), 124.5 (C-1), 124.6-124.7 (C-6'), 130.1 (C-6), 130.9 (C-2), 133.9 (C-1'), 148.3 (C-4'), 150.3-150.5 (C-3'), 156.9 (C-4), 166.0 ($\underline{C}O_2H$). LC-MS (m/z): negative mode XXXXXXXX $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. lit. mp 213-215 °C.

5.2.19.11 3-Fluor-4-(4-fluorobenzyloxy)benzoic acid 55 (*THB13)*[200]



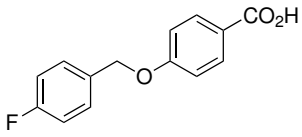
The compound was synthesized according to GP-9a by heating **54** (556 mg, 2.0 mmol), 5 mL MeOH and 7 mL KOH (1N) for 4 h at 120 °C in a pressure tube (ACE-glass), and was isolated as a white solid (457 mg, 86% yield). ^{1}H NMR: δ 5.24 (s, 2H, CH_2), 7.35 (t, J = 8.5 Hz, 1H, 5-H), 7.52 (ddd, J = 2.6, 5.34 Hz, 8.5 Hz, 2H, 2'-H, 6'-H), 7.67 (dd, J = 2.1, 11.8 Hz, 1H, 6-H), 7.74 (ddd, J = 1.0, 2.0, 8.6 Hz, 1H, 2-H). ^{13}C NMR: δ 69.8 (CH_2), 114.9 (C-5), 115.6 (C-3', C-5'), 116.8 (C-6), 123.88 (C-1), 126.8 (C-2), 130.4 (C-2', C-6'), 132.4 (C-1'), 150.2-152.2 (C-3), 161.2 (C-4), 163.1 (C-4'), 166.2 ($\underline{C}O_2H$). LC-MS (m/z): negative mode 263 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. lit. mp 89-90 °C.

5.2.19.12 4-(3-Cyclohexylpropoxy)benzoic acid 291 (*ANM24*)[231]



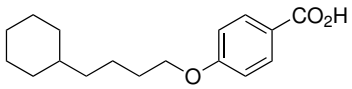
The compound was synthesized according to GP-9a using methyl 4-(3-cyclohexylpropoxy)benzoate (553 mg, 2.0 mmol), and was isolated as a white solid (488 mg, 97% yield). ^{1}H NMR: δ 0.83-0.91 (q, J = 9.9 Hz, 2H, 3'-H), 1.16-1.25 (m, 6H, Cyclohexyl), 1.61-1.72 (m, 7H, Cyclohexyl, 2'-H), 4.00 (t, J = 6.6 Hz, 2H, 1'-H), 6.98 (d, J = 9.0 Hz, 2H, 3-H, 5-H), 7.86 (d, J = 8.9 Hz, 2H, 2-H, 6-H), 12.53 (s, 1H, OH). ^{13}C NMR: δ 26.0 (C-3", C-5"), 26.1 (C-4"), 26.3 (C-2'), 32.9 (C-1", C-6"), 33.3 (C-3'), 36.9 (C-1"), 68.3 (C-1'), 114.4 (C-3, C-5), 122.9 (C-1), 131.5 (C-2, C-6), 162.4 (C-4), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 263 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 182-183 °C.

5.2.19.13 4-((4-Fluorobenzyl)oxy)benzoic acid 47 (*ANM55*)[196]



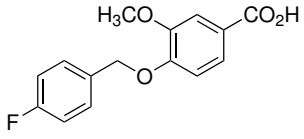
The compound was synthesized according to GP-9a using **46** (520 mg, 2.0 mmol), and was isolated as a white solid (418 mg, 85% yield). ^{1}H NMR: δ 5.15 (s, 2H, CH_2), 7.02-7.09 (m, 2H, 3-H, 5-H), 7.17-7.27 (m, 2H, 3'-H, 5'-H), 7.45-7.55 (m, 2H, 2'-H, 6'-H), 7.88 (m, 2H, 2-H, 6-H), 12.59 (s, 1H, OH). ^{13}C NMR: δ 68.9 (CH_2), 114.7 (C-2, C-6), 115.5-115.3 (C-3', C-5'), 123.4 (C-1), 130.3 (C-2', C-6'), 131.5 (C-3, C-5), 132.9 (C-1'), 161.9 (C-1, C-4), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 245 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 212-213 °C.

5.2.19.14 4-(4-Cyclohexylbutoxy)benzoic acid 77 (*ANM109*)



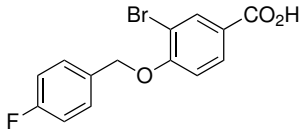
The compound was synthesized according to GP-9a using **76** (439 mg, 1.5 mmol), and was isolated as a white solid (377 mg, 91% yield). ^{1}H NMR: δ 1.29-1.55 (m, 17H, 2'-H, 3'-H, 4'-H, 1"-H, 2"-H, 3'-H, 4"-H, 5"-H, 6"-H), 4.01 (t, J = 6.5 Hz, 2H, 1'-H), 6.94-7.02 (m, 2H, 3-H, 5-H), 7.82-7.90 (m, 2H, 2-H, 6-H), 12.69 (s, 1H, OH). ^{13}C NMR: δ 22.8 (C-3'), 26.0 (C-3", C-5"), 26.3 (C-4"), 29.0 (C-2'), 33.0 (C-2", C-6"), 36.7 (C-4'), 37.1 (C-1"), 67.9 (C-1'), 114.3 (C-3, C-5), 122.9 (C-1), 131.4 (C-2, C-6), 162.4 (C-4), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 277 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 141-142 °C.

5.2.19.15 4-((4-Fluorobenzyl)oxy)-3-methoxybenzoic acid 57 (*ANM120*)[232]



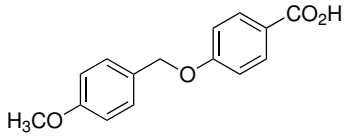
The compound was synthesized according to GP-9a using **56** (581 mg, 2.0 mmol), and was isolated as a white solid (514 mg, 93% yield). ^{1}H NMR: δ 3.80 (s, 3H, OCH_3), 5.14 (s, 2H, CH_2), 7.12 (d, J = 8.5 Hz, 1H, 5-H), 7.22 (m, 2H, 3'-H, 5'-H), 7.46 (d, J = 2.0 Hz, 1H, 2-H), 7.48-7.52 (m, 2H, 2'-H, 6'-H), 7.53 (dd, J = 8.4, 2.0 Hz, 1H, 6-H), 12.64 (s, 1H, OH). ^{13}C NMR: δ 55.7 (OCH_3), 69.3 (CH_2), 112.4 (C-5), 112.7 (C-3', C-5'), 123.1 (C-6), 123.5 (C-1), 130.2 (C-2', C-6'), 133.0 (C-1'), 148.8 (C-3), 151.6 (C-4), 161.0-163.0 (C-4'), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 277 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. lit. mp 208-209 °C.

5.2.19.16 3-Bromo-4-((4-fluorobenzyl)oxy)benzoic acid 59 (*ANM121*)



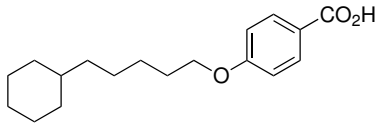
The compound was synthesized according to GP-9a using **58** (678 mg, 2.0 mmol), and was isolated as a white solid (585 mg, 90% yield). ^{1}H NMR: δ 5.27 (s, 2H, CH_2), 7.21-7.27 (m, 2H, 3'-H, 5'-H), 7.29 (d, J = 8.7 Hz, 1H, 5-H), 7.49-7.55 (m, 2H, 2'-H, 6'-H), 7.92 (dd, J = 8.6, 2.10 Hz, 1H, 6-H), 8.07 (d, J = 2.1 Hz, 1H, 2-H), 12.92 (s, 1H, OH). ^{13}C NMR: δ 69.9 (CH_2), 111.1 (C-3), 113.8 (C-5), 115.4-115.6 (C-3', C-5'), 124.8 (C-1), 129.9 (C-2', C-6'), 130.7 (C-6), 132.5 (C-1'), 134.0 (C-2), 158.0 (C-4), 161.1-163.0 (C-4'), 165.94 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 326 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.5%. mp 218-219 °C.

5.2.19.17 4-((4-Methoxybenzyl)oxy)benzoic acid 292 (*ANM131*)[232]



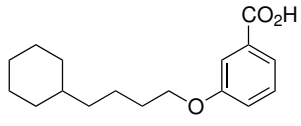
The compound was synthesized according to GP-9a using **293** (545 mg, 2.0 mmol), and was isolated as a white solid (495 mg, 95% yield). ^{1}H NMR: δ 3.75 (s, 3H, OCH_3), 5.08 (s, 2H, CH_2), 6.90-6.99 (m, 2H, 3'-H, 5'-H), 7.04-7.09 (m, 2H, 3-H, 5-H), 7.30-7.40 (m, 2H, 2'-H, 6'-H), 7.82-7.90 (m, 2H, 2-H, 6-H), 12.55 (s, 1H, OH). ^{13}C NMR: δ 55.3 (OCH_3), 69.4 (CH_2), 114.0 (C-3', C-5'), 114.8 (C-2, C-6), 123.2 (C-1), 128.5 (C-1'), 129.8 (C-2', C-6'), 131.4 (C-3, C-5), 159.3 (C-4'), 162.1 (C-4), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 259 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.9%. lit. mp 189-191 °C.

5.2.19.18 4-((5-Cyclohexylpentyl)oxy)benzoic acid 79 (*ANM154*)



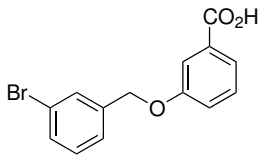
The compound was synthesized according to GP-9a using **78** (517 mg, 1.7 mmol), and was isolated as a white solid (297 mg, 60% yield). ^{1}H NMR: δ 0.81-0.86 (m, 2H, Cyclohexyl), 1.12-1.19 (m, 6H, 5'-H, Cyclohexyl), 1.30-1.38 (m, 4H, 3'-H, 4'-H), 1.61-1.70 (m, 7H, 2'-H, Cyclohexyl), 4.01 (t, J = 6.5 Hz, 2H, 1'-H), 6.94-7.01 (m, 2H, 3-H, 5-H), 7.84-7.90 (m, 2H, 2-H, 6-H). ^{13}C NMR: δ 25.9-26.1 (C-4', C-5'), 26.0 (C-3", C-5"), 26.3 (C-4"), 28.7 (C-3'), 33.0 (C-2", C-6"), 37.0 (C-2'), 37.1 (C-1"), 67.9 (C-1'), 114.3 (C-3, C-5), 121.9 (C-1), 131.4 (C-2, C-6), 162.4 (C-4), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 292 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 180-181 °C.

5.2.19.19 3-(4-Cyclohexylbutoxy)benzoic acid 101 (*ANM174*)



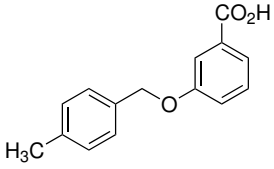
The compound was synthesized according to GP-9a using **100** (522 mg, 1.8 mmol), and was isolated as a white solid (312 mg, 63% yield). ^{1}H NMR: δ 0.81-0.86 (m, 2H, Cyclohexyl), 1.11-1.22 (m, 6H, 4'-H, Cyclohexyl), 1.37-1.45 (m, 2H, 3'-H), 1.60-1.69 (m, 7H, 2'-H, Cyclohexyl), 3.94-4.03 (m, 2H, 1'-H), 7.15 (ddd, J = 1.0, 2.7, 8.2 Hz, 1H, 4-H), 7.39-7.48 (m, 2H, 2-H, 5-H), 7.50 (dq, J = 1.3, 7.5 Hz, 1H, 6-H). ^{13}C NMR: δ 22.8 (C-4'), 26.0 (C-3", C-5"), 26.3 (C-4"), 29.0 (C-3'), 32.9 (C-2", C-6"), 36.7 (C-2'), 37.1 (C-1"), 67.8 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.5 (C-6), 129.8 (C-5), 132.3 (C-1), 158.8 (C-4), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 294 $[M+NH_4]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 93-94 °C.

5.2.19.20 3-((3-Bromobenzyl)oxy)benzoic acid 67 (*ANM226*)



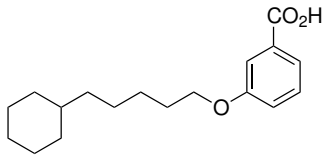
The compound was synthesized according to GP-9b using **66** (964 mg, 3.0 mmol), and was isolated as a white solid (660 mg, 72% yield). ^{1}H NMR: δ 5.17 (s, 2H, CH_2), 7.26 (ddd, J = 1.0, 2.7, 8.2 Hz, 1H, 4-H), 7.36 (t, J = 7.8 Hz, 1H, 5'-H), 7.41 (t, J = 7.9 Hz, 1H, 5-H), 7.42-7.48 (m, 1H, 6'-H), 7.51-7.55 (m, 3H, 2-H, 6-H, 2'-H), 7.62-7.68 (m, 1H, 4'-H), 12.96 (s, 1H, OH). ^{13}C NMR: δ 68.5 (CH_2), 115.1 (C-2), 119.9 (C-6), 121.8 (C-3'), 122.1 (C-4), 126.7 (C-6'), 129.9 (C-5'), 130.3 (C-5), 130.8 (C-4'), 132.4 (C-1), 139.8 (C-1'), 158.2 (C-3), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 305 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. mp 144-145 °C.

5.2.19.21 3-((4-Methylbenzyl)oxy)benzoic acid 69 (*ANM221)*[205]



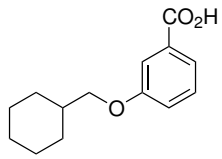
The compound was synthesized according to GP-9b using **68** (768 mg, 3.0 mmol), and was isolated as a white solid (687 mg, 95% yield). ^{1}H NMR: δ 2.29 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 7.18 (d, J = 7.9 Hz, 2H, 2'-H, 6'-H), 7.23 (ddd, J = 1.4, 2.7, 8.3 Hz, 1H, 4-H), 7.31-7.35 (m, 2H, 5'-H, 3'-H), 7.39 (t, J = 7.9 Hz, 1H, 5-H), 7.48-7.53 (m, 2H, 2-H, 6-H), 12.93 (s, 1H, OH). ^{13}C NMR: δ 20.9 (CH_3), 69.4 (CH_2), 115.1 (C-2), 119.8 (C-6), 121.8 (C-4), 127.8 (C-2', C-6'), 129.1 (C-3', C-5'), 129.8 (C-5), 132.3 (C-1'), 133.9 (C-1), 137.2 (C-4'), 158.4 (C-3), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 243 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 149-150 °C.

5.2.19.22 3-((5-Cyclohexylpentyl)oxy)benzoic acid 102 (*ANM240)*



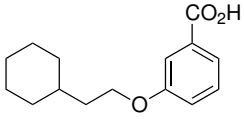
The compound was synthesized according to GP-9b using **45** (912 mg, 3.0 mmol), and was isolated as a white solid (843 mg, 97% yield). ^{1}H NMR: δ 0.80-0.86 (m, 2H, Cyclohexyl), 1.03-1.25 (m, 6H, 5'-H, Cyclohexyl), 1.25-1.44 (m, 4H, 3'-H, 4'-H), 1.47-1.82 (m, 7H, 2'-H, Cyclohexyl), 3.99 (t, J = 6.5 Hz, 2H, 1'-H), 7.15 (ddd, J = 8.2, 2.6, 1H, 1.0 Hz, 4-H), 7.35-7.42 (m, 2H, 2-H, 5-H), 7.47-7.56 (m, 1H, 6-H), 12.92 (s, 1H, OH). ^{13}C NMR: δ 25.9 (C-4', C-5'), 26.0 (C-3", C-5"), 26.4 (C-4"), 28.7 (C-3'), 33.0 (C-2", C-6"), 37.0 (C-2'), 37.0 (C-1"), 67.8 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.5 (C-6), 129.8 (C-5), 132.3 (C-1), 158.8 (C-4), 167.2 ($\underline{C}O_2Me$). LC-MS (m/z): positive mode 291 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.1%. mp 93-94 °C.

5.2.19.23 3-(Cyclohexylmethoxy)benzoic acid 95 (*ANM253)*[212]



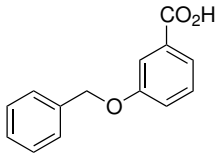
The compound was synthesized according to GP-9b using **94** (911 mg, 3.7 mmol), and was isolated as a white solid (788 mg, 91% yield). ^{1}H NMR: δ 1.00-1.08 (m, 2H, Cyclohexyl), 1.11-1.29 (m, 3H, Cyclohexyl), 1.60-1.84 (m, 7H, Cyclohexyl), 3.80 (d, J = 6.3 Hz, 2H, C1?H), 7.16 (ddd, J = 8.2, 2.7, 1.0 Hz, 1H, 4-H), 7.34-7.42 (m, 2H, 2-H, 5-H), 7.50 (dt, J = 7.7, 1.2 Hz, 1H, 6-H), 12.92 (s, 1H, OH).^{13}C NMR: δ 25.3 (C-3", C-5"), 26.1 (C-4"), 29.3 (C-2", C-6"), 37.1 (C-1"), 73.0 (C-1'), 114.5 (C-2), 119.4 (C-4), 121.5 (C-6), 129.7 (C-5), 132.2 (C-1), 158.9 (C-4), 167.2 (CO_2H). LC-MS (m/z): positive mode 235 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 123-124 °C.

5.2.19.24 3-(2-Cyclohexylethoxy)benzoic acid 97 (*ANM254*)[196]



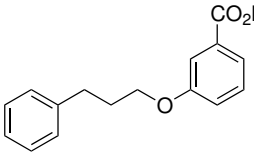
The compound was synthesized according to GP-9b using **96** (1.80 g, 7.0 mmol), and was isolated as a white solid (1.42 g, 82% yield). ^{1}H NMR: δ 0.89-1.00 (m, 2H, Cyclohexyl), 1.07-1.25 (m, 2H, Cyclohexyl), 1.44-1.48 (m, 2H, Cyclohexyl), 1.55-1.75 (m, 7H, 2'-H, Cyclohexyl), 4.03 (t, J = 6.6 Hz, 2H, 1'-H), 7.16 (ddd, J = 8.3, 2.7, 1.0 Hz, 1H, 4-H), 7.35-7.43 (m, 2H, 2-H, 5-H), 7.49-7.54 (m, 1H, 6-H), 12.92 (s, 1H, OH). ^{13}C NMR: δ 25.8 (C-3", C-5"), 26.1 (C-4"), 32.8 (C-2", C-6"), 34.1 (C-1"), 36.0 (C-2'), 65.9 (C-1'), 114.6 (C-2), 119.4 (C-4), 121.5 (C-6), 129.7 (C-5), 132.2 (C-1), 158.7 (C-4), 167.18 (CO_2H). LC-MS (m/z): positive mode 249 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.6%. lit. mp 119-121 °C.

5.2.19.25 3-(Benzyloxy)benzoic acid 71 (*ANM255*)[196]



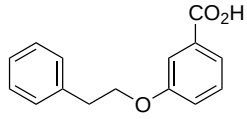
The compound was synthesized according to GP-9b using **70** (1.70 g, 7.0 mmol), and was isolated as a white solid (1.95 g, 82% yield). ^{1}H NMR: δ 5.16 (s, 2H, 1'-H), 7.25 (ddd, J = 8.2, 2.7, 1.0 Hz, 1H, 4-H), 7.30-7.35 (m, 1H, 4"-H), 7.36-7.43 (m, 3H, 2-H, 3"-H, 5"-H), 7.43-7.47 (m, 2H, 2"-H, 6"-H), 7.50-7.55 (m, 2H, 5-H, 6-H), 12.95 (s, 1H, OH). ^{13}C NMR: δ 69.5 (C-1'), 115.0 (C-2), 119.8 (C-4), 121.9 (C-6), 127.7 (C-2", C-6"), 128.0 (C-4"), 128.5 (C-3", C-5"), 129.8 (C-5), 132.3 (C-1), 136.9 (C-1"), 158.4 (C-4), 167.15 (CO_2H). LC-MS (m/z): positive mode 229 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 136-138 °C.

5.2.19.26 3-(3-Phenylpropoxy)benzoic acid 83 (*ANM257*)[196]



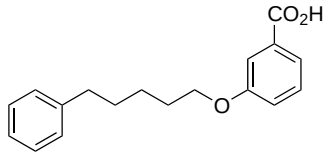
The compound was synthesized according to GP-9b using **82** (1.62 g, 6.0 mmol), and was isolated as a white solid (1.29 g, 84% yield). ^{1}H NMR: δ 1.81-2.21 (m, 2H, 2'-H), 2.72-2.76 (m, 2H, 3'-H), 4.00 (t, J = 6.3 Hz, 2H, 1'-H), 7.15-7.19 (m, 2H, 4-H, 4"-H), 7.20-7.24 (m, 2H, 2"-H, 6"-H), 7.27 (t, J = 7.5 Hz, 2H, 3"-H, 5"-H), 7.39 (t, J = 7.9 Hz, 1H, 5-H), 7.42 (dd, J = 2.7, 1.2 Hz, 1H, 2-H), 7.49-7.53 (m, 1H, 6-H). ^{13}C NMR: δ 30.4 (C-2'), 31.5 (C-3'), 67.0 (C-1'), 114.6 (C-2), 119.4 (C-4), 121.6 (C-6), 125.9 (C-4"), 128.4 (C-2", C-3", C-5", C-6"), 129.8 (C-5), 132.3 (C-1), 141.4 (C-1"), 158.7 (C-4), 167.2 (CO_2H). LC-MS (m/z): positive mode 257 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. lit. mp 118-120 °C.

5.2.19.27 3-Phenethoxybenzoic acid 81 (*ANM258)*[196]



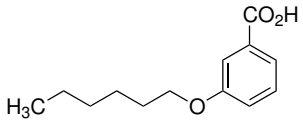
The compound was synthesized according to GP-9b using **80** (1.28 g, 5.0 mmol), and was isolated as a white solid (1.10 g, 90% yield). ^{1}H NMR: δ 3.04 (t, J = 6.8 Hz, 2H, 2'-H), 4.23 (t, J = 6.8 Hz, 2H, 1'-H), 7.15-7.19 (m, 1H, 4-H), 7.19-7.24 (m, 1H, 4'-H), 7.27-7.34 (m, 4H, 2"-H, 3"-H, 5"-H, 6"-H), 7.38 (t, J = 7.9 Hz, 1H, 5-H), 7.42 (dd, J = 1.5, 2.7 Hz, 2-H), 7.49-7.54 (m, 1H, 6-H). ^{13}C NMR: δ 35.0 (C-2'), 68.5 (C-1'), 114.8 (C-2), 119.4 (C-4), 121.7 (C-6), 126.4 (C-4"), 128.4 (C-2", C-6"), 129.1 (C-3", C-5"), 129.9 (C-5), 132.3 (C-1), 138.4 (C-1"), 158.5 (C-4), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 243 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. lit. mp 112-113 °C.

5.2.19.28 3-((5-Phenylpentyl)oxy)benzoic acid 87 (*ANM273)*



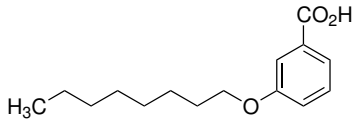
The compound was synthesized according to GP-9b using **86** (2.10 g, 7.0 mmol), and was isolated as a white solid (1.70 g, 86% yield). ^{1}H NMR: δ 1.40-1.46 (m, 2H, 3'-H), 1.60-1.64 (m, 2H, 4'-H), 1.70-1.76 (m, 2H, 2'-H), 2.54-2.62 (m, 2H, 5'-H), 3.99 (t, J = 6.4 Hz, 2H, 1'-H), 7.17-7.20 (m, 4H, 2"-H, 4"-H, 6"-H, 4-H), 7.25 (t, J = 7.6 Hz, 2H, 3"-H, 5"-H), 7.35-7.42 (m, 2H, 2-H, 5-H), 7.50 (d, J = 7.5 Hz, 1H, 6-H), 12.93 (s, 1H, OH). ^{13}C NMR: δ 25.2 (C-3'), 28.6 (C-2'), 30.8 (C-5'), 35.2 (C-4'), 67.7 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.5 (C-4), 125.7 (C-4"), 128.3-128.4 (C-2", C-3", C-5", C-6"), 129.8 (C-5), 132.3 (C-1), 142.3 (C-1"), 158.8 (C-4), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 285 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 89-92 °C.

5.2.19.29 3-(Hexyloxy)benzoic acid 89 (*ANM325)*[196]



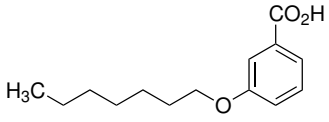
The compound was synthesized according to GP-9b using **88** (1.06 g, 4.05 mmol), and was isolated as a white solid (725 mg, 73% yield). ^{1}H NMR: δ 0.81-0.91 (m, 2H, 6'-H), 1.25-1.32 (m, 4H, 4'-H, 5'-H), 1.38-1.45 (m, 2H, 3'-H), 1.64-1.76 (m, 2H, 2'-H), 3.99 (t, J = 6.5 Hz, 2H, 1'-H), 7.13-7.18 (m, 1H, 4-H), 7.35-7.42 (m, 2H, 2-H, 6-H), 7.47-7.53 (m, 1H, 5-H), 12.92 (s, 1H, OH). ^{13}C NMR: δ 14.0 (C-6'), 22.2 (C-5'), 25.3 (C-4'), 28.7 (C-3'), 31.1 (C-2'), 67.8 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.6 (C-6), 129.8 (C-5), 132.3 (C-1), 158.8 (C-3), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 223 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. lit. mp 74-75 °C.

5.2.19.30 3-(Octyloxy)benzoic acid 93 (*ANM326*)[196]



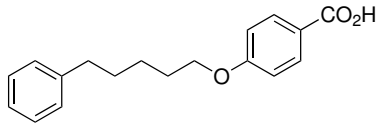
The compound was synthesized according to GP-9b using **92** (1.06 g, 4.00 mmol), and was isolated as a white solid (1.09 g, 72% yield). ^{1}H NMR: δ 0.85-0.90 (m, 3H, 6'-H), 1.28-1.40 (m, 6H, 5'-H, 4'-H, 3'-H), 1.68-1.72 (m, 2H, C2?H); 3.98-4.05 (m, 2H, C1?H); 7.15-7.16 (m, 1H, C4H); 7.36-7.51 (m, 3H, C2H, C5H, C6H); 12.92 (s, 1H, OH). ^{13}C NMR: δ 14.1 (CH, C-8'), 22.2 (C-7'), 25.6 (C-6'), 28.7 (C-3'), 28.8 (C-4'), 28.8 (C-3'), 31.3 (C-2'), 67.8 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.5 (C-6), 129.8 (C-5), 132.3 (C-1), 158.8 (C-3), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 251 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. lit. mp 76-79 °C.

5.2.19.31 3-(Heptyloxy)benzoic acid 91 (*ANM328*)[196]



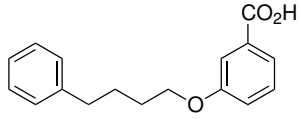
The compound was synthesized according to GP-9b using **90** (751 mg, 3.00 mmol), and was isolated as a white solid (879 mg, 84% yield). ^{1}H NMR: δ 0.80-0.90 (m, 3H, 7'-H), 1.20-1.45 (m, 8H, 6'-H, 5'-H, 4'-H, 3'-H), 1.65-1.75 (m, 2H, 2'-H), 3.95-4.05 (m, 2H, 1'-H), 7.10-7.20 (m, 1H, 4-H), 7.30-7.55 (m, 2H, 2-H, 6-H), 7.50-7.53 (m, 1H, 5-H), 12.7 (s, 1H, OH). ^{13}C NMR: δ 14.1 (C-7'), 22.2 (C-6'), 25.6 (C-5'), 28.5 (C-4'), 28.7 (C-3'), 31.4 (C-2'), 67.8 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.5 (C-6), 129.8 (C-5), 132.3 (C-1), 158.8 (C-3), 167.3 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 237 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. lit. mp 81-82 °C.

5.2.19.32 4-((5-Phenylpentyl)oxy)benzoic acid 75 (*THB20*)[32]



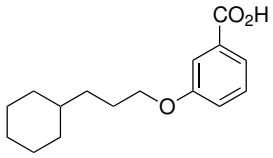
The compound was synthesized according to GP-9b using **74** (706 mg, 2.35 mmol), and was isolated as a white solid (649 mg, 97% yield). ^{1}H NMR: δ 1.42 (p, J = 7.6 Hz, 2H, 3'-H), 1.62 (p, J = 7.6 Hz, 2H, 4'-H), 1.74 (dt, J = 6.6, 14.3 Hz, 2H, 2'-H), 2.41-2.66 (m, 2H, 5'-H), 4.02 (t, J = 6.5 Hz, 2H, 1'-H), 6.89-7.03 (m, 2H, 3-H, 5-H), 7.07-7.32 (m, 5H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H), 7.77-7.95 (m, 2H, 2-H, 6-H). ^{13}C NMR: δ 25.3 (C-2'), 28.5 (C-3'), 30.8 (C-4'), 35.2 (C-5'), 67.9 (C-1'), 114.3 (C-3, C-5), 123.1 (C-1), 125.7 (C-4'), 128.3 (C-3", C-5"), 128.4 (C-2", C-6"), 131.4 (C-2, C-6), 142.3 (C-1"), 162.4 (C-4), 167.1 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 285 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. lit. mp 151-152 °C.

5.2.19.33 3-(4-Phenylbutoxy)benzoic acid 85 (*THB33)*[209]



The compound was synthesized according to GP-9b using **84** (816 mg, 2.86 mmol), and was isolated as a white solid (185 mg, 23% yield). ^{1}H NMR: δ 1.68-1.75 (m, 4H, 2'-H, 3'-H), 2.49 (p, J = 1.8 Hz, 2H, 4'-H), 3.99-4.05 (m, 2H, 1'-H), 7.12-7.30 (m, 5H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H), 7.34-7.44 (m, 2H, 2-H, 6-H), 7.50 (dt, J = 1.1, 7.6 Hz, 2H, 5-H, 6-H), 12.91 (s, 1H, OH). ^{13}C NMR: δ 27.5 (C-3'), 28.3 (C-2'), 34.9 (C-4'), 67.6 (C-1'), 114.6 (C-2), 119.5 (C-4), 121.6 (C-6), 125.8 (C-4'), 128.4 (3"-H, 5"-H), 128.4 (2"-H, 6"-H), 129.8 (5'-H), 132.3 (C-1), 142.1 (C-1"), 158.8 (C-3), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 271 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.0%. lit. mp 78-79 °C.

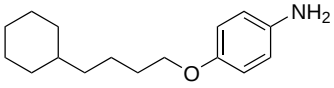
5.2.19.34 3-(3-Cyclohexylpropoxy)benzoic acid 99 (*THB60)*



The compound was synthesized according to GP-9b using **98** (723 mg, 2.60 mmol), and was isolated as a white solid (641 mg, 94% yield). ^{1}H NMR: δ 0.81-0.92 (m, 2H, 3'-H), 1.05-1.33 (m, 8H, 2"-H, 3"-H, 5"-H, 6"-H), 1.57-1.76 (m, 5H, 2'-H, 1"-H, 4"-H), 3.97 (t, J = 6.5 Hz, 2H, 1'-H), 7.15 (ddd, J = 1.0, 2.7, 8.2 Hz, 1H, 4-H), 7.34-7.43 (m, 2H, 5-H, 6-H), 7.50 (dt, J = 1.2, 7.6 Hz, 1H, 2-H). ^{13}C NMR: δ 25.9 (C-3", C-5"), 26.1 (C-4"), 26.3 (C-2'), 32.9 (C-2", C-6"), 33.3 (C-3'), 36.9 (C-1"), 68.1 (C-1'), 114.6 (C-5), 119.4 (C-6), 121.5 (C-4), 129.8 (C-2), 132.3 (C-1), 158.8 (C-3), 167.2 ($\underline{C}O_2H$). LC-MS (m/z): positive mode 263 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.0%. mp 113-114 °C.

5.2.20 4-(4-Cyclohexylbutoxy)aniline

5.2.20.1 4-(4-Cyclohexylbutoxy)aniline 187 (*ANM180)*



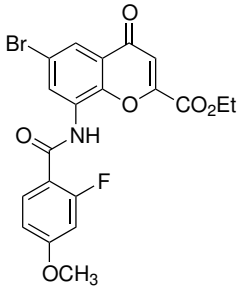
Compound **186** (555 mg, 2.00 mmol) was hydrogenated (45 psi) with Pd/C 5% (150 mg) in 10 mL of MeOH for 1 h at rt, filtrated over Celite® and isolated as a colourless oil (403 mg, 82% yield). The compound is unstable and therefore was further reacted without further purification and characterization.

5.2.21 Ethyl 8-benzamido-4-oxo-4*H*-chromene-2-carboxylates

5.2.21.1 General procedure for the synthesis of the ethyl 8-benzamido-4-oxo-4*H*-chromene-2-carboxylates (GP-10)

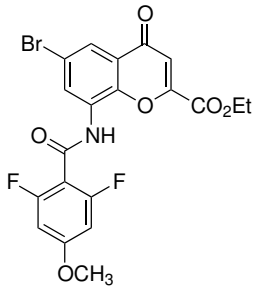
To form the acid chloride, the appropriate acid (1.2 mmol) and three drops of anhydrous DMF were dissolved in DCM (5 mL). Then 1 mL of freshly distilled thionyl chloride was added and the reaction mixture was stirred under an argon atmosphere at rt for 30 min. The DCM and excess of thionyl chloride were distilled off under reduced pressure using a glass filter pump. Cooling by an ice-bath induced the crystallization of the desired acid chloride. In the meantime, the appropriate ethyl 8-amino-4-oxo-4H-chromene-2-carboxylate (0.9 mmol) and DIPEA (0.20 mL, 1.2 mmol) were dissolved in DCM (4.5 mL) and anhydrous THF (1.5 mL). This solution was added to the formed acid chloride, which had been dissolved in 4 mL of DCM. The reaction mixture was then stirred at rt from 1 to 2 days under an argon atmosphere. For work-up conditions, see individual compounds.

5.2.21.2 Ethyl 6-bromo-8-(2-fluoro-4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 104 (*ANM3*)[65]



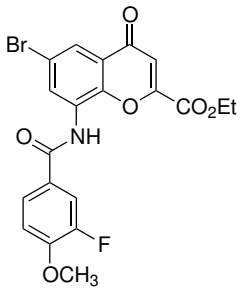
The compound was synthesized according to GP-10 using 2-fluoro-4-methoxybenzoic acid (204 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The product was isolated as a white solid (267 mg, 64% yield). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.88 (s, 3H, OCH_3), 4.41 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (dd, J = 8.9, 2.4 Hz, 1H, 5'-H), 7.04 (s, 1H, 3-H), 7.07 (dd, J = 13.8, 2.3 Hz, 1H, 3'-H), 7.89 (d, J = 2.4 Hz, 1H, 5-H), 7.94 (dd, J = 8.9 Hz, 1H, 6'-H), 8.68 (d, J = 2.4 Hz, 1H, 7-H), 9.82 (s, 1H, CONH). ^{13}C NMR: δ 14.2 ($CH_2\underline{C}H_3$) 56.7 (OCH_3), 63.4 ($\underline{C}H_2CH_3$),102.5 (d, J = 28.6 Hz, C-3'), 111.9 (C-5'), 113.6 (d, J = 13.2 Hz, C-1'), 114.4 (C-3), 118.6 (C-6), 122.3 (C-5), 125.7 (C-4a), 129.3 (C-7), 130.5 (C-8), 133.0 (d, J = 3.3 Hz, C-6'), 146.6 (C-8a), 152.3 (C-2), 160.0 (CO_2Et), 161.7 (d, J = 248.7 Hz, C-2'), 162.0 (C-4'), 164.1 (d, J = 12.1 Hz, CONH), 176.4 (C4). LC-MS (m/z): negative mode 464 $[M-H]^-$, positive mode 464 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 216 °C.

5.2.21.3 Ethyl 6-bromo-8-(2,6-difluoro-4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 105 (*ANM4*)[65]



The compound was synthesized according to GP-10 using 2,6-difluoro-4-methoxybenzoic acid (226 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The filtrate was concentrated under reduced pressure and separated by column chromatography on a column of silica gel (5% EtOAc in DCM), yielding further product. The amide was isolated as a white powder (221 mg, 51% yield). ^{1}H NMR: δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.87 (s, 3H, OCH_3), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.85 (d, J = 10.0 Hz, 2H, 3'-H, 5'-H), 7.00 (s, 1H, 3-H), 7.97 (d, J = 2.4 Hz, 1H, 5-H), 8.33 (d, J = 2.4 Hz, 1H, 7-H), 10.27 (s, 1H, CONH). ^{13}C NMR: δ 14.2 ($CH_2\underline{C}H_3$), 57.0 (OCH_3), 63.3 ($\underline{C}H_2CH_3$), 99.1 (d, J = 28.6 Hz, C-3', C-5'), 106.8-107.1 (m, C-1'), 114.4 (C-3), 118.3 (C- 6), 124.0 (C-5), 126.1 (C-4a), 129.7 (C-8), 132.1 (C-7), 148.1 (C-8a), 152.6 (C-2), 159.3 (C-4'), 160.1 (CO_2Et), 160.0-161.7 (m, C-2', C- 6'), 162.6 (t, J = 14.3 Hz, CONH), 176.4 (C4). LC-MS (m/z): negative mode 482 $[M-H]^-$, positive mode 484 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.6%. mp 263 °C.

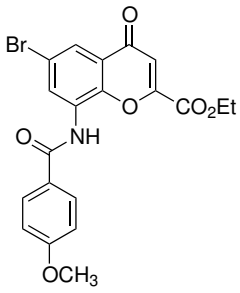
5.2.21.4 Ethyl 6-bromo-8-(3-fluoro-4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 106 (*ANM5*)[65]



The compound was synthesized according to GP-10 using 3-fluoro-4-methoxybenzoic acid (204 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The filtrate was concentrated under reduced pressure and separated by column chromatography on a column of silica gel (2 % EtOAc in DCM), yielding further product. The amide was isolated as a white solid (309 mg, 74% yield). ^{1}H NMR: δ 1.29 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.96 (s, 3H, OCH_3), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.34 (dd, J = 8.5 Hz, 1H, 5'-H), 7.83-7.89 (m, 2H, 2'-H, 6'-H), 7.97 (d, J = 2.4 Hz, 1H, 5-H), 8.32 (d, J = 2.4 Hz, 1H, 7-H), 10.05 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 56.4 (OCH_3), 62.6 ($\underline{C}H_2CH_3$), 113.6 (C-3 or C-6'), 113.7 (C-3 or C-6'), 115.1 (d, J = 19.2 Hz, C-2'), 117.6 (C-6), 123.0 (C-5), 124.8

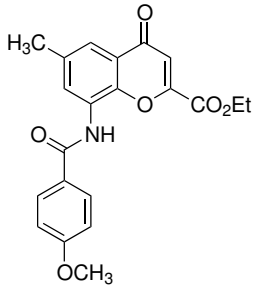
(d, J = 2.7 Hz, C-5'), 125.3 (C-4a), 126.0 (d, J = 5.5 Hz, C-1'), 130.0 (C-8), 131.8 (C-7), 147.9 (C-8a), 150.5 (d, J = 10.1 Hz, C-4'), 151.0 (d, J = 245.6 Hz, C-3'), 151.8 (C- 2), 159.4 (CO_2Et), 163.7 (CONH), 175.7 (C4). LC-MS (m/z): negative mode 461 $[M-H]^-$, positive mode 466 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.2%. mp 251 °C.

5.2.21.5 Ethyl 6-bromo-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 103 (*ANM28*)[95]



The compound was synthesized according to GP-10 using 4-methoxybenzoic acid (409 mg, 2.4 mmol) and **17** (562 mg, 1.8 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The filtrate was concentrated under reduced pressure and separated by column chromatography on a column of silica gel (2 % EtOAc in DCM), yielding further product. The amide was isolated as a white solid (832 mg, 78% yield). ^{1}H NMR: δ 1.26 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.86 (s, 3H, OCH_3), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.09-7.13 (m, 2H, 3'-H, 5'-H), 7.95 (d, J = 2.4 Hz, 1H, 5-H), 7.98-8.03 (m, 2H, 2'-H, 6'-H), 8.33 (d, J = 2.4 Hz, 1H, 7-H), 10.18 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 55.7 (OCH_3), 63.0 ($\underline{C}H_2CH_3$), 114.0 (C-3', C-5'), 118.1 (C-6), 123.0 (C-5), 125.6 (C-4a), 125.8 (C-1'), 129.9 (C-2', C-6'), 130.5 (C-8), 132.2 (C-7), 148.2 (C-8a), 152.0 (C-2), 159.8 (CO_2Et), 162.6 (C-4'), 164.9 (CONH), 176.2 (C4). LC-MS (m/z): negative mode 446 $[M-H]^-$, positive mode 448 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 251-252 °C.

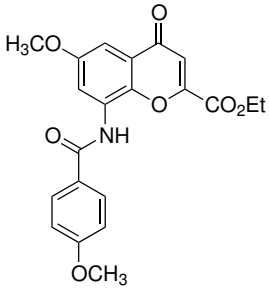
5.2.21.6 Ethyl 8-(4-methoxybenzamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylate 107 (*ANM33*)[65]



The compound was synthesized according to GP-10 using **23** (226 mg, 0.9 mmol) and 4-methoxybenzoylchloride (204 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The product was isolated as a white powder (278 mg, 81% yield). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.46 (s, 3H, Ar-CH_3), 3.87 (s,

3H, OCH_3), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.93 (s, 1H, 3-H), 7.07-7.10 (m, 2H, 3'-H, 5'-H), 7.68-7.69 (m, 1H, 5-H), 7.98 (d, J = 2.1 Hz, 1H, 7-H), 8.00-8.03 (m, 2H, 2'-H, 6'-H), 9.85 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 20.5 (Ar-CH_3), 55.4 (OCH_3), 62.4 ($\underline{C}H_2CH_3$), 113.3 (C-3), 113.7 (C-3', C-5'), 120.2 (C-5), 123.9 (C-4a), 126.1 (C-1'), 128.2 (C-8), 129.4 (C-2', C-6'), 130.9 (C-7), 135.3 (C-6), 147.2 (C-8a), 151.5 (C-2), 159.7 (CO_2Et), 162.1 (C-4'), 164.7 (CONH), 176.9 (C4). LC-MS (m/z): negative mode 380 $[M-H]^-$, positive mode 382 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.2%. mp 219 °C.

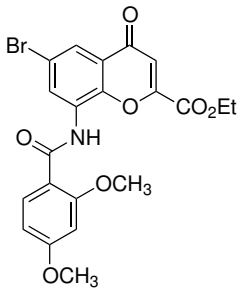
5.2.21.7 Ethyl 6-methoxy-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 108 (*ANM39*)[65]



The compound was synthesized according to GP-10 using **24** (237 mg, 0.9 mmol) and 4-methoxybenzoylchloride (204 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The product was isolated as a white solid (304 mg, 85% yield). ^{1}H NMR: δ 1.30 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.87 (s, 3H, C6-OCH_3 or 4'-OCH_3), 3.90 (s, 3H, C6-OCH_3 or 4'-OCH_3), 4.36 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.93 (s, 1H, 3-H), 7.09 (d, J = 8.8 Hz, 2H, 3'-H, 5'-H), 7.27 (d, J = 3.1 Hz, 1H, 5-H), 7.85 (d, J = 3.0 Hz, 1H, 7-H), 8.00 (d, J = 9.1 Hz, 2H, 2'-H, 6'-H), 9.76 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 55.4 (C6-$O\underline{C}H_3$ or C4'-$O\underline{C}H_3$), 55.8 (C6-$O\underline{C}H_3$ or C4'-$O\underline{C}H_3$), 62.4 ($\underline{C}H_2CH_3$), 101.1 (C-5), 112.5 (C-3), 113.8 (C-3', C-5'), 118.0 (C-7), 124.7 (C-4a), 126.0 (C-1'), 129.3 (C-2', C-6'), 129.7 (C-8), 143.5 (C-8a), 151.3 (C-2), 156.6 (C-6), 159.7 (CO_2Et), 162.3 (C-4'), 164.6 (CONH), 176.5 (C4). LC-MS (m/z): negative mode 396 $[M-H]^-$, positive mode 398 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.9%. mp 217-218 °C.

5.2.21.8 Ethyl 6-bromo-8-(2,4-dimethoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 109

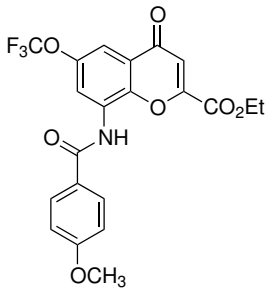
(*ANM43*)



The compound was synthesized according to GP-10 using **17** (281 mg, 0.9 mmol) and 2,4-dimethoxybenzoyl chloride (240 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each, and dried in vacuum at 50 °C. The product was isolated as a white solid (257 mg, 69% yield). ^{1}H NMR: δ 1.37 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.90 (s, 3H, C4'-$OC\underline{H}_3$), 4.18 (s, 3H, C2'$OC\underline{H}_3$), 4.46 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.68 (s, 1H, CONH), 6.77 (dd, J = 8.8, 2.3 Hz, 1H, 5'-H), 6.82 (d, J = 2.2 Hz, 1H, 3'-H), 7.03 (s, 1H, 3-H), 7.82 (d, J = 2.5 Hz, 1H, 5-H), 8.08 (d, J = 8.8 Hz, 1H, 6'-H), 9.08 (d, J = 2.4 Hz, 1H, 7-H), 10.56 (s, 1H, OH). ^{13}C NMR: δ 13.6 ($CH_2\underline{C}H_3$), 55.6 (C2'-$O\underline{C}H_3$), 56.9 (C4'-$O\underline{C}H_3$), 62.7 ($\underline{C}H_2CH_3$), 99.3 (C-3'), 107.0 (C-5'), 112.8 (C-6), 113.9 (C-3), 118.2 (C-1'), 120.2 (C-7), 125.0 (C-4a), 126.4 (C-5), 130.4 (C-8), 133.3 (C-6'), 144.7 (C-8a), 152.9 (C-2), 159.2 (C-2'), 160.7 (CO_2Et), 162.9 (C-4'), 164.3 (CONH), 175.8 (C-4). LC-MS (m/z): positive mode 450 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 284-285 °C.

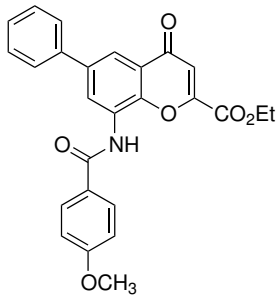
5.2.21.9 Ethyl 8-(4-methoxybenzamido)-4-oxo-6-(trifluoromethoxy)-4*H*-chromene-2-carboxylate 110

(*ANM62*)



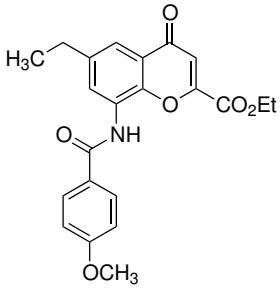
The compound was synthesized according to GP-10 using **18** (206 mg, 0.65 mmol) and 4-methoxybenzoyl chloride (205 mg, 1.2 mmol) and was isolated as a white solid (50 mg, 17 % yield). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.86 (s, 3H, OCH_3), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.98-7.02 (m, 2H, 3'-H, 5'-H), 7.04 (s, 1H, 3-H), 7.70-7.74 (m, 1H, 5-H), 7.86-7.90 (m, 2H, 2'-H, 6'-H), 8.21-9.25 (m, 1H, 7-H), 10.20 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 55.6 (OCH_3), 63.0 ($\underline{C}H_2CH_3$), 111.8 (C-5), 113.9 (C-3', C-5'), 114.1 (C-3), 119.2-121.2 (OCF_3), 122.3 (C-7), 124.9 (C-4a), 125.6 (C-1'), 131.0 (C-8), 131.5 (C-2', C-6'), 145.1 (C-8a), 147.3 (C-6), 152.1 (C-2), 159.7 (CO_2Et), 164.9 (C-4'), 167.1 (CONH), 176.6 (C-4). LC-MS (m/z): positive mode 452 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 176-177 °C.

5.2.21.10 Ethyl 8-(4-methoxybenzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 111 (*ANM74*)



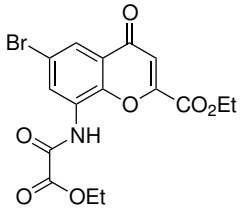
The compound was synthesized according to GP-10 using **26** (185 mg, 0.60 mmol) and 4-methoxybenzoyl chloride (135 mg, 0.8 mmol) and was isolated as a white solid (252 mg, 95% yield). ^{1}H NMR: δ 1.31 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.88 (s, 3H, OCH_3), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.09-7.13 (m, 2H, 3'-H, 5'-H), 7.43-7.48 (m, 1H, 4"-H), 7.51-7.55 (m, 2H, 3"-H, 5"-H), 7.72-7.76 (m, 2H, 2"-H, 6"-H), 8.01-8.05 (m, 2H, 2'-H, 6'-H), 8.07 (d, J = 2.3 Hz, 1H, 5-H), 8.48 (d, J = 2.3 Hz, 1H, 7-H), 9.89 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 55.4 (OCH_3), 62.5 ($\underline{C}H_2CH_3$), 113.4 (C-3), 113.8 (C-3', C-5'), 117.9 (C-5), 124.4 (C-4a), 126.0 (C-1'), 126.7 (C-2", C-6"), 127.9 (C-4"), 128.0 (C-7), 128.1 (C-8), 128.1 (C-3", C-5"), 129.4 (C-2', C-6'), 137.7 (C-6), 138.2 (C-1"), 148.1 (C-8a), 151.6 (C-2), 159.6 (C-4'), 162.3 (CO_2Et), 164.7 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 444 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 237-238 °C.

5.2.21.11 Ethyl 6-ethyl-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 112 (*ANM92*)



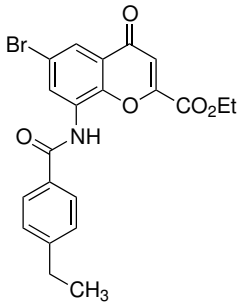
The compound was synthesized according to GP-10 using **20** (234 mg, 0.9 mmol) and 4-methoxybenzoyl chloride (205 mg, 1.2 mmol) and was isolated as a white solid (321 mg, 90% yield). ^{1}H NMR: δ 1.22-1.26 (m, 6H, $CO_2CH_2C\underline{H}_3$, C6-$CH_2C\underline{H}_3$), 2.76 (q, J = 7.6 Hz, 2H, C6-$C\underline{H}_2CH_3$), 3.85 (s, 3H, OCH_3), 4.32 (q, J = 7.1 Hz, 2H, CO_2-$C\underline{H}_2CH_3$), 6.95 (s, 1H, 3-H), 7.07-7.11 (m, 2H, 3'-H, 5'-H), 7.71 (d, J = 2.0 Hz, 1H, 5-H), 7.97 (d, J = 2.2 Hz, 1H, 7-H), 7.98-8.02 (m, 2H, 2'-H, 6'-H), 10.03 (s, 1H, CONH). ^{13}C NMR: δ 13.8 (CO_2-$CH_2\underline{C}H_3$), 15.4 (C6-$CH_2\underline{C}H_3$), 27.8 (C6-$\underline{C}H_2CH_3$), 55.6 (OCH_3), 62.8 (CO_2-$\underline{C}H_2CH_3$), 113.6 (C-3), 113.9 (C-3', C-5'), 119.5 (C-5), 124.2 (C-4a), 126.1 (C-1'), 128.5 (C-8), 129.7 (C-2', C-6'), 130.8 (C-7), 141.8 (C-6), 147.8 (C-8a), 151.7 (C-2), 160.0 (CO_2Et), 162.4 (C-4'), 164.9 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 369 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.9%. mp 168-170 °C.

5.2.21.12 Ethyl 6-bromo-8-(2-ethoxy-2-oxoacetamido)-4-oxo-4*H*-chromene-2-carboxylate 120 (*ANM181)*



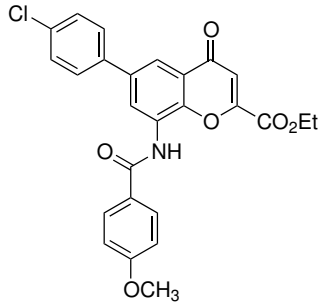
The compound was synthesized according to GP-10 using **17** (280 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (271 mg, 73% yield). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 6H, $CH_2C\underline{H}_3$), 4.38 (dq, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.02 (s, 1H, 3-H), 7.94 (d, J = 2.4 Hz, 1H, 5-H), 8.38 (d, J = 2.5 Hz, 1H, 7-H), 10.39 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.0-63.2 ($\underline{C}H_2CH_3$), 114.04 (C-3), 118.1 (C-6), 123.6 (C-7), 125.5 (C-4a), 128.6 (C-8), 129.9 (C-5), 146.8 (C-8a), 151.8 (C2), 155.3 (CONH), 159.6-159.7 (CO_2Et), 174.9 (C-4). LC-MS (m/z): positive mode 415 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.7%. mp 172-173 °C.

5.2.21.13 Ethyl 6-bromo-8-(4-ethylbenzamido)-4-oxo-4*H*-chromene-2-carboxylate 113 (*ANM186)*



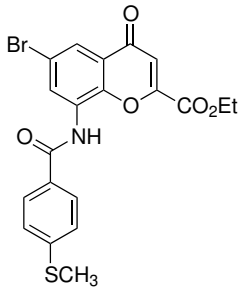
The compound was synthesized according to GP-10 using **17** (280 mg, 0.9 mmol) and 4-ethylbenzoyl chloride (0.18 mL, 1.2 mmol) and was isolated as a white solid (363 mg, 91% yield). ^{1}H NMR: δ 1.26 (t, J = 7.1 Hz, 3H, C4'-$CH_2C\underline{H}_3$), 1.31 (t, J = 7.1 Hz, 3H, CO_2-$CH_2C\underline{H}_3$), 2.75 (q, emphJ = 7.6 Hz, 2H, C4'-$C\underline{H}_2CH_3$), 4.39 (q, J = 7.1 Hz, 2H, CO_2-$C\underline{H}_2CH_3$), 7.02 (s, 1H, 3-H), 7.41-7.45 (m, 2H, 3'-H, 5'-H), 7.95-7.99 (m, 3H, 5-H, 2'-H, 6'-H), 8.40 (dd, J = 2.4 Hz, 1H, 7-H), 9.99 (s, 1H, CONH). ^{13}C NMR: δ 13.4 (CO_2-$CH_2\underline{C}H_3$), 14.8 (C4'-$CH_2\underline{C}H_3$), 27.9 (C4'-$\underline{C}H_2CH_3$), 62.6 (CO_2-$\underline{C}H_2CH_3$), 113.5 (C-3), 117.7 (C-6), 122.6 (C-5), 125.2 (C-4a), 127.6-127.7 (C-2', C-3', C-5', C-6'), 130.2 (C-8), 130.9 (C-1'), 131.1 (C-7), 147.6 (C-4'), 148.5 (C-8a), 151.8 (C-2), 159.4 (CO_2Et), 165.1 (CONH), 175.7 (C-4). LC-MS (m/z): positive mode 430 $[M+H]^+$ (methylester). Purity by HPLC-UV (254 nm)-ESI-MS: 95.9%. mp 208-210 °C.

5.2.21.14 Ethyl 6-(4-chlorophenyl)-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylate 114 (*ANM195)*



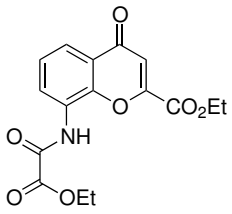
The compound was synthesized according to GP-10 using **27** (152 mg, 0.44 mmol) and 4-methoxybenzoyl chloride (100 mg, 0.59 mmol) and was isolated as a white solid (181 mg, 86% yield). ^{1}H NMR: δ 1.30 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.88 (s, 3H, OCH_3), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.01 (s, 2H, 3-H), 7.09-7.13 (m, 2H, 3'-H, 5'-H), 7.56 (d, J = 8.6 Hz, 2H, 3"-H, 5"-H), 7.78 (d, J = 8.6 Hz, 2H, 2"-H, 6"-H), 8.01-8.05 (m, 2H, 2'-H, 6'-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 8.47 (d, J = 2.3 Hz, 1H, 7-H), 9.92 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 55.4 (OCH_3), 62.5 ($\underline{C}H_2CH_3$), 113.4 (C-3), 113.7 (C-3', C-5'), 118.0 (C-5), 124.4 (C-4a), 126.0 (C-1"), 127.8 (C-7), 128.5 (C-2', C-6'), 128.9 (C-3", C-5"), 129.2 (C-8), 129.4 (C-2", C-6"), 136.2 (C-6), 137.0 (C-1'), 148.3 (C-8a), 151.6 (C-2), 159.6 (CO_2Et), 162.3 (C-4"), 164.7 (CONH), 176.8 (C-4). LC-MS (m/z): positive mode 478 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.4%. mp 250-251 °C.

5.2.21.15 Ethyl 6-bromo-8-(4-(methylthio)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 115 (*THB8)*



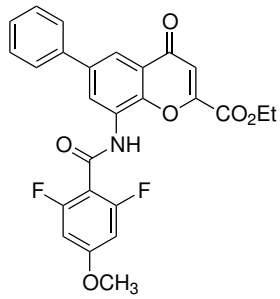
The compound was synthesized according to GP-10 using **17** (280 mg, 0.9 mmol) and 4-(methylthio)benzoyl chloride (224 mg, 1.2 mmol) and was isolated as a white solid (209 mg, 50% yield). ^{1}H NMR: δ 1.25 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.55 (s, 3H, S-CH_3), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.02 (s, 1H, 3-H), 7.43 (d, J = 8.1 Hz, 2H, 3'-H, 5'-H), 7.96 (d, J = 8.1 Hz, 3H, 5-H, 2'-H, 6'-H), 8.31 (s, 1H, 7-H), 10.29 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 14.3 (S-CH_3), 63.0 ($\underline{C}H_2CH_3$), 113.9 (CH, C-3', C-5'), 118.1 (C-6), 123.4 (C-5), 125.2 (C-4a), 125.6 (C-1'), 128.4 (C-2', C-6'), 129.5 (C-8), 130.4 (C-7), 132.5 (C-8a), 144.4 (C-2), 148.4 (C-4'), 152.07 (CO_2Et), 159.78 (CONH), 176.21 (C-4). LC-MS (m/z): positive mode 464 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.9%. mp 231-232 °C.

5.2.21.16 Ethyl 8-(2-ethoxy-2-oxoacetamido)-4-oxo-4*H*-chromene-2-carboxylate 121 (*THB11)*



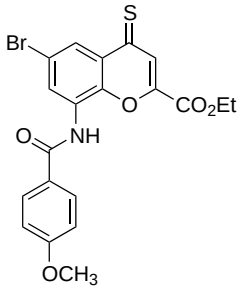
The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (299 mg, 99% yield). ^{1}H NMR: δ 1.30 (dt, J = 7.1, 11.1 Hz, 6H, $CH_2C\underline{H}_3$), 4.37 (dq, J = 7.1, 11.9 Hz, 4H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.55 (t, J = 7.9 Hz, 1H, 6-H), 7.89 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.20 (dd, J = 1.5, 7.8 Hz, 1H, 7-H), 10.40 (s, 1H, CONH). ^{13}C NMR: δ 13.8-13.9 ($CH_2\underline{C}H_3$), 62.9-63.0 ($\underline{C}H_2CH_3$), 113.9 (C-3), 121.8 (C-5), 124.3 (C-4a), 126.0 (C-7), 126.7 (C-8), 128.5 (C-6), 147.9 (C-8a), 151.7 (C-2), 155.3 (C-1'), 159.8 (CONH), 160.0 (CO_2Et). 177.09 (C-4). LC-MS (m/z): positive mode 334 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.6%. mp 174-175 °C.

5.2.21.17 Ethyl 8-(2,6-difluoro-4-methoxybenzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 116 (*THB25)*



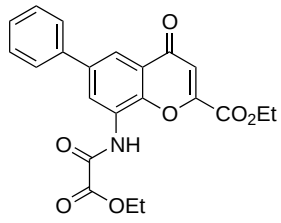
The compound was synthesized according to GP-10 using **26** (278 mg, 0.9 mmol) and 2,6-difluoro-4-methoxybenzoic acid (226 mg, 1.2 mmol) and was isolated as a white solid (236 mg, 55% yield). ^{1}H NMR: δ 1.35 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.87 (s, 3H, O-CH_3), 4.41 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.85 (d, J = 10.0 Hz, 2H, 3"-H, 5"-H), 7.00 (s, 1H, 3-H), 7.41?7.48 (m, 1H, 4'-H), 7.53 (t, J = 7.6 Hz, 2H, 3'-H, 5'-H), 7.7 (d, J = 7.9 Hz, 2H, 2'-H, 6'-H), 8.10 (d, J = 2.1 Hz, 1H, 7-H), 8.39 (s, 1H, 5-H), 10.26 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 56.3 (O-CH_3), 62.5 ($\underline{C}H_2CH_3$), 98.5-98.7 (C-3', C-5'), 106.8 (C-1'), 113.6 (C-3), 118.6 (C-5), 124.6 (C-4a), 126.7 (C-2", C-6"), 127.8 (C-7), 128.0 (C-8), 128.1 (C-4"), 129.0 (C-3", C-5"), 137.7 (C-6), 138.0 (C-1"), 147.9 (C-8a), 151.9 (C-2), 158.7 (C-4'), 159.6 (C-2', C-6'), 161.4 (CO_2Et), 162.3 (CONH), 177.8 (C-4). LC-MS (m/z): positive mode 480 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. mp 210-211 °C.

5.2.21.18 Ethyl 6-bromo-8-(4-methoxybenzamido)-4-thioxo-4*H*-chromene-2-carboxylate 119 (*ANM278)*



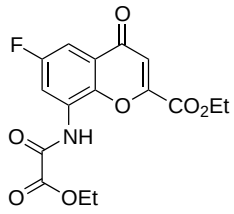
The compound was synthesized according to GP-10 using **34** (295 mg, 0.9 mmol) and 4-methoxybenzoyl chloride (205 mg, 1.2 mmol)and was isolated as a green solid (195 mg, 47% yield). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.86 (s, 3H, OCH_3), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.11 (d, J = 8.8 Hz, 2H, 3'-H, 5'-H),7.69 (s, 1H, 3-H), 7.95-8.05 (m, 2H, 2'-H, 6'-H), 8.24 (d, J = 2.4 Hz, 1H, 5-H), 8.38 (d, J = 2.4 Hz, 1H, 7-H), 10.21 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 55.7 (OCH_3), 63.0 ($\underline{C}H_2CH_3$), 114.0 (C-3', C-5'), 119.7 (C-6), 125.6 (C-5, C-3), 126.6 (C-1'), 129.9 (C-2', C-6'), 132.0 (C-8), 132.0 (C-4a), 132.4 (C-7), 142.5 (C-8a), 143.8 (C-2), 160.2 (CO_2Et), 162.6 (C-4'), 164.9 (CONH), 201.4 (C-4). LC-MS (m/z): positive mode 463 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 85.9%. mp 231-232 °C.

5.2.21.19 Ethyl 8-(2-ethoxy-2-oxoacetamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 122 (*ANM282)*



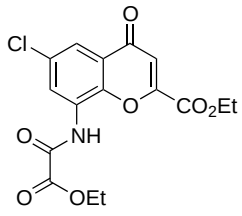
The compound was synthesized according to GP-10 using **26** (278 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (282 mg, 77% yield). ^{1}H NMR: δ 1.35 (td,J = 7.2, 1.1 Hz, 6H, $CH_2C\underline{H}_3$), 4.38 (dq, J = 7.1 Hz, 4H, $C\underline{H}_2CH_3$), 7.02 (s, 1H, 3-H), 7.41-7.48 (m, 1H, 4'-H), 7.52 (dd, J = 8.0, 7.0 Hz, 2H, 3'-H, 5'-H), 7.69-7.75 (m, 2H, 2'-H, 6'-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 8.48 (d, J = 2.3 Hz, 1H, 7-H), 10.52 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 62.95 ($\underline{C}H_2CH_3$), 113.9 (C-3), 119.1 (C-5), 124.6 (C-4a), 127.0 (C-7, C-2', C-6'), 127.4 (C-8), 128.6 (C-4'), 129.4 (C-3', C-5'), 137.8 (C-6), 138.1 (C-1'), 147.6 (C-8a), 151.7 (CONH), 155.5 (C-2), 159.8-160.0 (CO_2Et), 177.1 (C-4). LC-MS (m/z): positive mode 410 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 158-159 °C.

5.2.21.20 Ethyl 8-(2-ethoxy-2-oxoacetamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylate 123 (*ANM289)*



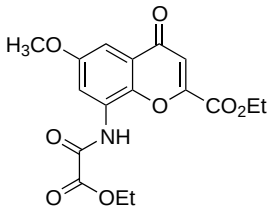
The compound was synthesized according to GP-10 using **21** (226 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (234 mg, 74% yield). ^{1}H NMR: δ 1.35 (td, J = 7.1, 3.6 Hz, 6H, $CH_2C\underline{H}_3$), 4.38 (dq, J = 7.1 Hz, 4H, $C\underline{H}_2CH_3$), 7.01 (s, 1H, 3-H), 7.59 (dd, J = 3.1, 7.9 Hz, 1H, 5-H), 8.16 (dd, J = 9.6, 3.1 Hz, 1H, 7-H), 10.43 (s, 1H, CONH). ^{13}C NMR: δ 13.8-13.9 ($CH_2\underline{C}H_3$), 63.0-63.2 ($\underline{C}H_2CH_3$), 106.1- 106.2 (C-5), 113.2 (C-3), 115.6-115.8 (C-7), 125.1 (C-4a), 128.8-128.9 (C-8), 143.3 (C-8a), 151.8 (C-2), 155.3-157.8 (C-6), 159.6 (CONH), 159.7 (CO_2Et), 176.4 (C-4). LC-MS (m/z): positive mode 352 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 164-166 °C.

5.2.21.21 Ethyl 6-chloro-8-(2-ethoxy-2-oxoacetamido)-4-oxo-4*H*-chromene-2-carboxylate 124 (*ANM290)*



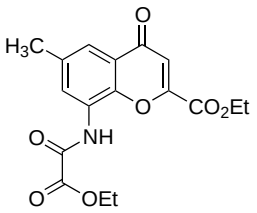
The compound was synthesized according to GP-10 using **22** (241 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (77 mg, 23% yield). ^{1}H NMR: δ 1.30-1.33 (m, 6H, $CH_2C\underline{H}_3$), 4.38 (dq, J = 7.1 Hz, 4H, $C\underline{H}_2CH_3$), 7.03 (s, 1H, 3-H), 7.84 (d, J = 2.6 Hz, 1H, 5-H), 8.27 (d, J = 2.6 Hz, 1H, 7-H), 10.45 (s, 1H, CONH). ^{13}C NMR: δ 13.9-14.0 ($CH_2\underline{C}H_3$), 63.0 ($\underline{C}H_2CH_3$), 114.0 (C-3), 120.6 (C-5), 125.2 (C-4a), 127.4 (C-7), 128.6 (C-8), 130.3 (C-6), 146.6 (C-8a), 151.9 (C-2), 155.3 (CONH), 159.6 (CO_2Et), 176.1 (C-4). LC-MS (m/z): positive mode 368 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 148-150 °C.

5.2.21.22 Ethyl 8-(2-ethoxy-2-oxoacetamido)-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate 125 (*ANM291)*



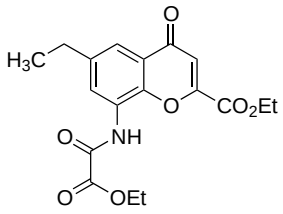
The compound was synthesized according to GP-10 using **24** (237 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (74 mg, 23% yield). ^{1}H NMR: δ 1.34 (td, J = 7.1, 1.8 Hz, 6H, $CH_2C\underline{H}_3$), 3.87 (s, 3H, OCH_3), 4.37 (dq, J = 7.1, 1.8 Hz, 4H, $C\underline{H}_2CH_3$), 6.95 (s, 1H, 3-H), 7.26 (d, J = 3.0 Hz, 1H, 5-H), 7.86 (d, J = 3.0 Hz, 1H, 7-H), 10.31 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 56.1 (OCH_3), 62.9 ($\underline{C}H_2CH_3$), 101.5 (C-5), 113.0 (C-3), 117.0 (C-7), 124.9 (C-4a), 128.0 (C-8), 142.6 (C-8a), 151.4 (C-2), 155.2 (CONH), 156.7 (C-6), 159.8 (CO_2Et), 176.7 (C-4). LC-MS (m/z): positive mode 364 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 147-149 °C.

5.2.21.23 Ethyl 8-(2-ethoxy-2-oxoacetamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylate 126 (*ANM292)*



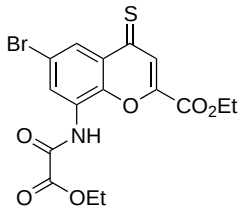
The compound was synthesized according to GP-10 using **23** (222 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (121 mg, 39% yield). ^{1}H NMR: δ 1.31 (m, 6H, $CH_2C\underline{H}_3$), 2.43 (s, 3H, CH_3), 4.36 (dq, J = 7.1 Hz, 4H, $C\underline{H}_2CH_3$), 6.95 (s, 1H, 3-H), 7.68 (t, J = 1.5 Hz, 1H, 5-H), 8.02 (d, J = 2.0 Hz, 1H, 7-H), 10.36 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 20.9 (CH_3), 62.9 ($\underline{C}H_2CH_3$), 113.8 (C-3), 121.8 (C-5), 124.0 (C-4a), 126.4 (C-8), 129.5 (C-7), 135.9 (C-6), 146.3 (C-8a), 151.6 (C-2), 155.6 (CONH), 159.9 (CO_2Et), 177.0 (C-4). LC-MS (m/z): positive mode 348 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.6%. mp 133-135 °C.

5.2.21.24 Ethyl 8-(2-ethoxy-2-oxoacetamido)-6-ethyl-4-oxo-4*H*-chromene-2-carboxylate 127 (*ANM293)*



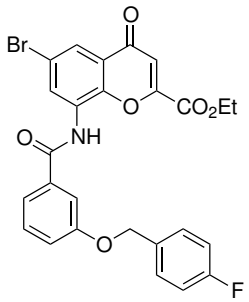
The compound was synthesized according to GP-10 using **23** (222 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a white solid (121 mg, 39% yield). ^{1}H NMR: δ 1.22 (t, J = 7.6 Hz, 3H, C6-$CH_2C\underline{H}_3$), 1.34 (td, J =7.1, 1.8 Hz, 6H, $CH_2C\underline{H}_3$), 2.75 (q, J = 7.6 Hz, 2H, C6-$C\underline{H}_2CH_3$), 4.37 (dq, J = 7.1 Hz, 4H, $C\underline{H}_2CH_3$), 6.96 (s, 1H, 3-H), 7.71 (d, J = 2.1 Hz, 1H, 5-H), 8.05 (d, J = 2.1 Hz, 1H, 7-H), 10.39 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 15.3 (C6-$CH_2\underline{C}H_3$), 27.8 (C6-$\underline{C}H_2CH_3$), 62.9 ($\underline{C}H_2CH_3$), 113.8 (C-3), 120.1 (C-5), 124.1 (C-4a), 126.6 (C-8), 128.7 (C-7), 141.9 (C-6), 146.5 (C-8a), 151.6 (C-2), 155.4 (CONH), 159.8 (CO_2Et), 177.1 (C-4). LC-MS (m/z): positive mode 362 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 133-135 °C.

5.2.21.25 Ethyl 6-bromo-8-(2-ethoxy-2-oxoacetamido)-4-thioxo-4*H*-chromene-2-carboxylate 128 (*ANM294)*



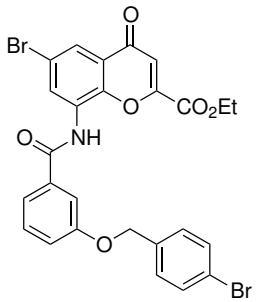
The compound was synthesized according to GP-10 using **34** (295 mg, 0.9 mmol) and ethyl chlorooxoacetate (0.13 mL, 1.2 mmol) and was isolated as a green solid (113 mg, 29% yield). ^{1}H NMR: δ 1.35 (td, J = 7.1, 3.1 Hz, 6H, $CH_2C\underline{H}_3$), 4.38 (dq, J = 7.1 Hz, 4H, $C\underline{H}_2CH_3$), 7.66 (s, 1H, 3-H), 8.22 (d, J = 2.4 Hz, 1H, 5-H), 8.43 (d, J = 2.4 Hz, 1H, 7-H), 10.44 (s, 1H, CONH). ^{13}C NMR: δ 13.9 ($CH_2\underline{C}H_3$), 63.1 ($\underline{C}H_2CH_3$), 119.7 (C-6), 125.0 (C-7), 125.7 (C-5), 129.2 (C-8), 130.2 (C-3), 131.8 (C-4a), 142.3 (C-8a), 155.3 (CONH), 159.6 (C-2), 160.1 (CO_2Et), 201.3 (C-4). LC-MS (m/z): positive mode 428 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 91.6%. mp 171-173 °C.

5.2.21.26 Ethyl-6-bromo-8-(3-(4-fluorobenzyloxy)benzamido)oxo-4*H*-chromene-2-carboxylate 146 (*ANM16)*



The compound was synthesized according to GP-10 using **61** (295 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (229 mg, 35% yield). ^{1}H NMR: δ 1.26 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.20 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.17-7.24 (m, 2H, 3″-H, 5″-H), 7.30 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H, 4′-H), 7.50 (t, J = 7.9 Hz, 1H, 5′-H), 7.51-7.55 (m, 2H, 2″-H, 6″-H), 7.60-7.64 (m, 1H, 6′-H), 7.96 (d, J = 2.4 Hz, 1H, 5-H), 8.36 (d, J = 2.4 Hz, 1H, 7-H), 10.06 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 62.6 ($\underline{C}H_2CH_3$), 69.0 (CH_2-O), 113.6 (C-3), 114.3 (C-2′), 114.9 (C-3″, C-5″), 117.6 (C-6), 118.6 (C-6′), 120.0 (C-4′), 122.8 (C-5), 125.3 (C-4a), 129.6 (C-2″, C-6″), 129.6 (C-5′), 130.1 (C-8), 131.5 (C-7), 132.9 (C-1″), 135.0 (C-1′), 147.5 (C-8a), 151.8 (C-2), 158.4 (C-3′), 159.4 (CO_2Et), 164.9 (CONH), 175.7 (C-4). LC-MS (m/z): negative mode 538 $[M-H]^-$, positive mode 542 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 186-187 °C.

5.2.21.27 Ethyl-6-bromo-8-(3-(4-bromobenzyloxy)benzamido)oxo-4*H*-chromene-2-carboxylate 147 (*ANM17)*

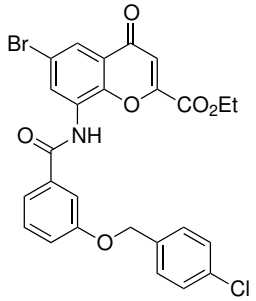


The compound was synthesized according to GP-10 using **63** (369 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (331 mg, 46% yield). ^{1}H NMR: δ 1.28 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 7.02 (s, 1H, 3-H), 7.31 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H, 4′-H), 7.43-7.48 (m, 2H, 3′-H, 5′-H), 7.51 (t, J = 7.9 Hz, 1H, 5′-H), 7.59-7.63 (m, 2H, 2″-H, 6″-H), 7.63-7.66 (m, 1H, 2′-H), 7.67-7.69 (m, 1H, 6′-H), 7.99 (dd, J = 2.4 Hz, 1H, 5-H), 8.37 (d, J = 2.4 Hz, 1H, 7-H), 10.10 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 62.6 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.6 (C-3), 114.3 (C-2′), 117.6 (C-6), 118.7 (C-6′), 120.1 (C-4′), 120.8 (C-4″), 122.9 (C-5), 125.3 (C-4a), 129.5 (C-2″, C-6″), 129.6 (C-5′), 130.0 (C-8), 131.2

(C-3", C-5"), 131.5 (C-7), 135.0 (C-1'), 136.2 (C-1"), 147.8 (C-8a), 151.8 (C-2), 158.3 (C-3'), 159.4 (CO_2Et), 164.9 (CONH), 175.6 (C-4). LC-MS (m/z): negative mode 600 $[M-H]^-$, positive mode 602 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 195-196 °C.

5.2.21.28 Ethyl-6-bromo-8-(3-(4-chlorobenzyloxy)benzamido)oxo-4*H*-chromene-2-carboxylate 148

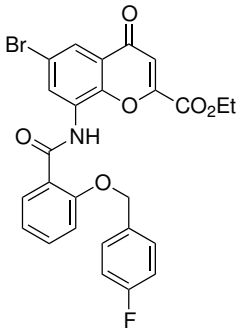
(ANM18)



The compound was synthesized according to GP-10 using **65** (315 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The product was dissolved in 1 mL of DCM, separated by column chromatography on a column of silica gel (2% EtOAc in DCM) and dried in vacuum at 50 °C. The product was isolated as a white solid (185 mg, 33% yield). ^{1}H NMR: δ 1.26 (t, J = 7.0 Hz, 3H, $CH_2C\underline{H}_3$), 4.36 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.30 (dd, J = 8.3, 2.6 Hz, 1H, 4'-H), 7.44-7.52 (m, 5H, 5'-H, 2"-H, 3"-H, 5"-H, 6"-H), 7.61-7.67 (m, 2H, 2'-H, 6'-H), 7.97 (d, J = 2.5 Hz, 1H, 5-H), 8.35 (d, J = 2.4 Hz, 1H, 7-H), 10.06 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 62.6 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.6 (C-3), 114.3 (C-2'), 117.7 (C-6), 118.7 (C-6'), 118.6 (C-4"), 120.1 (C-4'), 122.9 (C-5), 125.3 (C-4a), 128.3 (C-3", C-5"), 129.2 (C-2", C-6"), 129.6 (C-5'), 130.1 (C-8), 131.5 (C-7), 132.4 (C-1'), 135.0 (C-4"), 135.7 (C-1"), 147.8 (C-8a), 151.8 (C-2), 158.3 (C-3'), 159.4 (CO_2Et), 164.9 (CONH), 175.7 (C-4). LC-MS (m/z): positive mode 558 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 186-187 °C.

5.2.21.29 Ethyl-6-bromo-8-(2-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 153

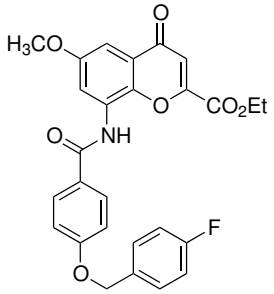
(ANM19)



The compound was synthesized according to GP-10 using **73** (297 mg, 1.2 mmol) and **17** (281 mg, 0.9 mmol). The precipitate was filtered off and the filtrate was concentrated under reduced pressure und separated by column chromatography on a column of silica gel (2% EtOAc in DCM) yielding further product. The product was isolated as a white solid (364 mg, 56% yield). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.30 (q, J = 7.1 Hz,

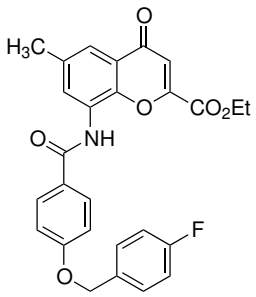
2H, C<u>H</u>$_2$CH$_3$), 5.54 (s, 2H, CH$_2$-O), 6.98 (s, 1H, 3-H), 7.02-7.08 (m, 2H, 3″-H, 5″-H), 7.18 (t, J = 7.5 Hz, 1H, 5′-H), 7.38 (d, J = 8.3 Hz, 1H, 3′-H), 7.45-7.49 (m, 2H, 2″-H, 6″-H), 7.57-7.62 (m, 1H, 4″-H), 7.86 (d, J = 2.4 Hz, 1H, 5-H), 8.04 (dd, J = 7.8, 1.7 Hz, 1H, 6′-H), 8.93 (s, 1H, 7-H), 10.68 (s, 1H, CONH). ^{13}C NMR: δ 13.8 (CH$_2$<u>C</u>H$_3$), 63.0 (<u>C</u>H$_2$CH$_3$), 70.4 (CH$_2$-O), 113.9 (C-3), 115.2 (C-3′), 115.3 (C-2″, C-5″), 118.5 (C-6), 121.2 (C-5′), 121.8 (C-1′), 122.0 (C-5), 125.3 (C-4a), 127.4 (C-7), 130.0 (C-2″, C-6″), 130.1 (C-8), 131.6 (C-6′), 132.6 (C-1″), 134.3 (C-4′), 145.2 (C-8a), 151.7 (C-2), 156.3 (C-2′), 159.4 (CO$_2$Et), 160.9 (C-4″), 163.6 (CONH), 176.0 (C-4). LC-MS (m/z): negative mode 538 [M-H]$^-$, positive mode 542 [M+H]$^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 190-191 °C.

5.2.21.30 Ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate 133 (*ANM56)*



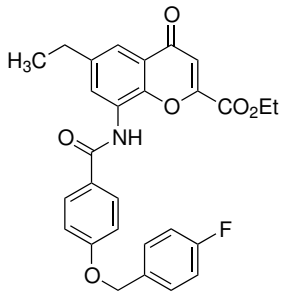
The compound was synthesized according to GP-10 using **47** (297 mg, 1.2 mmol) and **24** (237 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (175 mg, 79% yield). ^{1}H NMR: δ 1.29 (t, J = 7.1 Hz, 3H, CH$_2$C<u>H</u>$_3$), 3.89 (s, 3H, O-CH$_3$), 4.36 (q, J = 7.1 Hz, 2H, C<u>H</u>$_2$CH$_3$), 5.22 (s, 2H, CH$_2$-O), 6.93 (s, 1H, 3-H), 7.15-7.23 (m, 4H, 3′-H, 5′-H, 3″-H, 5″-H), 7.27 (d, J = 3.1 Hz, 1H, 5-H), 7.50-7.55 (m, 2H, 2″-H, 6″-H), 7.84 (d, J = 3.1 Hz, 1H, 7-H), 7.97-8.02 (m, 2H, 2′-H, 6′-H), 9.78 (s, 1H, CONH). ^{13}C NMR: δ 13.5 (CH$_2$<u>C</u>H$_3$), 55.8 (OCH$_3$), 62.4 (<u>C</u>H$_2$CH$_3$), 68.9 (CH$_2$-O), 101.1 (C-5), 112.5 (C-3), 114.7 (C-3′, C-5′), 115.1 (C-3″, C5″), 118.1 (C-7), 124.7 (C-4a), 126.3 (C-1′), 129.3 (C-2′, C-6′), 129.7 (C-2″, C-6″), 129.7 (C-8), 132.8 (C-1″), 143.5 (C-8a), 151.3 (C-2), 156.6 (C-6), 159.7 (CO$_2$Et), 161.2 (C-4), 162.7 (C-4′), 164.6 (CONH), 176.5 (C-4). LC-MS (m/z): negative mode 490 [M-H]$^-$, positive mode 492 [M+H]$^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 253-254 °C.

5.2.21.31 Ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylate 134 (*ANM57)*



The compound was synthesized according to GP-10 using **47** (297 mg, 1.2 mmol) and **23** (223 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (166 mg, 78% yield). ^{1}H NMR: δ 1.22 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.45 (s, 3H, CH_3), 4.32 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.20 (s, 2H, CH_2-O), 6.95 (s, 1H, 3-H), 7.15-7.19 (m, 2H, 3'-H, 5'-H), 7.20-7.26 (m, 2H, 3"-H, 5"-H), 7.51-7.56 (m, 2H, 2"-H, 6"-H), 7.69 (dd, J = 2.2 Hz, 1H, 5-H), 7.92 (d, J = 2.1 Hz, 1H, 7-H), 7.98-8.03 (m, 2H, 2'-H, 6'-H), 10.10 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 20.8 (OCH_3), 62.8 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.6 (C-3), 114.8 (C-3', C-5'), 115.4-115.5 (C-3", C-5"), 120.8 (C-5), 124.1 (C-4a), 126.4 (C-1'), 128.3 (C-8), 128.3 (C-2', C-6'), 129.8-130.2 (C-2", C-6"), 131.9 (C-7), 133.0 (C-1"), 135.7 (C-6), 147.7 (C-8a), 151.7 (C-2), 160.0 (CO_2Et), 161.0 (C-4'), 162.9 (C-4"), 162.9 (C-4'), 164.9 (CONH), 177.3 (C-4). LC-MS (m/z): negative mode 474 $[M-H]^-$, positive mode 476 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 63.0%. mp 234-235 °C.

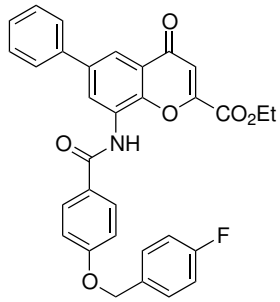
5.2.21.32 Ethyl-6-ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 135 (*ANM90)*



The compound was synthesized according to GP-10 using **47** (297 mg, 1.2 mmol) and **20** (235 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (321 mg, 73% yield). ^{1}H NMR: δ 1.24-1.30 (m, 6H, CO_2-$CH_2C\underline{H}_3$, C6-$CH_2C\underline{H}_3$), 2.78 (q, J = 7.6 Hz, 2H, C6-$C\underline{H}_2CH_3$), 4.35 (q, J = 7.1 Hz, 2H, CO_2-$C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 6.94 (s, 1H, 3-H), 7.14-7.24 (m, 4H, 3'-H, 5'-H, 3"-H, 5"-H), 7.50-7.55 (m, 2H, 2"-H, 6"-H), 7.71 (d, J = 2.2 Hz, 1H, 5-H), 7.96-8.02 (m, 2H, 2'-H, 6'-H), 8.03 (d, J = 2.2 Hz, 1H, 7-H), 9.78 (s, 1H, CONH). ^{13}C NMR: δ 13.4 (CO_2-$CH_2\underline{C}H_3$), 14.8 (C6-$CH_2\underline{C}H_3$), 27.5 (C6-$\underline{C}H_2CH_3$), 62.4 (CO_2-$\underline{C}H_2CH_3$), 68.8 (CH_2-O), 113.2 (C-3), 114.6 (C-3', C-5'), 114.9-115.1 (C-3", C-5"), 118.9 (C-5), 123.9 (C-4a), 126.4 (C-1'), 128.2 (C-8), 129.3 (C-2', C-6'), 129.7

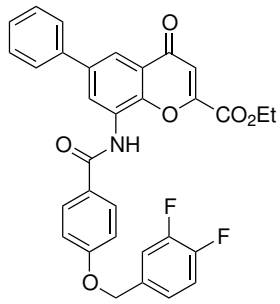
(C-2", C-6", C-7), 132.8 (C-1"), 141.4 (C-8), 147.2 (C-6), 147.2 (C-8a), 151.5 (C-2), 159.7 (CO_2Et), 161.1 (C-4'), 162.7 (C-4"), 164.1 (CONH), 176.9 (C-4). LC-MS (m/z): negative mode 488 $[M-H]^-$, positive mode 490 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.4%. mp 210-211 °C.

5.2.21.33 Ethyl-8-(4-(4-fluorobenzyloxy)benzamivdo)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 136 (*ANM91*)



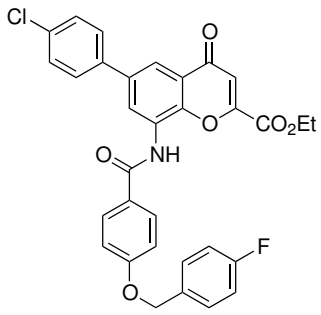
The compound was synthesized according to GP-10 using **47** (148 mg, 0.6 mmol) and **26** (139 mg, 0.45 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (220 mg, 91% yield). ^{1}H NMR: δ 1.30 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.38 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.16-7.24 (m, 4H, 3'-H, 5'-H, 3"-H, 5"-H), 7.42-7.47 (m, 1H, 4"'-H), 7.50-7.56 (m, 4H, 2"-H, 6"-H, 3"'-H, 5"'-H), 7.72-7.77 (m, 2H, 2"'-H, 6"'-H), 8.01-8.05 (m, 2H, 2'-H, 6'-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 8.48 (d, J = 2.3 Hz, 1H, 7-H), 9.90 (s. 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$), 68.8 (CH_2-O), 113.4 (C-3), 114.7 (C-3', C-5'), 114.9-115.1 (C-3", C-5"), 117.9 (C-5), 124.4 (C-4a), 126.4 (C-1"'), 126.6 (C-2"', C-6"'), 127.9 (C-7), 128.0 (C-4"'), 129.0 (C-2"', C-6"'), 129.0 (C-8), 129.4 (C-2', C-6'), 129.6-129.7 (C-2", C-6"), 132.7 (C-1"), 137.6 (C-6), 138.2 (C-1'), 148.1 (C-8a), 151.6 (C-2), 159.6 (CO_2Et), 160.8-162.7 (C-4"), 161.2 (C-4'), 164.7 (CONH), 176.9 (C-4). LC-MS (m/z): negative mode 536 $[M-H]^-$, positive mode 538 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 86.3%. mp 246-247 °C.

5.2.21.34 Ethyl-8-(4-(3,4-difluorobenzyloxy)benzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 137 (*ANM125)*



The compound was synthesized according to GP-10 using **49** (159 mg, 0.6 mmol) and **26** (139 mg, 0.45 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (173 mg, 69% yield). ^{1}H NMR: δ 1.25 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 7.01 (s, 1H, 3-H), 7.17-7.21 (m, 2H, 3'-H, 5'-H), 7.32-7.65 (m, 6H, 6"-H, 4"'-H, 5"-H, 5"'-H, 3"'-H, 2"-H), 7.73-7.78 (m, 2H, 2"'-H, 6"'-H), 8.01-8.06 (m, 2H, 2?-H, 6?-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 8.41 (d, J = 2.3 Hz, 1H, 7-H), 10.20 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 62.9 ($\underline{C}H_2CH_3$), 68.3 (CH_2-O), 113.7 (C-3), 114.8 (C-3', C-5'), 117.0-117.1 (C-2"), 117.7-117.8 (C-5"), 118.5 (C-5), 124.7 (C-4a), 124.9 (C-6"), 126.5 (C-1"'), 127.0 (C-2"', C-6"'), 128.4 (C-7), 129.2 (C-4"'), 129.3 (C-8), 129.4 (C-3"', C-5"'), 129.9 (C-2', C-6'), 134.6 (C-1"), 137.7 (C-6),138.3 (C-1'), 148.2-148.5 (C-4"), 148.8 (C-8a), 150.3-150.5 (C-3"), 151.9 (C-2), 160.0 (CO_2Et), 161.2 (C-4'), 165.0 (CONH), 177.3 (C-4). LC-MS (m/z): negative mode 554 $[M-H]^-$, positive mode 556 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 63.9%. mp 215-218 °C.

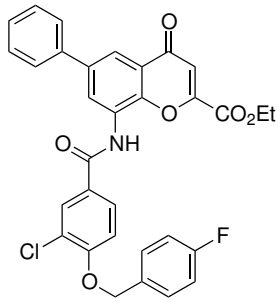
5.2.21.35 Ethyl-6-(4-chlorophenyl)-8-(4-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 138 (*ANM132)*



The compound was synthesized according to GP-10 using **47** (134 mg, 0.51 mmol) and **27** (130 mg, 0.38 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (185 mg, 85% yield). ^{1}H NMR: δ 1.29 (t, J = 7.2 Hz, 3H, $CH_2C\underline{H}_3$), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.23 (s, 2H, CH_2-O), 7.01 (s, 1H, 3-H), 7.17-7.24 (m, 4H, 3'-H, 5'-H, 3"-H,5'-H), 7.50-7.59 (m, 4H, 2"-H, 6"-H, 5"'-H, 3"'-H), 7.75-7.80 (m, 2H, 2"'-H, 6"'-H), 8.01-8.05 (m, 2H, 2'-H, 6'-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 9.47 (d, J = 2.5 Hz, 1H, 7-H), 9.93 (s, 2H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$),

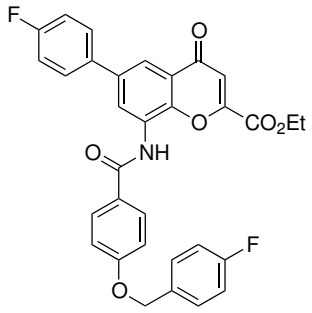
68.8 (CH_2-O), 113.4 (C-3), 114.7 (C-3', C-4'), 115.0-115.1 (C-3", C-5"), 118.0 (C-5), 124.4 (C-4a), 126.3 (C-1"'), 127.8 (C-7), 128.5 (C-2', C-6'), 129.0 (C-3"', C-5"'), 129.1 (C-8),129.4 (C-2"', C-6"'), 129.6-129.7 (C-2", C-6"), 133.1 (C-1"), 136.2 (C-6), 137.0 (C-1'), 148.3 (C-8a), 151.7 (C-2), 159.6 (CO_2Et), 161.2 (C-4"'), 162.7 (C-4'), 164.7 (CONH), 176.8 (C-4). LC-MS (m/z): negative mode 570 $[M-H]^-$, positive mode 572 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.6%. mp 247-248 °C.

5.2.21.36 Ethyl-8-(3-chloro-4-(4-fluorobenzyloxy)benzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 139 (*ANM133)*



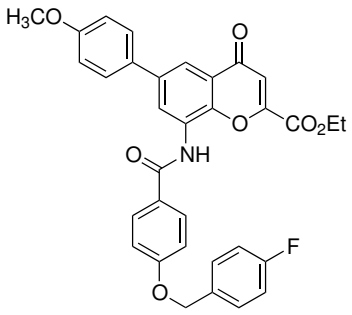
The compound was synthesized according to GP-10 using **51** (168 mg, 0.6 mmol) and **26** (139 mg, 0.45 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (115 mg, 45% yield). ^{1}H NMR: δ 1.28 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.33 (s, 2H, CH_2-O), 7.01 (s, 1H, 3-H), 7.20-7.26 (m, 2H, 3"-H, 5"-H), 7.40-7.47 (m, 2H, 4"'-H, 5'-H), 7.50-7.58 (m, 4H, 2"-H, 6"-H, 3"'-H, 5"'-H), 7.73-7.77 (m, 2H, 2"'-H, 6"'-H), 8.03 (dd, J = 2.3, 8.6 Hz, 1H, 5-H), 8.15 (d, J = 2.2 Hz, 1H, 2'-H), 8.39 (d, J = 2.3 Hz, 1H, 7-H), 10.15 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$), 69.8 (CH_2-O), 113.4 (C-3), 114.1 (C-5'), 115.0-115.2 (C-3", C-5"), 118.5 (C-5), 121.8 (C-3'), 124.5 (C-4a), 126.7 (C-2"', C-6"'), 127.3 (C-1"'), 128.1 (C-7), 128.7 (C-8), 128.8 (C-4"'), 129.0 (C-3"', C-5"'), 129.3 (C-2', C-6'), 129.5-129.6 (C-2", C-6"), 132.3 (C-1"), 137.6 (C-6), 138.0 (C-1'), 148.6 (C-8a), 151.7 (C-2), 156.3 (C-4'), 159.6 (CO_2Et), 160.9-162.8 (C-4"), 163.7 (CONH), 176.8 (C-4). LC-MS (m/z): negative mode 570 $[M-H]^-$, positive mode 572 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 242-243 °C.

5.2.21.37 Ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylate 140 (*ANM135)*



The compound was synthesized according to GP-10 using **47** (214 mg, 0.87 mmol) and **30** (213 mg, 0.65 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (225 mg, 62% yield). ^{1}H NMR: δ 1.29 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.16-7.24 (m, 4H, 3'-H, 5'-H, 3"-H, 5"-H), 7.30-7.35 (m, 2H, 3"'-H, 5"'-H), 7.51-7.55 (m, 2H, 2"-H, 6"-H), 7.77-7.81 (m, 2H, 2"'-H, 6"'-H), 8.01-8.04 (m, 2H, 2'-H, 6'-H), 8.05 (d, J = 2.3 Hz, 1H, 5-H), 8.45 (d, J = 2.3 Hz, 1H, 7-H), 9.92 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.4 (C-3), 114.7 (C-3', C-5'), 115.0-115.1 (C-3"', C-5"'), 115.7-115.9 (C-3", C-5"), 117.9 (C-5), 124.4 (C-4a), 126.3 (C-1"'), 127.9 (C-7), 128.8 (C-2"', C-6"'), 129.1 (C-8), 129.4 (C-2', C-6'), 129.6-129.7 (C-2", C-6"), 132.8 (C-1"), 134.7 (C-6), 136.5 (C-8), 148.1 (C-8a), 151.6 (C-2), 159.6 (CO_2Et), 160.8-161.2 (C-4"), 162.7-163.2 (C-4'), 164.7 (CONH), 176.8 (C-4). LC-MS (m/z): negative mode 554 $[M-H]^-$, positive mode 556 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.3%. mp 219-220 °C.

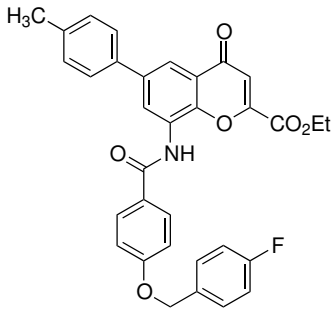
5.2.21.38 Ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-6-(4-methoxyphenyl)-4-oxo-4*H*-chromene-2-carboxylate 141 (*ANM136)*



The compound was synthesized according to GP-10 using **47** (214 mg, 0.87 mmol) and **29** (221 mg, 0.65 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (280 mg, 75% yield). ^{1}H NMR: δ 1.29 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.83 (sm, 3H, OCH_3), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.05-7.12 (m, 2H, 3"'-H,5"'-H), 7.15-7.25 (m, 4H, 3'-H, 5'-H, 3"-H, 5"-H), 7.48-7.56 (m, 2H, 2"-H, 6"-H), 7.64-7.72 (m, 2H, 2"'-H, 6"'-H), 7.98-8.07 (m, 3H, 5-H, 2'-H, 6'-H), 8.42 (d, J = 2.2 Hz, 1H, 7-H), 9.89 (s, 1H, CONH). ^{13}C NMR: δ 13.5

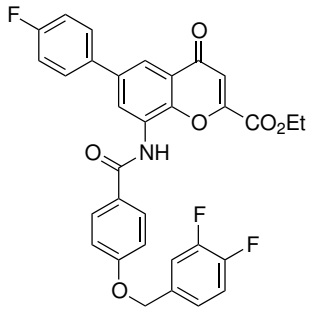
($CH_2\underline{C}H_3$), 55.2 (OCH_3), 62.5 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.3 (C-3), 114.7 (C-3‴, C-5‴, C-3′, C-5′), 115.0-115.1 (C-3″, C-5″), 117.2 (C-5), 124.4 (C-4a), 126.4 (C-1‴), 127.6 (C-7), 127.9 (C-2‴, C-6‴), 128.9 (C-8), 129.1 (C-2′, C-6′), 129.6-129.8 (C-2″, C-6″), 130.5 (C-6′) 132.8 (C-1″) 137.3 (C-4‴), 147.8 (C-8a), 151.6 (C-2), 159.5 (CO_2Et), 160.8-162.8 (C-4″), 161.2 (C-4′), 164.7 (CONH), 176.9 (C-4). LC-MS (m/z): negative mode 566 $[M-H]^-$, positive mode 568 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 225-226 °C.

5.2.21.39 Ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-4-oxo-6-p-tolyl-4*H*-chromene-2-carboxylate 142 (*ANM137*)



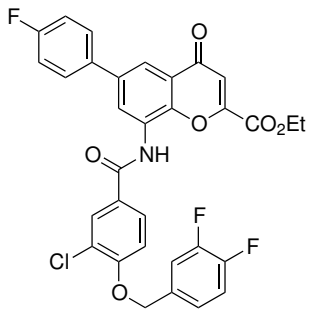
The compound was synthesized according to GP-10 using **47** (165 mg, 0.67 mmol) and **28** (160 mg, 0.5 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (226 mg, 82% yield). ^{1}H NMR: δ 1.29 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.23 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.11-7.27 (m, 4H, 3′-H, 5′-H, 3″-H, 5″-H), 7.44-7.62 (m, 4H, 3‴-H, 5‴-H, 2″-H, 6″-H), 7.73-7.82 (m, 2H, 2‴-H, 6‴-H), 8.00-8.06 (m, 2H, 2′-H, 6′-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 8.47 (d, J = 2.3 Hz, 1H, 7-H), 9.93 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 55.1 (C4‴-CH_3), 62.9 ($C\underline{H}_2CH_3$), 68.9 (CH_2-O), 113.3 (C-3), 115.8 (C-3′, C-5′), 115.6 (C-3″, C-5″), 124.7 (C-5), 124.7 (C-4a), 126.4 (C-1‴), 126.6 (C-2‴, C.6‴″), 126.9 (C-7), 129.3 (C-8), 129.8 (C-2′, C-6′), 130.0 (C-3‴, C-5‴), 130.2 (C-2″, C-6″), 133.0 (C-1″), 135.4 (C-6), 137.4 (C-1′), 138.0 (C-8), 148.7 (C-8a), 151.7 (C-2), 160.0 (C-4′), 160.3 (CO_2Et), 161.4 (C-4″), 165.0 (CONH), 177.4 (C-4). LC-MS (m/z): negative mode 550 $[M-H]^-$, positive mode 552 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 87.4%. mp 223-224 °C.

5.2.21.40 Ethyl-8-(4-(3,4-difluorobenzyloxy)benzamido)-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylate 143 (*ANM147)*



The compound was synthesized according to GP-10 using **49** (282 mg, 1.07 mmol) and **30** (262 mg, 0.8 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (252 mg, 55% yield). ^{1}H NMR: δ 1.29 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.23 (s, 2H, CH_2-O), 6.99 (s, 1H, 3-H), 7.16-7.22 (m, 2H, 3'-H, 5'-H), 7.28-7.37 (m, 3H, 5"'-H, 3"'-H, 6"-H), 7.43 (dt, J = 8.3 Hz, 1H, 5"-H), 7.52 (ddd, J = 2.1, 7.9 Hz, 1H, 2"-H), 7.75-7.82 (m, 2H, 2"'-H, 6"'-H), 8.00-8.07 (m, 3H, 5-H, 2'-H, 6'-H), 8.45 (d, J = 2.3 Hz, 1H, 7-H), 9.92 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 62.5 ($C\underline{H}_2CH_3$), 68.2 (CH_2-O), 113.4 (C-3), 114.7 (C-3', C-5'), 115.7-115.9 (C-3", C-5"), 116.4-116.6 (C-2"), 117.3-117.4 (C-5"), 117.9 (C-7), 124.2 (C-6"), 124.4 (C-4a), 126.5 (C-1'), 127.9 (C-5), 128.8 (C-2"', C-6"'), 129.0 (C-8), 129.4 (C-2', C-6'), 134.3 (C-1"), 134.6 (C-6), 136.5 (C-1"'), 146.9-147.3 (C-4"), 148.1 (C-8a), 149.5-150.2 (C-3"), 151.6 (C-2), 159.6 (CO_2Et), 161.0 (C-4"'), 161.2 (C-4'), 164.7 (CONH), 176.8 (C-4). LC-MS (m/z): positive mode 574 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.1%. mp 223-224 °C.

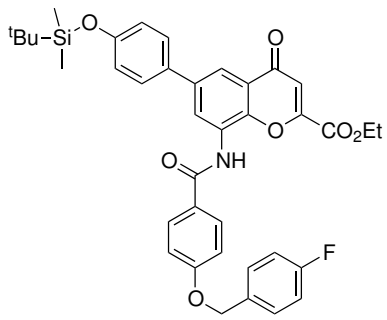
5.2.21.41 Ethyl-8-(3-chloro-4-(3,4-difluorobenzyloxy)benzamido)-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylate 144 (*ANM149)*



The compound was synthesized according to GP-10 using **53** (358 mg, 1.2 mmol) and **30** (294 mg, 0.9 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (407 mg, 75% yield). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.34 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.27-7.39 (m, 3H, 3"'-H, 5"'-H, 6"-H), 7.39-7.50 (m, 2H, 5'-H, 5"-H), 7.53 (ddd, J = 2.1, 7.8 Hz, 1H, 2"-H), 7.71-7.86 (m, 2H, 2"'-H, 6"'-H), 8.02 (dd, J = 2.2, 8.6 Hz, 1H, 6'-H), 8.08 (d, J = 2.4 Hz, 1H, 5-H), 8.16 (d, J = 2.2 Hz, 1H, 2'-H), 8.36 (d, J = 2.4 Hz, 1H, 7-H),

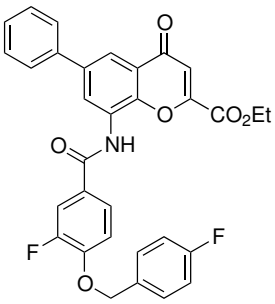
10.17 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 62.5 ($C\underline{H}_2CH_3$), 69.2 (CH_2-O), 113.4 (C-3), 114.1 (C-5'), 115.7-115.9 (C-3"', C-5"'), 116.3-116.5 (C-2"), 117.4-117.5 (C-5"), 118.5 (C-7), 121.8 (C-3'), 124.1-124.2 (C-6"), 124.5 (C-4a), 127.4 (C-1'), 128.1-129.4 (C-2', C-6', C-5), 128.8 (C-2"', C-6"', C-8), 132.8 (C-1"), 134.5 (C-6), 136.5 (C-1"'), 148.1 (C-4"), 148.5 (C-8a), 150.0 (C-3"), 151.7 (C-2), 159.6 (C-4"), 161.2 (CO_2Et), 163.2 (C-4'), 163.7 (CONH), 176.8 (C-4). LC-MS (m/z): positive mode 608 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 88.6%. mp 210-212 °C.

5.2.21.42 Ethyl-6-(4-((*tert*-butoxycarbonyl)amino)phenyl)-8-(4-((4-fluorobenzyl)-oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 129 (*ANM197)*



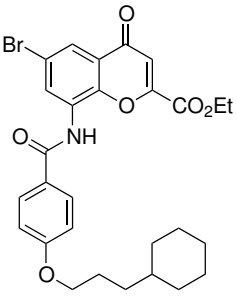
The compound was synthesized according to GP-10 using **47** (194 mg, 0.79 mmol) and **31** (264 mg, 0.6 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (330 mg, 82% yield). ^{1}H NMR: δ 0.23 (s, 6H, Si($C\underline{H}_3$)$_2$), 0.97 (s, 9H, Si(C-$\underline{H}_3$)$_3$), 1.26 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.21 (s, 2H, CH_2-O), 6.95-7.01 (m, 3H, 3"'-H, 5"'-H, 3-H), 7.15-7.26 (m, 4H, 3"-H, 5"-H, 3'-H, 5'-H), 7.50-7.56 (m, 2H, 2H, 2"'-H, 6"'-H), 7.63-7.68 (m, 2H, 2"-H, 6"-H), 7.99-8.05 (m, 3H, 5-H, 2'-H, 6'-H), 8.37 (d, J = 2.3 Hz, 1H, 7-H), 10.14 (s, 1H, CONH). ^{13}C NMR: δ 4.4 (Si($\underline{C}H_3$)$_2$), 13.8 ($CH_2\underline{C}H_3$), 18.1 (Si$\underline{C}$), 25.7 (SiC($\underline{C}H_3$)$_3$), 62.8 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.6 (C-3), 114.7 (C-3', C-5'), 115.4-115.5 (C-3", C-5"), 117.7 (C-5), 120.7 (C-3"', C-5"'), 124.6 (C-4a), 126.4 (C-1'), 128.8 (C-2"', C-6"'), 128.5 (C-7), 129.1 (C-8), 129.8 (C-2', C-6'), 130.2 (C-2", C-6"), 131.5 (C-1"), 133.0 (C-6, C-1"'), 148.3 (C-8a), 151.8 (C-2), 155.7 (C-4"'), 160.0 (C-4'), 161.0-162.9 (C-4"), 161.4 (CO_2Et), 164.9 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 668 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 83.7%. mp 172-173 °C.

5.2.21.43 Ethyl-8-(3-fluor-4-(4-fluorobenzyloxy)benzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylate 145 (*THB24)*



The compound was synthesized according to GP-10 using **26** (278 mg, 1.2 mmol) and **55** (317 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (363 mg, 72% yield). ^{1}H NMR: δ 1.28 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.30 (s, 2H, CH_2-O), 7.00 (s, 1H, 3-H), 7.22 (t, J = 8.9 Hz, 2H, 3‴-H, 5‴-H), 7.41-7.47 (m, 2H, 2‴-H, 6‴-H), 7.49-7.57 (m, 5H, 2′-H, 3′-H, 4′-H, 5′-H, 6′-H), 7.70-7.77 (m, 2H, 5″-H, 6″-H), 7.86-7.92 (m, 1H, 2″-H), 8.10 (d, J = 2.3 Hz, 1H, 7-H), 8.41 (d, J = 2.3 Hz, 1H, 5-H), 10.09 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$), 69.9 (CH_2-O), 113.4 (C-3), 115.0 (C-5″), 115.2-115.3 (C-3‴, C-5‴), 118.4 (C-5), 124.5 (C-3″), 124.6 (C-7), 126.7 (C-2′, C-6′), 127.9 (C-4a), 128.0 (C-4′), 128.7 (C-1′, C-8), 129.0 (C-3′, C-5′), 129.8 (C-2″, C-6″), 129.9 (C-2‴, C-6‴), 137.6 (C-1‴), 138.1 (C-6), 151.7 (C-1″), 152.3 (C-8a), 153.0 (C-2), 154.2 (C-4″), 159.6 (CO_2Et), 163.8 (C-4‴), 166.1 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 556 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 89.0%. mp 249-250 °C.

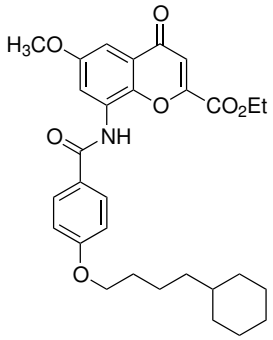
5.2.21.44 Ethyl 6-bromo-8-(4-(3-cyclohexylpropoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 172 (*ANM25)*



The compound was synthesized according to GP-10 using **17** (281 mg, 0.9 mmol) and **291** (314 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (249 mg, 37% yield). ^{1}H NMR: δ 0.91-1.79 (m, 18H, Cyclohexyl-H, 2″-H, 3″-H, $CH_2C\underline{H}_3$), 4.08 (t, J = 6.5 Hz, 2H, 1″-H), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.05-7.10 (m, 2H, 3′-H, 5′-H), 7.94 (d, J = 2.4 Hz, 1H, 5-H), 7.96-8.01 (m, 2H, 2′-H, 6′-H), 8.38 (d, J = 2.4 Hz, 1H, 7-H), 9.87 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 25.5-26.0 (C-2″, C-3‴, C-4‴, C-5‴), 32.6 (C-2‴, C-6‴), 32.8 (C-3″), 62.6 ($\underline{C}H_2CH_3$), 68.3 (C-1″), 113.5 (C-3), 114.3 (C-3′, C-5′), 117.7 (C-6), 122.4 (C-5), 125.2 (C-1′), 126.0 (C-4a), 129.4 (C-2′,

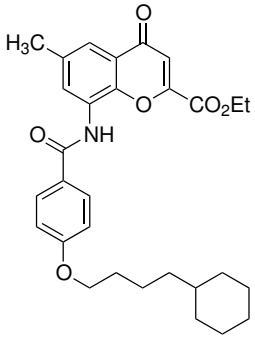
C-6'), 130.3 (C-8), 131.0 (C-7), 148.3 (C-8a), 151.8 (C-2), 159.4 (C-4'), 162.0 (CO_2Et), 169.3 (CONH), 177.5 (Cq, C-4). LC-MS (m/z): positive mode 557 $[M+H]^+$, negative mode 554 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.4%. mp 268-271 °C.

5.2.21.45 Ethyl 8-(4-(4-cyclohexylbutoxy)benzamido)-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate 173 (*ANM111)*



The compound was synthesized according to GP-10 using **24** (177 mg, 0.68 mmol) and **77** (250 mg, 0.91 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (142 mg, 40% yield). ^{1}H NMR: δ 0.84-0.89 (m, 2H, cyclohexyl), 1.07-1.27 (m, 6H, 4"-H, cyclohexyl), 1.30 (t, J = 7.7 Hz, 3H, $CH_2C\underline{H}_3$), 1.40-1.51 (m, 2H, 3"-H), 1.55-1.80 (m, 7H, 2"-H, cyclohexyl), 3.90 (s, 3H, OCH_3), 4.09 (t, J = 6.5 Hz, 2H, 1"-H), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.93 (s, 1H, 3-H), 7.06-7.08 (m, 2H, 3'-H, 5'-H), 7.27 (d, J = 3.1 Hz, 1H, 5-H), 7.85 (d, J = 3.0 Hz, 1H, 7-H), 7.96-8.00 (m, 2H, 2'-H, 6'-H), 9.75 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 22.5 (C-3"), 25.6 (C-3"', C-5"'), 26.0 (C-4"'), 28.7 (C-2"', C-6"'), 36.2 (C-4"), 36.8 (C-1"'), 55.8 (OCH_3), 62.4 ($\underline{C}H_2CH_3$), 67.9 (C-1"), 101.0 (C-5), 112.5 (C-3), 114.3 (C-3', C-5'), 118.0 (C-7), 124.6 (C-4a), 125.8 (C-1'), 129.3 (C-2', C-6'), 129.7 (C-8), 143.5 (C-8a), 151.6 (C-6), 156.6 (C-2), 159.7 (CO_2Et), 161.8 (C-4'), 164.6 (CONH), 176.5 (C-4). LC-MS (m/z): positive mode 522 $[M+H]^+$, negative mode 520 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 174-175 °C.

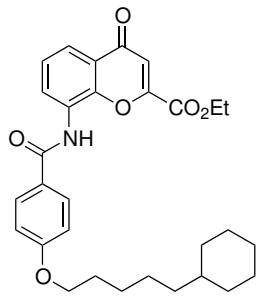
5.2.21.46 Ethyl 8-(4-(4-cyclohexylbutoxy)benzamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylate 174 (*ANM110)*



The compound was synthesized according to GP-10 using **23** (167 mg, 0.68 mmol) and **77** (250 mg, 0.91 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (291 mg, 64% yield). ^{1}H NMR: δ 0.84-0.89 (m, 2H, cyclohexyl), 1.20-1.35 (m, 9H, 4"-H, cyclohexyl, $CH_2C\underline{H}_3$), 1.42-1.46 (m, 2-H, 3"-H), 1.65-1.75 (m, 7H, 2"-H, cyclohexyl), 2.46 (s,

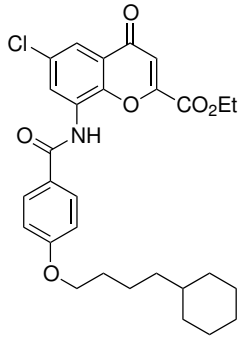
3H, CH_3), 4.09 (t, J = 6.5 Hz, 2H, 1″-H), 4.36 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.93 (s, 1H, 3-H), 7.04-7.08 (m, 2H, 3′-H, 5′-H), 7.68 (dd, J = 2.1 Hz, 1H, 5-H), 7.96-8.00 (m, 2H, 2′-H, 6′-H), 8.00 (d, J = 2.2 Hz, 1H, 7-H), 9.75 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 20.5 (CH_3), 22.5 (C-4″), 25.6 (C-3‴, C-5‴), 26.0 (C-3″), 28.7 (C-2‴, C-6‴), 32.6 (C-2‴, C-6‴), 36.2 (C-2″), 36.8 (C-1‴), 62.4 ($\underline{C}H_2CH_3$), 67.9 (C-1″), 113.3 (C-3), 114.2 (C-3′, C-5′), 120.1 (C-5), 123.8 (C-4a), 125.9 (C-1′), 128.1 (C-8), 129.3 (C-2′, C-6′), 130.7 (C-7), 135.3 (C-6), 147.0 (C-8a), 151.5 (C-2), 159.7 (CO_2Et), 161.6 (C-4′), 164.6 (CONH), 176.8 (C-4). LC-MS (m/z): positive mode 506 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 92.0%. mp 165-166 °C.

5.2.21.47 Ethyl 8-(4-((5-cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 178 (*ANM155)*



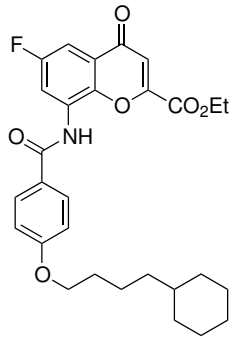
The compound was synthesized according to GP-10 using **19** (179 mg, 0.68 mmol) and **79** (250 mg, 0.91 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (207 mg, 60% yield). ^{1}H NMR: δ 0.86-0.95 (m, 2H, Cyclohexyl), 1.08-1.27 (m, 6H, Cyclohexyl, 5″-H), 1.28 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.32-1.50 (m, 4H, 4″-H, 3″-H), 1.60-1.78 (m, 7H, 2″-H, Cyclohexyl), 4.00-4.22 (m, 2H, 1″-H), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.96 (s, 1H, 3-H), 7.04-7.14 (m, 2H, 3′-H, 5′-H), 7.54 (t, J = 7.9 Hz, 1H, 6-H), 7.88 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 7.95-8.01 (m, 2H, 2′-H, 6′-H), 8.16 (dd, J = 1.58, 7.8 Hz, 1H, 7-H), 9.79 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CH_2\underline{C}H_3$), 25.6 (C-3‴, C-5‴), 25.8-26.0 (C-5″, C-4″), 28.4 (C-3″), 32.7 (C-2‴, C-6‴), 36.5 (C-2″), 36.8 (C-1‴), 62.4 ($\underline{C}H_2CH_3$), 67.9 (C-1″), 113.1 (C-3), 114.2 (C-3′, C-5′), 120.6 (C-5), 124.1 (C-4a), 125.2 (C-7), 126.0 (C-1′), 128.4 (C-8), 129.3 (C-2′, C-6′), 129.5 (C-6), 148.7 (C-8a), 152.8 (C-2), 160.9 (CO_2H), 161.6 (C-4′), 164.7 (CONH), 177.2 (C-4). LC-MS (m/z): positive mode 507 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 186-188 °C.

5.2.21.48 Ethyl 6-chloro-8-(4-(4-cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 175 (*ANM166)*



The compound was synthesized according to GP-10 using **22** (203 mg, 0.76 mmol) and **79** (270 mg, 1.01 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (263 mg, 50% yield). ^{1}H NMR: δ 0.86-0.95 (m, 2H, Cyclohexyl), 1.08-1.30 (m, 9H, Cyclohexyl, 4''-H, $CH_2C\underline{H}_3$), 1.32-1.50 (m, 2H, 3''-H), 1.60-1.78 (m, 7H, 2''-H, Cyclohexyl), 4.09 (t, J = 6.5 Hz, 2H, 1''-H), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.04-7.14 (m, 2H, 3'-H, 5'-H), 7.80 (d, J = 2.5 Hz, 1H, 5-H), 7.95-8.01 (m, 2H, 2'-H, 6'-H), 8.27 (d, J = 2.6 Hz, 1H, 7-H), 9.87 (s, 1H, CONH). ^{13}C NMR: δ 13.6 ($CH_2\underline{C}H_3$), 22.5 (C-4''), 25.6 (C-3''', C-5'''), 26.2 (C-3''), 28.7 (C-3''), 32.7 (C-2''', C-6'''), 36.2 (C-2''), 36.8 (C-1'''), 62.6 ($\underline{C}H_2CH_3$), 67.9 (C-1''), 113.4 (C-3), 114.3 (C-3', C-5'), 119.2 (C-7), 124.9 (C-4a), 125.2 (C-7), 125.5 (C-1'), 128.2 (C-5), 129.4 (C-2', C-6'), 130.0-130.3 (C-6, C-8), 147.1 (C-8a), 151.8 (C-2), 159.4 (CO_2H), 161.9 (C-4'), 164.6 (CONH), 175.8 (C-4). LC-MS (m/z): positive mode 526 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.6%. mp 162-163 °C.

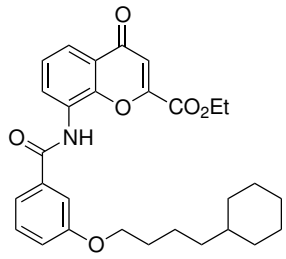
5.2.21.49 Ethyl 8-(4-(4-cyclohexylbutoxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylate 176 (*ANM175)*



The compound was synthesized according to GP-10 using **21** (176 mg, 0.70 mmol) and **79** (258 mg, 1.01 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (214 mg, 60% yield). ^{1}H NMR: δ 0.86-0.95 (m, 2H, Cyclohexyl), 1.08-1.30 (m, 9H, Cyclohexyl, 4''-H, $CH_2C\underline{H}_3$), 1.32-1.50 (m, 2H, 3''-H), 1.60-1.78 (m, 7H, 2''-H, Cyclohexyl), 4.09 (t, J = 6.5 Hz, 2H, 1''-H), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.04-7.14 (m, 2H, 3'-H, 5'-H), 7.53 (dd, J = 3.1, 8.1 Hz, 1H, 5-H), 7.95-8.01 (m, 2H, 2'-H, 6'-H), 8.15 (dd, J = 3.1, 9.8 Hz, 1H, 7-H), 9.86 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CH_2\underline{C}H_3$), 22.5 (C-4''), 25.6 (C-3''', C-5'''), 26.0 (C-3''), 28.7 (C-3''), 32.6 (C-2''', C-6'''), 36.2 (C-2''), 36.8 (C-1'''), 62.5

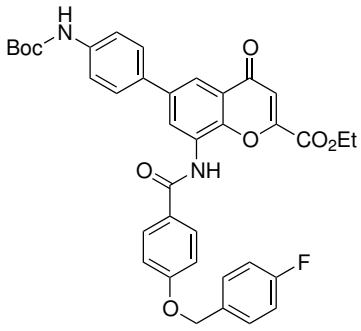
($\underline{C}H_2CH_3$), 67.9 (C-1''), 104.5-104.7 (C-5), 112.7 (C-3), 114.3 (C-3', C-5'), 116.3-116.5 (C-7), 124.7-124.8 (C-4a), 125.4 (C-1'), 129.4 (C-2', C-6'), 130.6-130.7 (C-8), 144.8 (C-8a), 151.7 (C-2), 157.5 (C-6), 159.5 (CO_2H), 161.9 (C-4'), 164.5 (CONH), 176.2 (C-4). LC-MS (m/z): positive mode 510 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.1%. mp 151-152 °C.

5.2.21.50 Ethyl 8-(3-(4-cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 159 (*ANM176)*



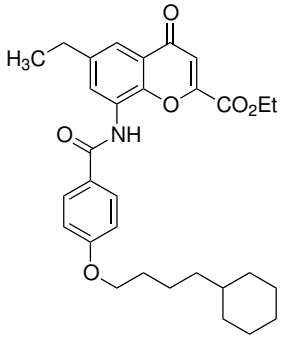
The compound was synthesized according to GP-10 using **19** (142 mg, 0.61 mmol) and **101** (225 mg, 0.81 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a yellow-white solid (183 mg, 61% yield). ^{1}H NMR: δ 0.83-0.89 (m, 2H, Cyclohexyl), 1.08-1.29 (m, 9H, Cyclohexyl, 4''-H, $CH_2C\underline{H}_3$), 1.32-1.50 (m, 2H, 3''-H), 1.60-1.78 (m, 7H, 2''-H, Cyclohexyl), 4.02-4.08 (m, 2H, 1''-H), 4.33 (q, J = 7.9 Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.19 (ddd, J = 2.5, 8.2 Hz, 1H, 2'-H), 7.46 (t, J = 7.9 Hz, 1H, 6-H), 7.50-7.60 (m, 3H, 4'-H, 5'-H, 6'-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.09 (dd, J = 1.6, 7.7 Hz, 1H, 7-H), 10.20 (s, 1H, CONH). ^{13}C NMR: δ 13.7 ($CH_2\underline{C}H_3$), 22.9 (C-4''), 25.6 (C-3''', C-5'''), 26.3 (C-4'''), 29.1 (C-3''), 33.0 (C-2''', C-6'''), 36.7 (C-2''), 37.1 (C-1'''), 62.5 ($\underline{C}H_2CH_3$), 67.9 (C-1''), 113.7 (C-3, C-2'), 118.2-119.9 (C-4', C-6'), 121.6 (C-5), 124.4 (C-8), 125.8 (C-7), 128.3 (C-4a), 129.8 (C-5'), 130.9 (C-6), 135.3 (C-1'), 149.4 (C-8a), 151.8 (C-2), 158.9 (C-3'), 159.9 (CO_2Et), 165.3 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 492 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.1%. mp 148-149 °C.

5.2.21.51 Ethyl 6-(4-((*tert*-butoxycarbonyl)amino)phenyl)-8-(4-((4-fluorobenzyl)-oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 130 (*ANM193)*



The compound was synthesized according to GP-10 using **32** (467 mg, 1.1 mmol) and **47** (361 mg, 1.47 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a yellow solid (515 mg, 72% yield). ^{1}H NMR: δ 1.24 (td, J = 7.12 Hz, 3H, $CO_2CH_2C\underline{H}_3$), 1.49 (s, 9H, $C(C\underline{H}_3)_3$), 4.33 (q, J = 7.1 Hz, 2H, $CO_2C\underline{H}_2CH_3$), 5.21 (s, 2H, CH_2), 7.00 (s, 1H, 3-H), 7.15-7.20 (m, 2H, 3'-H, 5'-H), 7.23 (td, J = 8.8 Hz, 2H, 3"-H, 5"-H), 7.51-7.58 (m, 2H, 2"-H, 6"-H), 7.62-7.67 (m, 4H, 2"'-H, 3"'-H, 5"'-H, 6"'-H), 8.01-8.85 (m, 3H, 5-H, 2'-H, 6'-H), 8.36 (d, J = 2.2 Hz, 1H, 7-H), 9.50 (s, 1H, CONH), 10.15 (C-4"'NH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 28.3 ($C(\underline{C}H_3)_3$), 62.8 ($CO_2\underline{C}H_2CH_3$), 68.9 (CH_2), 79.4 ($\underline{C}(CH_3)_3$), 113.6 (C-3), 114.8 (C-3', C-5'), 115.3-115.5 (C-3", C-5"), 117.6 (C-7), 118.7 (C-3"', C-5"'), 124.7 (C-4a), 126.3 (C-1'), 127.3 (C-2"', C-6"'), 128.5 (C-5), 129.2 (C-8), 129.8 (C-2', C-6'), 130.2 (C-2", C-6"), 131.7 (C-1"), 133.0 (C-6), 137.4 (C-1"'), 140.0 (CO_2NH), 148.4 (C-8a), 151.8 (C-4"'), 152.8 (C-2), 159.9 (CO_2Et), 161.0 (C-4"), 162.9 (C-4'), 164.9 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 653 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.9%. mp 232-233 °C.

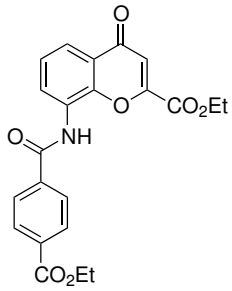
5.2.21.52 Ethyl 8-(4-(4-cyclohexylbutoxy)benzamido)-6-ethyl-4-oxo-4*H*-chromene-2-carboxylate 177 (*ANM216)*



The compound was synthesized according to GP-10 using **20** (235 mg, 0.9 mmol) and **77** (331 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (403 g, 86% yield). ^{1}H NMR: δ 0.88-0.92 (m, 2H, cyclohexyl), 1.24-1.29 (m, 12H, cyclohexyl, 4"-H, C6-$CH_2C\underline{H}_3$, $CO_2CH_2C\underline{H}_3$), 1.42-1.46 (m, 2H, 3"-H), 1.69-1.73 (m, 7H, 2"-H, cyclohexyl), 2.75-2.81 (m, 2H, C6-$C\underline{H}_2CH_3$), 4.07-4.10 (m, 2H, 1"-H), 4.34-4.38 (m, 2H, $CO_2C\underline{H}_2CH_3$), 6.94 (s, 1H, 3-H), 7.06 (dd, J = 1.7, 8.75 Hz, 2H, 3'-H, 5'-H), 7.68-

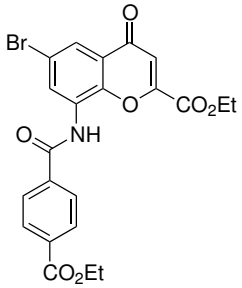
7.02 (m, 1H, 5-H), 7.96-8.00 (m, 2H, 2'-H, 6'-H), 8.01-8.04 (m, 1H, 7-H), 9.76 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CO_2CH_2\underline{C}H_3$), 14.8 (C6-$CH_2\underline{C}H_3$), 22.6 (C-4''), 25.6 (C-3''', C-5'''), 26.0 (C-4'''), 27.6 (C6-$\underline{C}H_2CH_3$), 28.7 (C-3''), 32.7 (C-2''', C-6'''), 36.2 (C-2''), 36.8 (C-1'''), 62.4 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1''), 113.3 (C-3), 114.2 (C-3', C-5'), 118.9 (C-5), 123.9 (C-4a), 126.0 (C-1'), 128.3 (C-8), 129.3 (C-2', C-6'), 129.7 (C-7), 141.5 (C-6), 147.2 (C-8a), 151.5 (C-2), 159.7 ($\underline{C}O_2$Et), 161.6 (C-4'), 164.7 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 520 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 147-148 °C.

5.2.21.53 Ethyl 8-(4-(ethoxycarbonyl)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 117 (*ANM220)*



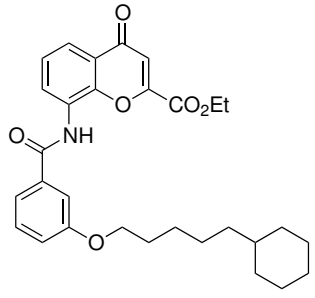
The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **290** (232 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (297 mg, 80% yield). ^{1}H NMR: δ 1.21 (t, J = 7.2 Hz, 3H, C4'-$CO_2CH_2C\underline{H}_3$), 1.36 (t, J = 7.1 Hz, 3H, C2-$CO_2CH_2C\underline{H}_3$), 4.34-4-36 (m, 4H, $C\underline{H}_2CH_3$), 6.99 (s, 1H, 3-H), 7.58 (t, J = 7.9 Hz, 1H, 6-H), 7.97-8.01 (m, 2H, 5-H, 7-H), 8.12-8.16 (m, 4H, 2'-H, 3'-H, 5'-H, 6'-H), 10.50 (s, 1H, CONH). ^{13}C NMR: δ 13.7 (CH, C2-$CO_2CH_2\underline{C}H_3$), 14.3 (C4'-$CO_2CH_2\underline{C}H_3$), 61.3 (C4'-$CO_2\underline{C}H_2CH_3$), 62.8 (C2-$CO_2\underline{C}H_2CH_3$), 113.8 (C-3), 122.2 (C-5), 124.5 (C-8), 125.9 (C-7), 128.0 (C-4a), 128.2 (C-2', C-6'), 129.4 (C-3', C-5'), 131.6 (C-6), 132.9 (C-4'), 138.0 (C-1'), 149.7 (C-8a), 151.9 (C-2), 159.9 (C2-$\underline{C}O_2$Et), 164.9 (CONH), 165.3 (C4'-$\underline{C}O_2$Et), 177.3 (C-4). LC-MS (m/z): positive mode 410 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. mp 201-202 °C.

5.2.21.54 Ethyl 6-bromo-8-(4-(ethoxycarbonyl)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 118 (*ANM223)*



The compound was synthesized according to GP-10 using **17** (280 mg, 0.9 mmol) and **290** (232 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (387 mg, 88% yield). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3H, C4'-$CO_2CH_2C\underline{H}_3$), 1.37 (t, J = 7.1 Hz, 3H, C2-$CO_2CH_2C\underline{H}_3$), 4.34-4-36 (m, 4H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.99 (d, J = 2.5 Hz, 1H, 5-H), 8.12-8.16 (m, 4H, 2'-H, 3'-H, 5'-H, 6'-H), 8.30-8.33 (m, 1H, 7-H), 10.32 (s, 1H, CONH). ^{13}C NMR: δ 13.4 (CH, C2-$CO_2CH_2\underline{C}H_3$), 13.9 (C4'-$CO_2CH_2\underline{C}H_3$), 60.9 (C4'-$CO_2\underline{C}H_2CH_3$), 62.6 (C2-$CO_2\underline{C}H_2CH_3$), 113.6 (C-3), 117.6 (C-6), 123.4 (C-5), 125.3 (C-8), 127.8 (C-2', C-6'), 129.0 (C-3', C-5'), 129.8 (C-4a), 132.2 (C-7), 133.0 (C-4'), 137.4 (C-1'), 148.1 (C-8a), 151.8 (C-2), 159.4 (C2-$\underline{C}O_2$Et), 164.6 (CONH), 164.9 (C4'-$\underline{C}O_2$Et), 175.6 (C-4). LC-MS (m/z): positive mode 490 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.0%. mp 207-208 °C.

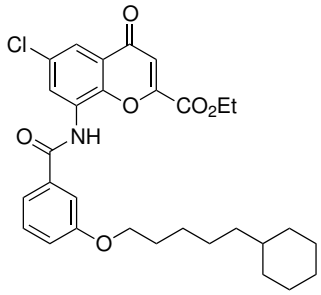
5.2.21.55 Ethyl 8-(3-((5-cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 162 (*ANM241)*



The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **102** (348 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (388 mg, 85% yield). ^{1}H NMR: δ 0.78-0.90 (m, 2H, cyclohexyl), 1.04-1.21 (m, 6H, 5"-H, cyclohexyl), 1.24 (t, J = 8.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.32-1.48 (m, 4H, 3"-H, 4"-H), 1.54-1.79 (m, 7H, 2"-H, cyclohexyl), 4.05 (t, J = 6.5 Hz, 2H, 1"-H), 4.33 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.99 (s, 1H, 3-H), 7.19 (ddd, J = 8.3, 2.4, 1.0 Hz, 1H, 4'-H), 7.46 (t, J = 7.9 Hz, 1H, 2'-H), 7.54-7.60 (m, 3H, 5'-H, 6'-H, 6-H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.08 (dd, J = 7.8, 1.6 Hz, 1H, 7-H), 10.21 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 26.0 (C-5", C-4", C-3"', C-5"'), 26.3 (C-4"'), 28.8 (C-3"), 33.0 (C-2"', C-6"'), 37.0 (C-2"), 37.1 (C-1"'), 62.8 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1"'), 113.7 (C-3, C-2'), 118.3 (C-4'), 119.9 (C-5),

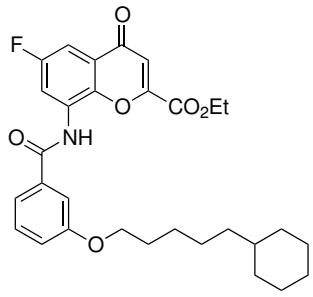
121.7 (C-6'), 124.5 (C-4a), 125.9 (C-7), 128.3 (C-8), 129.8 (C-6), 131.0 (C-5'), 135.4 (C-1'), 149.4 (C-8a), 151.9 (C-2), 158.9 (C-3'), 160.0 ($\underline{C}O_2Et$), 165.3 (CONH), 177.3 (C4). LC-MS (m/z): positive mode 506 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 144-145 °C.

5.2.21.56 Ethyl 6-chloro-8-(3-((5-cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 163 (*ANM242*)



The compound was synthesized according to GP-10 using **22** (160 mg, 0.6 mmol) and **102** (232 mg, 0.8 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (234 mg, 72% yield). ^{1}H NMR: δ 0.83-0.94 (m, 2H, cyclohexyl), 1.14-1.22 (m, 6H, cyclohexyl, 5''-H), 1.29 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.32-1.48 (m, 4H, 3''-H, 4''-H), 1.56-1.80 (m, 7H, 2''-H, cycylohexyl), 4.08 (t, J = 6.5 Hz, 2H, 1''-H), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.20 (dd, J = 8.3 Hz, 1H, 4'-H), 7.47 (t, J = 7.9 Hz, 1H, 2'-H), 7.53-7.60 (m, 2H, 5'-H, 6'-H), 7.80-7.84 (m, 1H, 5-H), 8.25 (d, J = 2.6 Hz, 1H, 7-H), 10.02 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CO_2CH_2\underline{C}H_3$), 25.6 (C-4'', C-5'', C-3''', C-5'''), 26.0 (C-4'''), 28.5 (C-3''), 32.7 (C-2''', C-6'''), 36.5 (C-2''), 36.8 (C-1'''), 62.6 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1''), 113.5 (C-2', C-3), 118.3 (C-4'), 119.6 (C-5, C-6'), 124.9 (C-4a), 128.6 (C-7), 129.6 (C-5'), 130.0 (C-8), 134.9 (C-1'), 147.3 (C-8a), 151.8 (C-2), 158.8 (C-3'), 159.4 ($\underline{C}O_2Et$), 165.0 (CONH), 175.8 (C-4). LC-MS (m/z): positive mode 541 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 148-149 °C.

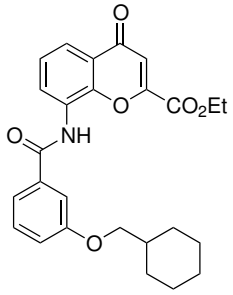
5.2.21.57 Ethyl 8-(3-((5-cyclohexylpentyl)oxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylate 164 (*ANM243*)



The compound was synthesized according to GP-10 using **21** (160 mg, 0.6 mmol) and **102** (232 mg, 0.8 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (198 mg, 63% yield). ^{1}H NMR: δ 0.83-0.93 (m, 2H, cyclohexyl), 1.08-1.27 (m, 6H, cyclohexyl,

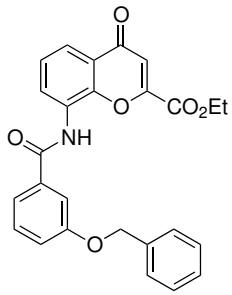
5″-H), 1.30 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.32-1.48 (m, 4H, 3″-H, 4″-H), 1.57-1.81 (m, 7H, 2″-H, cycylohexyl), 4.08 (t, J = 6.5 Hz, 2H, 1″-H), 4.38 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.20 (dd, J = 8.3 Hz, 1H, 4′-H), 7.47 (t, J = 7.9 Hz, 1H, 2′-H), 7.52-7.61 (m, 3H, 5′-H, 6′-H, 5-H), 8.13 (d, J = 3.1 Hz, 1H, 7-H), 10.02 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CO_2CH_2\underline{C}H_3$), 25.6 C-4″, C-5″, C-3‴, C-5‴), 26.0 (C-4‴), 28.5 (C-3″), 32.7 (C-2‴, C-6‴), 36.5 (C-2″), 36.8 (C-1‴), 62.5 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1″), 105.1-105.3 (C-7), 112.8 (C-2′), 113.7 (C-3), 116.8 (C-5), 118.4 (C-4′), 119.5 (C-6′), 124.8 (C-4a), 129.6 (C-5′), 130.3 (C-8), 134.9 (C-1′), 145.1 (C-8a), 151.7 (C-2), 157.5 (C-6), 158.8 (C-3′), 159.5 ($\underline{C}O_2Et$), 165.0 (CONH), 176.2 (C-4). LC-MS (m/z): positive mode 524 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.6%. mp 148-149 °C.

5.2.21.58 Ethyl 8-(3-(cyclohexylmethoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 154 (*ANM259)*



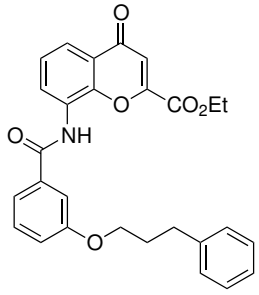
The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **95** (281 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (348 mg, 86% yield). ^{1}H NMR: δ 1.04-1.12 (m, 2H, cyclohexyl), 1.13-1.32 (m, 6H, cyclohexyl, $CH_2C\underline{H}_3$), 1.62-1.87 (m, 7H, cyclohexyl), 3.88 (d, J = 6.3 Hz, 2H, 1″-H), 4.33 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.99 (s, 1H, 3-H), 7.19 (ddd, J = 8.3, 2.6, 1.0 Hz, 1H, 4′-H), 7.46 (t, J = 7.9 Hz, 1H, 2′-H), 7.53-7.60 (m, 3H, 5′-H, 6′-H, 6-H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.07 (dd, J = 7.7, 1.5 Hz, 1H, 7-H), 10.22 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 25.4 (C-3‴, C-5‴), 26.2 (C-4‴), 29.4 (C-2‴, C-6‴), 37.3 (C-1‴), 62.8 ($CO_2\underline{C}H_2CH_3$), 73.1 (C-1″), 113.6 (C-3, C-2′), 118.3 (C-4′), 119.9 (C-5), 121.7 (C-6′), 124.5 (C-4a), 125.9 (C-8), 129.8 (C-6), 131.1 (C-5′), 135.4 (C-1′), 149.5 (C-8a), 151.9 (C-2), 159.0 (C-3′), 160.0 ($\underline{C}O_2Et$), 165.3 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 450 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%. mp 199-201 °C.

5.2.21.59 Ethyl 8-(3-(benzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 167 (*ANM260*)



The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **71** (274 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (268 mg, 67% yield). ^{1}H NMR: δ 1.25 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, 1″-H), 6.97 (s, 1H, 3-H), 7.26-7.31 (m, 1H, 4′-H), 7.31-7.36 (m, 1H, 4‴-H), 7.38-7.43 (m, 2H, 3‴-H, 5‴-H), 7.44-7.52 (m, 3H, 2‴-H, 6‴-H, 2′-H), 7.52-7.59 (m, 1H, 6-H), 7.58-7.64 (m, 1H, 5′-H), 7.66-7.69 (m, 1H, 6′-H), 7.91 (dd, J = 8.0, 1.6Hz, 1H, 5-H), 8.14 (dd, J = 7.7, 1.6 Hz, 1H, 7-H), 9.97 (s, 1H, CONH). ^{13}C NMR: δ 13.4 ($CO_2CH_2\underline{C}H_3$), 62.5 ($CO_2\underline{C}H_2CH_3$), 69.7 (C-1″), 113.46 (C-3), 114.2 (C-2′), 118.4 (C-4′), 119.9 (C-5), 121.2 (C-6′), 124.2 (C-4a), 125.5 (C-7), 127.4 (C-2‴, C-6‴), 127.7 (C-5‴), 128.1 (C-8), 128.2 (C-3‴, C-5‴), 129.5 (C-6), 130.1 (C-5′), 135.4 (C-1′), 136.7 (C-1‴), 148.9 (C-8a), 151.7 (C-2), 158.5 (C-3′), 159.7 ($\underline{C}O_2Et$), 165.0 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 444 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 195-197 °C.

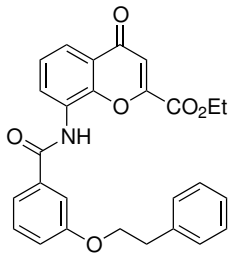
5.2.21.60 Ethyl 4-oxo-8-(3-(3-phenylpropoxy)benzamido)-4*H*-chromene-2-carboxylate 169 (*ANM261*)



The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **83** (308 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (172 mg, 41% yield). ^{1}H NMR: δ 1.24 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.94-2.20 (m, 2H, 2″-H), 4.08 (t, J = 6.3 Hz, 2H, 1″-H), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.17-7.24 (m, 2H, 4′-H, 4‴-H), 7.24-7.28 (m, 2H, 3‴-H, 5‴-H), 7.28-7.34 (m, 2H, 2‴-H, 6‴-H), 7.49 (t, J = 7.9 Hz, 1H, 6-H), 7.56-7.64 (m, 2H, 5′-H, 6′-H), 7.93 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.09 (dd, J = 7.7, 1.6 Hz, 1H, 7-H), 10.25 (s, 1H, CONH). ^{13}C NMR: δ 14.1 ($CO_2CH_2\underline{C}H_3$), 30.8 (C-2″), 31.9 (C-3″), 63.1 ($CO_2\underline{C}H_2CH_3$), 67.5 (C-1″), 114.1 (C-2′, C-3), 118.6 (C-4′), 120.4 (C-5), 122.1 (C-6′), 124.8 (C-7), 128.8 (C-8), 128.8 (C-2‴, C-3‴, C-4‴, C-5‴, C-6‴), 130.2 (C-6), 131.4 (C-5′), 135.7 (C-1‴), 141.8 (C-1′), 149.8 (C-8a), 152.2 (C-2), 159.2

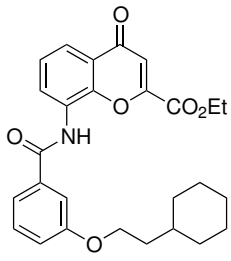
(C-3'), 160.3 ($\underline{C}O_2Et$), 165.6 (CONH), 177.7 (C-4). LC-MS (m/z): positive mode 472 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.9%. mp 157-159 °C.

5.2.21.61 Ethyl 4-oxo-8-(3-phenethoxybenzamido)-4*H*-chromene-2-carboxylate 168 (*ANM262)*



The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **81** (291 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (203 mg, 49% yield). ^{1}H NMR: δ 1.21 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.08 (t, J = 6.8 Hz, 2H, 2"-H), 4.12-4.46 (m, 3H, 1"-H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.17-7.25 (m, 2H, 4'-H, 4"'-H), 7.27-7.39 (m, 4H, 2"'-H, 3"'-H, 5"'-H, 6"'-H), 7.46 (t, J = 7.9 Hz, 1H, 6-H), 7.52-7.62 (m, 3H, 2'-H, 5'-H, 6'-H), 7.91 (dd, J = 8.1, 1.6 Hz, 1H, 5-H), 8.06 (dd, J =7.7, 1.6Hz, 1H, 7-H), 10.22 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 35.1 (C-2"), 62.8 ($CO_2\underline{C}H_2CH_3$), 68.6 (C-1"), 113.7 (C-2', C-3), 118.3 (C-4'), 120.1 (C-5), 121.8 (C-6'), 124.5 (C-4a), 125.9 (C-7), 126.5 (C-4"'), 128.3 (C-8), 128.5 (C-2"', C-6"'), 129.1 (C-3"', C-5"'), 129.9 (C-6), 131.2 (C-5'), 135.4 (C-1'), 138.4 (C-1"'), 149.5 (C-8a), 151.9 (C-2), 158.6 (C-3'), 159.9 ($\underline{C}O_2Et$), 165.9 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 458 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 168-169 °C.

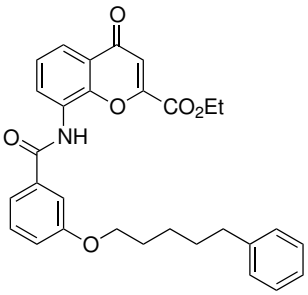
5.2.21.62 Ethyl 8-(3-(2-cyclohexylethoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 155 (*ANM266)*



The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **97** (298 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (232 mg, 78% yield). ^{1}H NMR: δ 0.97-1.10 (m, 2H, cyclohexyl), 1.14-1.27 (m, 2H, cyclohexyl), 1.27-1.33 (m, 3H, $CH_2C\underline{H}_3$), 1.50-1.60 (m, 2H, cyclohexyl), 1.60-1.84 (m, 7H, cyclohexyl, 2"-H), 4.14 (t, J = 6.6 Hz, 2H, 1"-H), 4.38 (t, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.99 (s, 1H, 3-H), 7.21 (ddd, J = 8.1, 2.6, 1.0 Hz, 1H, 4'-H), 7.48 (t, J = 7.9 Hz, 1H, 2'-H), 7.54-7.64 (m, 3H, 6-H, 5'-H, 6'-H), 7.93 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.16 (dd, J = 7.7, 1.6 Hz, 1H, 7-H), 9.97 (s, 1H, CONH).

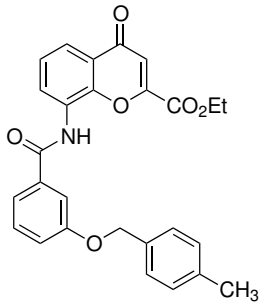
^{13}C NMR: δ 13.4 ($CO_2CH_2\underline{C}H_3$), 25.5 (C-3‴, C-5‴), 25.8 (C-4‴), 32.5 (C-2‴, C-6‴), 34.0 (C-1‴), 35.8 (C-2″), 62.4 ($CO_2\underline{C}H_2CH_3$), 66.0 (C-1″), 113.4-113.6 (C-2′, C-3), 118.0 (C-4′), 119.5 (C-5), 121.1 (C-6′), 124.2 (C-4a), 125.4 (C-7), 128.1 (C-8), 129.4 (C-6), 130.0 (C-5′), 135.3 (C-1′), 148.9 (C-8a), 151.6 (C-2), 158.7 (C-3′), 159.6 ($\underline{C}O_2$Et), 165.0 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 464 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 150-151 °C.

5.2.21.63 Ethyl 4-oxo-8-(3-((5-phenylpentyl)oxy)benzamido)-4*H*-chromene-2-carboxylate 171 (*ANM276)*



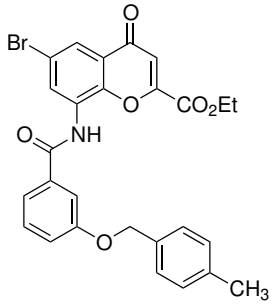
The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **87** (340 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (168 mg, 37% yield). ^{1}H NMR: δ 1.22 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.40-1.53 (m, 2H, 2″-H), 1.60-1.67 (m, 2H, 3″-H), 1.73-1.82 (m, 2H, 5″-H), 2.54-2.63 (m, 2H, 4″-H), 4.05 (t, J = 6.5 Hz, 2H, 1″-H), 4.31 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.12-7.22 (m, 4H, 4′-H, 3‴-H, 4‴-H, 5‴-H), 7.25 (t, J = 7.6 Hz, 2H, 2‴-H, 6‴-H), 7.46 (t, J = 7.9 Hz, 1H, 6-H), 7.52-7.61 (m, 3H, 5′-H, 6′-H, 2′-H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.08 (dd, J = 7.7, 1.5 Hz, 1H, 7-H), 10.21 (s, 1H, CONH). ^{13}C NMR: δ 13.7 ($CO_2CH_2\underline{C}H_3$), 25.3 (C-2″), 28.6 (C-3″), 30.8 (C-5″), 35.2 (C-4″), 62.8 ($CO_2\underline{C}H_2CH_3$), 67.8 (C-1″), 113.6 (C-3, C-2′), 118.2 (C-4′), 119.9 (C-5), 121.7 (C-6′), 124.4 (C-4a), 125.7 (C-7), 128.3 (C-8), 128.3 (C-2‴, C-3‴, C-4‴, C-5‴, C-6‴), 129.8 (C-6), 131.0 (C-5′), 135.3 (C-1′), 142.2 (C-1‴), 149.4 (C-8a), 151.8 (C-2), 158.8 (C-3′), 159.9 ($\underline{C}O_2$Et), 165.3 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 500 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 137-138 °C.

5.2.21.64 Ethyl 8-(3-((4-methylbenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 149 (*ANM229)*



The compound was synthesized according to GP-10 using **19** (209 mg, 0.9 mmol) and **69** (291 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (152 mg, 36% yield). ^{1}H NMR: δ 1.24 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.31 (s, 3H, CH_3), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.16 (s, 2H, CH_2), 6.96 (s, 1H, 2'-H), 7.20 (d, J = 7.7 Hz, 2H, 3"-H, 5"-H), 7.26 (dd, J = 2.6, 8.2 Hz, 1H, 4'-H), 7.36 (d, J = 7.7 Hz, 2H, 2"-H, 6"-H), 7.47 (t, J = 7.9 Hz, 1H, 6'-H), 7.55 (t, J = 7.9 Hz, 1H, 5'-H), 7.61 (d, J = 7.6 Hz, 1H, 6-H), 7.65 (s, 1H, 3-H), 7.90 (dd, J = 1.5, 8.2 Hz, 1H, 5-H), 8.13 (d, J = 7.7 Hz, 1H, 7H), 9.97 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CO_2CH_2\underline{C}H_3$), 20.5 (CH_3), 62.5 ($CO_2\underline{C}H_2CH_3$), 69.6 (CH_2), 113.5 (C-2'), 114.2 (C-4'), 118.4 (C-6'), 119.9 (C-3), 121.2 (C-5), 124.2 (C-4a), 125.5 (C-7), 127.5 (C-2", C-6"), 128.2 (C-8), 128.8 (C-3", C-5"), 129.6 (C-6), 130.1 (C-5'), 133.7 (C-1"), 135.4 (C-1'), 137.0 (C-4"), 149.0 (C-8a), 151.7 (C-3'), 158.5 (C-2), 159.7 ($\underline{C}O_2Et$), 165.1 (CONH), 177.0 (C-4). LC-MS (m/z): positive mode 558 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 196-198 °C.

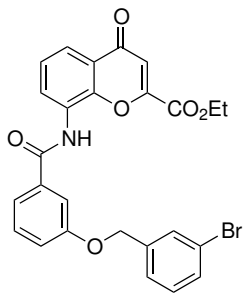
5.2.21.65 Ethyl 6-bromo-8-(3-((4-methylbenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 150 (*ANM232)*



The compound was synthesized according to GP-10 using **17** (281 mg, 0.9 mmol) and **69** (291 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a pale white solid (65 mg, 14% yield). ^{1}H NMR: δ 1.26 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.31 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.16 (s, 2H, CH_2), 7.00 (s, 1H, 2'-H), 7.21 (dt, J = 0.9, 7.7 Hz, 2H, 3"-H, 5"-H), 7.29 (ddd, J = 1.0, 2.6, 8.2 Hz, 1H, 4'-H), 7.36 (m, 2H, 2"-H, 6"-H) 7.48 (t, J = 7.9 Hz, 1H, 3-H), 7.60 (ddd, J = 1.0, 1.7, 7.6 Hz, 1H, 6'-H), 7.7 (dd, J = 1.6, 2.6 Hz, 1H, 5'-H), 7.96 (d, J = 2.4 Hz, 1H, 5-H), 8.36 (d, J = 2.4 Hz, 1H, 7-H), 10.04 (m, 1H, CONH). ^{13}C NMR: δ 13.5 ($CO_2CH_2\underline{C}H_3$), 20.5

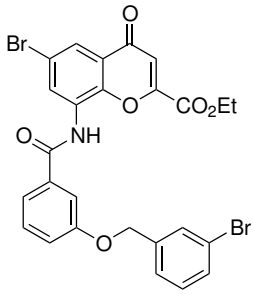
(CH_3), 62.7 ($CO_2\underline{C}H_2CH_3$), 69.7 (CH_2), 113.6 (C-2'), 114.3 (C-4'), 117.7 (C-6), 118.7 (C-6'), 119,9 (C-3), 122.9 (C-7), 125.3 (C-4a), 127.5 (C-2", C-6"), 128.8 (C-3", C-5"), 129.6 (C-5), 130.4 (C-8), 131.2 (C-5'), 133.7 (C-1"), 135.0 (C-1'), 137.0 (C-4"), 147.8 (C-8a), 151.9 (C-3'), 158.6 (C-2), 159.5 ($\underline{C}O_2Et$), 165.0 (CONH), 175.7 (C-4). LC-MS (m/z): positive mode 538 $[M+H]^+$, negative mode 534 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.8%. mp 194-196 °C.

5.2.21.66 Ethyl 8-(3-((3-bromobenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 151 (*ANM233)*



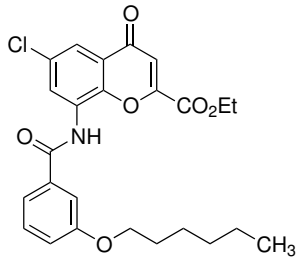
The compound was synthesized according to GP-10 using **19** (140 mg, 0.6 mmol) and **67** (249 mg, 0.8 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a pale white solid (197 mg, 63% yield). ^{1}H NMR: δ 1.21 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.32 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2), 6.99 (s, 1H, 2'-H), 7.30 (ddd, J = 0.9, 2.5, 8.3 Hz, 1H, 4'-H), 7.38 (t, J = 7.8 Hz, 1H, 5"-H), 7.53 (m, 4H, 3-H, 6-H, 2"-H, 6"-H), 7.66 (m, 3H, 5'-H, 6'-H, 4"-H), 7.92 (dd, J = 1.56, 8.0 Hz, 1H, 5-H), 8.08 (dd, J = 1.6, 7.7 Hz, 1H, 7-H), 10.25 (s, 1H, CONH). ^{13}C NMR: δ 14.1 ($CO_2CH_2\underline{C}H_3$), 63.2 ($CO_2\underline{C}H_2CH_3$), 69.0 (CH_2), 114.1 (C-2'), 114.7 (C-4'), 118.8 (C-6'), 120.8 (C-3), 122.1 (C-5), 122.2 (C-3"), 124.8 (C-4a), 126.3 (C-7), 127.0 (C-6"), 128.7 (C-8), 130.3 (C-6), 130.7 (C-5"), 131.2 (C-5'), 131.2 (C-4"), 131.4 (C-2"), 135.8 (C-1'), 140.1 (C-1"), 149.1 (C-8a), 152.2 (C-3'), 158.7 (C-2), 160.3 ($\underline{C}O_2Et$), 165.5 (CONH), 177.7 (C-4). LC-MS (m/z): positive mode 522 $[M+H]^+$, negative mode 520 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 175-176 °C.

5.2.21.67 Ethyl 6-bromo-8-(3-((3-bromobenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 152 (*ANM230)*



The compound was synthesized according to GP-10 using **17** (281 mg, 0.9 mmol) and **67** (368 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a pale white solid (402 mg, 74% yield). ^{1}H NMR: δ 1.22 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.33 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.22 (s, 2H, CH_2), 7.03 (s, 1H, 2'-H), 7.31 (ddd, J = 1.0, 2.6, 8.2 Hz, 1H, 4'-H), 7.38 (t, J = 7.8 Hz, 1H, 5"-H), 7.52 (m, 3H, 3-H, 2"-H, 6"-H), 7.65 (m, 3H, 5'-H, 6'-H, 4"-H), 7.98 (d, J = 2.4 Hz, 1H, 5-H), 8.31 (d, J = 2.4 Hz, 1H, 7-H), 10.34 (s, 1H, CONH). ^{13}C NMR: δ 13.7 ($CO_2CH_2\underline{C}H_3$), 62.9 ($CO_2\underline{C}H_2CH_3$), 68.7 (CH_2), 113.9 (C-2'), 114.4 (C-4'), 118.0 (C-6), 118.7 (C-6'), 120.5 (C-3), 121.9 (C-3"), 123.5 (C-6"), 125.6 (C-4a), 126.7 (C-7), 130.0 (C-5"), 130.2 (C-8), 130.3 (C-5), 130.8 (C-5'), 130.9 (C-4"), 132.5 (C-2"), 135.1 (C-1'), 139.7 (C-1"), 148.3 (C-8a), 152.0 (C-3'), 158.3 (C-2), 159.7 ($\underline{C}O_2$Et), 165.2 (CONH), 176.1 (C-24). LC-MS (m/z): negative mode 597 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.5%. mp 198-200 °C.

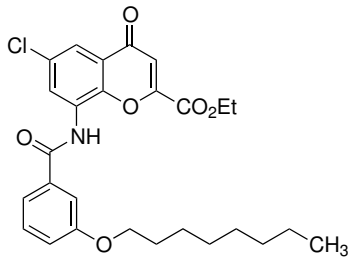
5.2.21.68 Ethyl 6-chloro-8-(3-(hexyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 165 (*ANM331)*



The compound was synthesized according to GP-10 using **22** (240 mg, 0.9 mmol) and **89** (268 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (40 mg, 9% yield). ^{1}H NMR: δ 0.84-0.92 (m, 2H, 6"-H), 1.24 (td, J = 7.1, 1.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.32 (m, 4H, 4"-H, 5"-H), 1.38-1.49 (m, 2H, 3"-H), 1.72-1.76 (m, 2H, 2"-H), 4.06 (t, J = 6.5 Hz, 2H, 1"-H), 4.21-4.50 (m, 2H, $C\underline{H}_2CH_3$), 7.02 (s, 1H, 3-H), 7.15-7.24 (m, 1H, 4'-H), 7.47 (t, J = 7.9 Hz, 1H, 5'-H), 7.54-7.63 (m, 2H, 2'-H, 6'-H), 7.84 (dd, J = 2.6 Hz, 1H, 5-H), 8.19 (dd, J = 2.5, 1.0 Hz, 1H, 7-H), 10.41 (s, 1H, CONH). ^{13}C NMR: δ 13.75 ($CO_2CH_2\underline{C}H_3$), 22.2 (C-5"), 25.3 (C-4"), 28.7 (C-3"), 31.1 (C-2"), 62.9 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1"), 113.7 (C-3, C-2'), 118.5 (C-4'), 120.0 (C-6'), 120.3 (C-5), 125.2 (C-4a), 129.9 (C-7, C-5'), 130.1

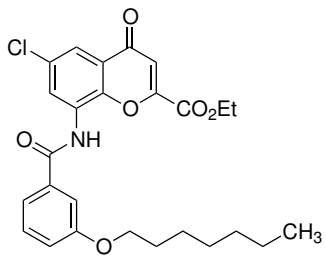
(C-6, C-1'), 134.9 (C-8), 148.0 (C-8a), 152.0 (C-2), 158.9 (C-3'), 159.7 ($\underline{C}O_2Et$), 165.3 (CONH), 176.3 (C-4). LC-MS (m/z): positive mode 472 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.9%. mp 149-150 °C.

5.2.21.69 Ethyl 6-chloro-8-(3-(octyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 294 (*ANM333)*



The compound was synthesized according to GP-10 using **22** (240 mg, 0.9 mmol) and **93** (301 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (312 mg, 70% yield). ^{1}H NMR: δ 0.84-0.90 (m, 2H, 8"-H), 1.23-1.40 (m, 13H, $CH_2C\underline{H}_3$, 4"-H, 5"-H, 6"-H, 7"-H), 1.38-1.49 (m, 2H, 3"-H), 1.72-1.76 (m, 2H, 2"-H), 4.08 (t, J = 6.5 Hz, 2H, 1"-H), 4.37 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.20 (ddd, J = 8.2, 2.6, 1.0 Hz, 1H, 4'-H), 7.47 (t, J = 7.9 Hz, 1H, 5'-H), 7.52-7.61 (m, 2H, 2'-H, 6'-H), 7.82 (d, J = 2.6 Hz, 1H, 5-H), 8.25 (d, J = 2.6 Hz, 1H, 7-H), 10.01 (s, 1H, CONH). ^{13}C NMR: δ 13.4-13.5 ($CO_2CH_2\underline{C}H_3$, C-8"), 21.8 (C-7"), 25.3 (C-6"), 28.5 (C-3", C-4", C-5"), 30.0 (C-2"), 62.6 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1"), 113.5-113.7 (C-3, C-2'), 118.3 (C-4'), 119.6 (C-6', C-5'), 124.9 (C-4a), 128.7 (C-7), 129.6 (C-5'), 130.0 (C-6, C-1'), 134.9 (C-8), 147.3 (C-8a), 151.8 (C-2), 158.8 (C-3'), 159.4 ($\underline{C}O_2Et$), 165.0 (CONH), 176.8 (C-4). LC-MS (m/z): positive mode 501 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.5%. mp 151-153 °C.

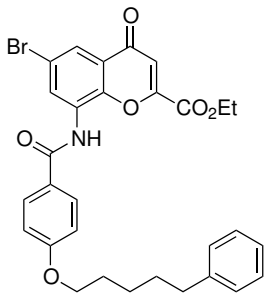
5.2.21.70 Ethyl 6-chloro-8-(3-(heptyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 166 (*ANM336)*



The compound was synthesized according to GP-10 using **22** (240 mg, 0.9 mmol) and **91** (283 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (234 mg, 54% yield). ^{1}H NMR: δ 0.84-0.91 (m, 2H, 7"-H), 1.24-1.41 (m, 11H, $CH_2C\underline{H}_3$, 4"-H, 5"-H, 6"-H), 1.42-1.51 (m, 2H, 3"-H), 1.71-1.81 (m, 2H, 2"-H), 4.08 (t, J = 7.9 Hz,

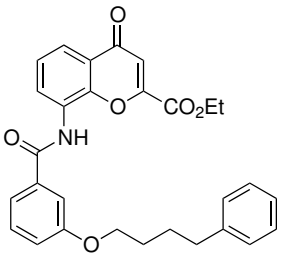
2H, 1″-H), 4.38 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$CH$_3$), 7.00 (s, 1H, 3-H), 7.20 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H, 4′-H), 7.47 (t, *J* = 7.9 Hz, 1H, 5′-H), 7.54-7.61 (m, 2H, 2′-H, 6′-H), 7.83 (d, *J* = 2.6 Hz, 1H, 5-H), 8.25 (d, *J* = 2.6 Hz, 1H, 7-H), 10.02 (s, 1H, CONH). ^{13}C NMR: *δ* 13.8 (CO$_2$CH$_2$<u>C</u>H$_3$, C-7″), 22.0 (C-6″), 25.6 (C-5″), 28.5 (C-3″, C-4″), 31.3 (C-2″), 62.9 (CO$_2$<u>C</u>H$_2$CH$_3$), 68.2 (C-1″), 113.8-114.0 (C-3, C-2′), 118.7 (C-4′), 119.9 (C-6′, C-5′), 125.2 (C-4a), 129.9 (C-7), 130.3 (C-5′), 130.3 (C-6, C-1′), 135.2 (C-8), 147.7 (C-8a), 152.1 (C-2), 159.1 (C-3′), 159.7 (<u>C</u>O$_2$Et), 165.4 (CONH), 176.1 (C-4). LC-MS (m/z): positive mode 486 [M+H]$^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.0%. mp 153-155 °C.

5.2.21.71 Ethyl 6-bromo-4-oxo-8-(4-((5-phenylpentyl)oxy)benzamido)-4*H*-chromene-2-carboxylate 179 (*THB31)*



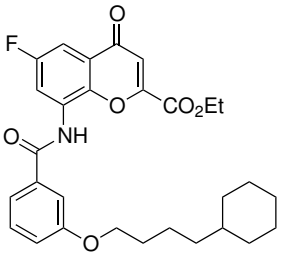
The compound was synthesized according to GP-10 using **17** (280 mg, 0.9 mmol) and **75** (341 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (229 mg, 44% yield). ^{1}H NMR: *δ* 1.29 (t, *J* = 7.1 Hz, 3H, CH$_2$C<u>H</u>$_3$), 1.48 (ddd, *J* = 6.5, 8.6, 15.1 Hz, 2H, 3″-H), 1.67 (p, *J* = 7.5 Hz, 2H, 4″-H), 1.76-1.83 (m, 2H, 2″-H), 2.60-2.64 (m, 2H, 5″-H), 4.09 (t, *J* = 6.5 Hz, 2H, 1″-H), 4.37 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$CH$_3$), 7.00 (s, 1H, 3-H), 7.05-7.08 (m, 2H, 3′-H, 5′-H), 7.14-7.21 (m, 4H, 2‴-H, 3‴-H, 5‴-H, 6‴-H), 7.25-7.28 (m, 1H, 4‴-H), 7.89-8.01 (m, 3H, 2′-H, 6′-H, 5-H), 8.38 (d, *J* = 2.5 Hz, 1H, 5-H), 9.89 (s, 1H, CONH). ^{13}C NMR: *δ* 13.4 (CO$_2$CH$_2$<u>C</u>H$_3$), 24.9 (C-2″), 28.2 (C-3″), 30.2 (C-5″), 34.9 (C-4″), 62.6 (CO$_2$<u>C</u>H$_2$CH$_3$), 67.8 (C-1″), 113.5 (C-3), 114.3 (C-3′, C-5′), 117.7 (C-6), 122.4 (C-5), 125.2-125.4 (C-4a, C-1′), 125.4 (C-4‴), 128.0 (C-2′, C-6′, C-2‴, C-6‴), 129.4 (C-3‴, C-5‴), 130.0 (C-8), 131.0 (C-7), 142.0 (C-1‴), 147.5 (C-8a), 151.8 (C-2), 159.4 (C-4′), 161.8 (CO$_2$Et), 164.6 (CONH), 175.7 (C-4). LC-MS (m/z): positive mode 580 [M+H]$^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.7%. mp 176-177 °C.

5.2.21.72 Ethyl 4-oxo-8-(3-(4-phenylbutoxy)benzamido)-4*H*-chromene-2-carboxylate 170 (*THB41)*



The compound was synthesized according to GP-10 using **19** (105 mg, 0.45 mmol) and **85** (162 mg, 0.6 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (206 mg, 94% yield). ^{1}H NMR: δ 1.21 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.74 (ddd, J = 3.2, 7.1 Hz, 2H, 2″-H), 2.49 (p, J = 1.8 Hz, 2H, 3″-H), 2.61-2.69 (m, 2H, 4″-H), 4.09 (t, J = 5.9 Hz, 2H, 1″-H), 4.31 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (s, 1H, 3-H), 7.13-7.34 (m, 6H, 2′-H, 2‴-H, 3‴-H, 4‴-H, 5‴-H, 6‴-H), 7.34-7.51 (m, 1H, 6-H), 7.53-7.61 (ddd, J = 3.2, 7.1 Hz, 3H, 4′-H, 5′-H, 6′-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.09 (dd, J = 1.6, 7.7 Hz, 1H, 7-H), 10.19 (s, 1H, CONH). ^{13}C NMR: δ 13.7 ($CO_2CH_2\underline{C}H_3$), 27.6 (C-2″), 28.4 (C-3″), 34.9 (C-4″), 62.8 ($CO_2\underline{C}H_2CH_3$), 67.7 (C-1″), 113.7 (C-3), 118.2-119.9 (C-4′, C-6′), 121.7 (C-5), 124.4 (C-8), 125.8 (C-4‴), 125.9 (C-2‴, C-6‴), 128.3 (C-4a), 128.4 (C-7, C-3‴, C-5‴), 129.8 (C-5′), 131.0 (C-6), 135.4 (C-1′), 142.1 (C-1‴), 149.4 (C-8a), 151.8 (C-2), 158.8 (C-3′), 159.9 (CO_2Et), 165.3 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 486 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 92.0%. mp 136-137 °C.

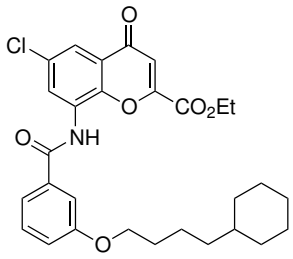
5.2.21.73 Ethyl 8-(3-(4-cyclohexylbutoxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylate 161 (*THB64)*



The compound was synthesized according to GP-10 using **21** (113 mg, 0.45 mmol) and **101** (176 mg, 0.6 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (109 mg, 48% yield). ^{1}H NMR: δ 0.80-0.90 (m, 2H, Cyclohexyl), 1.05-1.35 (m, 6H, Cyclohexyl, 4″-H), 1.27 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.39-1.48 (m, 2H, 3″-H), 1.57-1.77 (m, 7H, 2″-H, Cyclohexyl), 4.06 (t, J = 6.4 Hz, 2H, 1″-H), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.00 (s, 1H, 3-H), 7.18-7.24 (m, 1H, 2′-H), 7.34-7.43 (m, 1H, 5′-H), 7.44-7.63 (m, 3H, 5-H, 4′-H, 6′-H), 8.10 (dd, J = 3.1, 9.7 Hz, 1H, 7-H), 10.30 (s, 1H, CONH). ^{13}C NMR: δ 13.7 ($CO_2CH_2\underline{C}H_3$), 22.9 (C-4″), 26.0 (C-3‴, C-5‴), 26.3 (C-4‴), 29.1 (C-3″), 33.0 (C-2‴, C-6‴), 36.7 (C-2″), 37.2

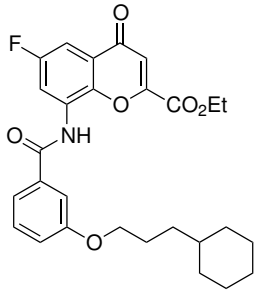
(C-1‴), 62.9 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1″), 105.8 (C-5), 113.1 (C-2′), 113.7 (C-3), 118.2 (C-7), 118.5-119.9 (C-4′, C-6′), 125.2 (C-4a), 129.9 (C-5′), 130.5 (C-8), 135.0 (C-1′), 145.7 (C-8a), 152.0 (C-2), 153.2 (C-2), 157.7 (C-6), 158.9 (C-3′), 158.9 (CO_2Et), 165.3 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 510 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 93.1%. mp 147-148 °C.

5.2.21.74 Ethyl 6-chloro-8-(3-(4-cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 160 (*THB65)*



The compound was synthesized according to GP-10 using **22** (240 mg, 0.9 mmol) and **101** (352 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a yellow solid (259 mg, 48% yield). ^{1}H NMR: δ 0.78-0.93 (m, 2H, cyclohexyl), 1.02-1.27 (m, 9H, $CH_2C\underline{H}_3$, cyclohexyl, 4″-H), 1.38-1.49 (m, 2H, 3″-H), 1.54-1.77 (m, 7H, 2″-H, cyclohexyl), 4.05 (t, J = 6.4 Hz, 2H, 1″-H), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.68-7.76 (m, 5H, 2′-H, 3-H, 4′-H, 5′-H, 6′-H), 7.84 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.31 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 22.9 (C-4″), 26.0 (C-3‴, C-5‴), 26.3 (C-4‴), 29.1 (C-3″), 33.0 (C-2‴, C-6‴), 36.7 (C-2″), 37.2 (C-1‴), 62.9 ($CO_2\underline{C}H_2CH_3$), 67.9 (C-1″), 105.9 (C-6), 113.1-113.7 (C-3, C-2′), 118.2 (C-7), 118.6-118.5 (C-4′, C-6′), 125.2 (C-4a), 129.9 (C-5), 130.5 (C-8), 130.1 (C-5′), 135.0 (C-1′), 145.7 (C-8a), 152.0 (C-2), 157.7 (C-3′), 158.9 (CO_2Et), 165.3 (CONH), 176.5 (C-4). LC-MS (m/z): positive mode 526 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 94.2%. mp 147-148 °C.

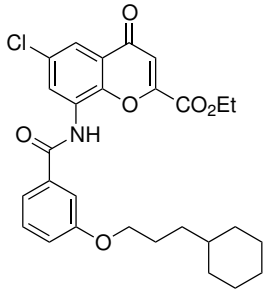
5.2.21.75 Ethyl 8-(3-(3-cyclohexylpropoxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylate 158 (*THB66)*



The compound was synthesized according to GP-10 using **21** (226 mg, 0.9 mmol) and **99** (314 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (256 mg, 53% yield).^{1}H NMR: δ 0.79-0.98 (m, 2H, Cyclohexyl), 1.05-1.37 (m, 9H, $CH_2C\underline{H}_3$, Cyclohexyl, 3″-H), 1.56-

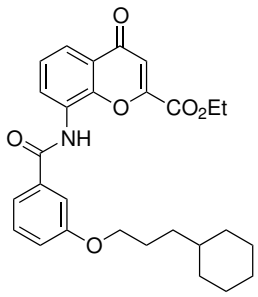
1.80 (m, 7H, 2''-H, Cyclohexyl), 4.04 (t, J = 6.5 Hz, 2H, 1''-H), 4.35 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.01 (s, 1H, 3-H), 7.18-7.24 (m, 1H, 2'-H), 7.37-7.53 (m, 1H, 5'-H), 7.54-7.63 (m, 3H, 5-H, 4'-H, 6'-H), 8.10 (dd, J = 3.1, 9.6 Hz, 1H, 7-H), 10.31 (s, 1H, CONH). ^{13}C NMR: δ 13.7 ($CO_2CH_2\underline{C}H_3$), 25.8-26.2 (C-3''', C-5''', C-4'''. C-3''), 32.9 (C-2''', C-6'''), 33.2 (C-2''), 36.8 (C-1'''), 62.8 ($CO_2\underline{C}H_2CH_3$), 68.2 (C-1''), 105.6 (C-5), 113.0 (C-2'), 113.7 (C-3), 117.9 (C-7), 118.4-119.8 (C-4', C-6'), 125.0 (C-4a), 129.9 (C-5'), 130.5 (C-8), 134.9 (C-1'), 145.6 (C-8a), 151.9 (C-2), 156.8 (C-6), 158.8 (C-3'), 159.7 (CO_2Et), 165.4 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 496 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.7%. mp 147-148 °C.

5.2.21.76 Ethyl 6-chloro-8-(3-(3-cyclohexylpropoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 157 (*THB67)*



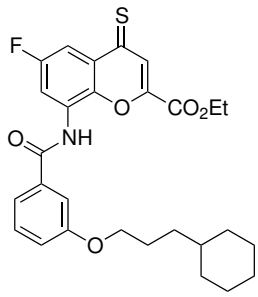
The compound was synthesized according to GP-10 using **22** (240 mg, 0.9 mmol) and **99** (314 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (259 mg, 58% yield). ^{1}H NMR: δ 0.84-0.94 (m, 2H, Cyclohexyl), 1.04-1.23 (m, 6H, Cyclohexyl, 3''-H), 1.26 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.28-1.80 (m, 8H, 2'''-H, 6'''-H, 3''-H, 2''-H, Cyclohexyl), 4.04 (t, J = 6.4 Hz, 2H, 1''-H), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.02 (s, 1H, 3-H), 7.20 (ddd, J = 2.5, 7.5 Hz, 1H, 2'-H), 7.49 (dt, J = 7.9 Hz, 3H, 5'-H, 4'-H, 6'-H), 7.84 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.30 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 25.9 (C-3''', C-5''', C-4'''. C-3''), 33.0 (C-2''', C-6'''), 33.3 (C-2''), 36.9 (C-1'''), 62.9 ($CO_2\underline{C}H_2CH_3$), 68.3 (C-1''), 113.8-114.6 (C-3, C-2'), 118.5 (C-7), 120.0-120.4 (C-4', C-6'), 125.2 (C-4a), 129.7 (C-5), 129.9 (C-8, C-6), 130.2 (C-5'), 135.0 (C-1'), 147.9 (C-8a), 152.0 (C-2), 158.9 (C-3'), 159.7 (CO_2H), 165.3 (CONH), 176.3 (C-4). LC-MS (m/z): positive mode 512 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.2%. mp 129-130 °C.

5.2.21.77 Ethyl 8-(3-(3-cyclohexylpropoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 156 (*THB68)*



The compound was synthesized according to GP-10 using **19** (210 mg, 0.9 mmol) and **99** (314 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (207 mg, 48% yield). ^{1}H NMR: δ 0.83-0.94 (m, 2H, cyclohexyl), 1.08-1.24 (m, 6H, Cyclohexyl, 3"-H), 1.24 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.26-1.80 (m, 7H, 2"-H, Cyclo- hexyl), 4.04 (t, J = 6.4 Hz, 2H, 1"-H), 4.33 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.99 (s, 1H, 3-H), 7.19 (dd, J = 1.7, 8.3 Hz, 1H, 2'-H), 7.46 (t, J = 7.9 Hz, 1H, 6-H), 7.54-7.61 (m, 3H, 4'-H, 5'-H, 6'-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.08 (dd, J = 1.7, 7.8 Hz, 1H, 7-H), 10.20 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 25.9-26.3 (C-3"', C-5"', C-4"'. C-3"), 33.0 (C-2"', C-6"'), 33.3 (C-2"), 36.9 (C-1"'), 62.9 ($CO_2\underline{C}H_2CH_3$), 68.2 (C-1"), 113.7-114.6 (C-3, C-2'), 118.2-119.9 (C-4', C-6'), 121.7 (C-5), 124.5 (C-8), 125.9 (C-7), 128.3 (C-4a), 129.8 (C-5'), 131.0 (C-6), 135.4 (C-1'), 149.4 (C-8a), 151.9 (C-2), 158.9 (C-3'), 159.9 (CO_2H), 165.3 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 478 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 85.1%. mp 129-130 °C.

5.2.21.78 Ethyl 8-(3-(3-cyclohexylpropoxy)benzamido)-6-fluoro-4-thioxo-4*H*-chromene-2-carboxylate 180 (*ANM319)*

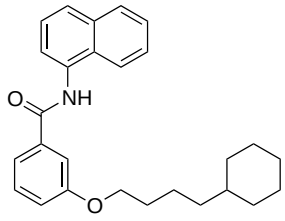


The compound was synthesized according to GP-10 using **36** (200 mg, 0.76 mmol) and **99** (280 mg, 1.01 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a green solid (115 mg, 30% yield). ^{1}H NMR: δ 0.88-0.99 (m, 2H, 4"'-H), 1.12-1.40 (m, 11H, $CH_2C\underline{H}_3$, 2"'-H, 3"'-H, 5"'-H, 6"'-H), 1.57-1.82 (m, 5H, 1"'-H, 3"-H, 2"-H), 4.07 (t, J = 6.5 Hz, 2H, 1"-H), 4.39 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.21 (ddd, J = 8.2, 2.6, 1.0 Hz, 1H, 4'-H), 7.48 (t, J = 7.9 Hz, 1H, 5'-H), 7.53-7.62 (m, 2H, 2'-H, 6'-H), 7.69 (s, 1H, 3-H), 7.85 (dd, J = 9.1, 3.1 Hz, 1H, 5-H), 8.19 (dd, J = 9.4, 3.1 Hz, 1H, 7-H), 10.06 (s, 1H, CONH). ^{13}C NMR: δ 13.5 ($CO_2CH_2\underline{C}H_3$), 25.6 (C-3"', C-5"'), 26.0 (C-3"), 32.5 (C-2"', C-6"'), 32.6 (C-2"), 36.6

(C-1‴), 62.7 ($CO_2\underline{C}H_2CH_3$), 68.3 (C-1″), 107.2-107.4 (C-7), 113.7 (C-2′), 117.1-117.4 (C-5), 118.4 (C-4′), 119.6 (C-6′), 124.0 (C-3), 129.6 (C-6), 130.9 (Cq, C4a); 131.4 (Cq, C1?), 134.8 (C-8a), 140.6 (C-2), 142.2 (C-8), 158.8-165.0 (C-3′), 160.0 ($\underline{C}O_2Et$), 160.6 (CONH), 201.7 (C-4). LC-MS (m/z): positive mode 512 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 78.0%. mp 145-147 °C.

5.2.22 3-(4-Cyclohexylbutoxy)-*N*-(naphthalen-1-yl)benzamide

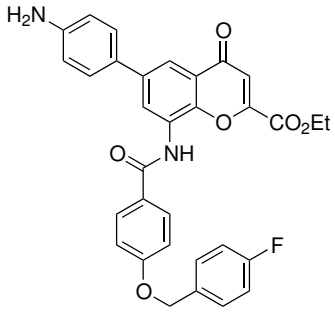
5.2.22.1 3-(4-Cyclohexylbutoxy)-*N*-(naphthalen-1-yl)benzamide 295 (*ANM277*)



The compound was synthesized according to GP-10 using naphthalen-1-amine (129 mg, 0.9 mmol) and **102** (332 mg, 1.2 mmol). The precipitate was filtered off, washed three times with 1 mL of DCM each and dried in vacuum at 50 °C. The product was isolated as a white solid (298 mg, 82% yield). ^{1}H NMR: δ 0.82-0.80 (m, 2H, cyclohexyl), 1.03-1.31 (m, 6H, cyclohexyl, 4″-H), 1.39-1.52 (m, 2H, 3″-H), 1.54-1.80 (m, 7H, 2″-H, cyclohexyl), 4.06 (t, J = 6.5 Hz, 2H, 1″-H), 7.16 (dd, J = 8.1, 2.5 Hz, 1H, 2′-H), 7.45 (t, J = 7.9 Hz, 1H, 4′-H), 7.50-7.60 (m, 4H, 2-H, 3-H, 5′-H, 6′-H), 7.60-7.68 (m, 2H, 6-H, 7-H), 7.86 (d, J = 7.9 Hz, 1H, 4-H), 7.93-7.99 (m, 2H, 5-H, 8-H), 10.37 (s, 1H, CONH). ^{13}C NMR: δ 25.9 (C-3″), 26.0 (C-3‴, C-5‴), 26.3 (C-4‴), 29.1 (C-4″), 33.0 (C-2‴, C-6‴), 36.7 (C-2″), 37.1 (C-1‴), 67.8 (C-1″), 113.6 (C-2′), 118.1 (C-4′), 120.0 (C-6′), 123.5 (C-2), 124.1 (C-4), 125.6 (C-8), 126.1 (C-7), 126.2 (C-6), 126.4 (C-3), 128.2 (C-5), 129.4 (C-8a), 129.7 (C-5′), 133.9-134.0 (C-4a, C-1′), 135.9 (C-1), 158.8 (C-3′), 166.0 (CONH). LC-MS (m/z): positive mode 402 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 136-137 °C.

5.2.23 Ethyl 6-(4-aminophenyl)-8-(4-((4-fluorobenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate

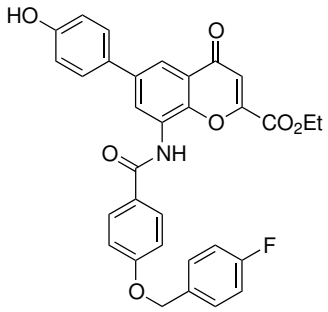
5.2.23.1 Ethyl 6-(4-aminophenyl)-8-(4-((4-fluorobenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylate 132 (*ANM198)*



130 (487 mg, 0.75 mmol) was supended in 5 mL of THF and heated to 45 °C. Then a solution of HCl in Dioxan (4N, 10 mL) was added and the reaction mixture stirred overnight at rt. After removing the solvent under reduced pressure the solid was filtrated, washed twice with cold THF (5 mL each) an dried in vacuum at 45 °C. The product was isolated as an orange solid (393 mg, 95% yield). ^{1}H NMR: δ 1.23-1.26 (m, 3H, $CO_2CH_2C\underline{H}_3$), 4.34 (q, J = 7.1 Hz, 2H, $CO_2C\underline{H}_2CH_3$), 5.21 (s, 2H, C4‴-NH), 7.02 (s, 1H, 3-H), 7.16-7.21 (m, 2H, 3′-H, 5′-H), 7.21-7.25 (m, 2H, 3″-H, 5″-H), 7.35 (d, J = 8.0 Hz, 2H, 2‴-H, 6‴-H), 7.51-7.57 (m, 2H, 2″-H, 6″-H), 7.78-7.82 (m, 2H, 3‴-H, 5‴-H), 8.01-8.07 (m, 2H, 2′-H, 6′-H), 8.07 (d, J = 2.3 Hz, 1H, 5-H), 8.40 (d, J = 2.3 Hz, 1H, 7-H), 10.22 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CO_2CH_2\underline{C}H_3$), 62.8 ($CO_2\underline{C}H_2CH_3$), 113.7 (C-3), 114.8 (C-3′, C-5′), 115.3-115.5 (C-3″, C-5″), 118.1 (C-7), 122.1 (C-3‴, C-5‴), 124.7 (C-4a), 126.3 (C-1′), 128.2 (C-2‴, C-6‴), 128.6 (C-5), 129.3 (C-8), 129.8 (C-2′, C-6′), 130.1-130.2 (C-2″, C-6″), 133.0 (C-1″, C-6), 136.8 (C-1‴), 148.7 (C-8a), 151.8 (C-2), 159.9 (CO_2Et), 161.0 (C-4″), 162.9 (C-4′), 164.9 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 553 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.1%. mp 215-216 °C.

5.2.24 Ethyl-8-(4-((4-fluorobenzyl)oxy)benzamido)-6-(4-hydroxyphenyl)-4-oxo-4*H*-chromene-2-carboxylate

5.2.24.1 Ethyl-8-(4-((4-fluorobenzyl)oxy)benzamido)-6-(4-hydroxyphenyl)-4-oxo-4*H*-chromene-2-carboxylate 131 (*ANM199*)

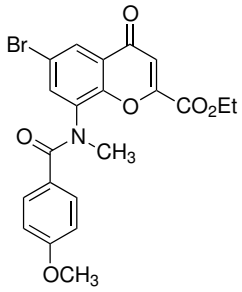


129 (294 mg, 0.44 mmol) was suspended in 10 mL of THF. After addition of 2 mL of conc. HCl the reaction mixture was refluxed for 4 hours. The solvent was removed under reduced pressure and after addition of 5 mL of water the obtained precipitate was filtered of, washed with water and dried in vacuum at 45 °C. The product was isolated as a yellow solid (236 mg, 89% yield). ^{1}H NMR: δ 1.25 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.34 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 5.21 (s, 2H, CH_2-O), 6.86-6.92 (m, 2H, 3‴-H, 5‴-H), 6.99 (s, 1H, 3-H), 7.15-7.21 (m, 2H, 3′-H, 5′-H), 7.23 (t, J = 8.9 Hz, 2H, 3″-H, 5″-H), 7.54 (dd, J = 5.1 Hz, 2H, 2″-H, 6″-H), 7.56-7.61 (m, 2H, 2‴-H, 6‴-H), 7.98 (d, J = 2.3 Hz, 1H, 5-H), 8.00-8.05 (m, 2H, 2′-H, 6′-H), 8.33 (d, J = 2.3 Hz, 1H, 7-H), 9.69 (s, 1H, OH), 10.13 (s, 1H, CONH). ^{13}C NMR: δ 13.8 ($CH_2\underline{C}H_3$), 62.8 ($\underline{C}H_2CH_3$), 68.9 (CH_2-O), 113.5 (C-3), 114.8 (C-3′, C-5′), 115.3-115.5 (C-3″, C-5″), 116.2 (C-3‴, C-5‴), 117.2 (C-5), 124.6 (C-4a), 126.4 (C-1′), 128.2 (C-2‴, C-6‴), 128.4 (C-7), 128.9-129.0 (C-1″, C-8), 129.8 (C-2′, C-6′), 130.2 (C-2″, C-6″), 133.0 (C-6), 137.8 (C-1‴), 148.1 (C-8a), 151.7 (C-2), 160.0 (C-4‴), 161.0 (C-4′), 161.3 (CO_2Et), 162.9 (C-4″), 164.9 (CONH), 177.3 (C-4). LC-MS (m/z): positive mode 554 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 89.3%. mp 291-292 °C.

5.2.25 Ethyl 6-bromo-8-(4-methoxy-N-methylbenzamido)-4-oxo-4*H*-chromene-2-carboxylate

5.2.25.1 Ethyl 6-bromo-8-(4-methoxy-N-methylbenzamido)-4-oxo-4*H*-chromene-2-carboxylate 181

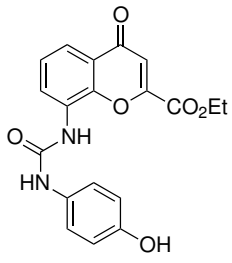
(ANM76)



103 (312 mg, 0.7 mmol) and K_2CO_3 (193 mg, 1.4 mmol) were suspended in 10 mL of DMF. After addition of methyliodide (0.056 mL, 0.9 mmol) the reaction mixture was stirred for 2 days at rt under an argon atmosphere. The reaction was stopped by addition of 22 mL of water and the mixture was extracted 3 times with 25 mL EtOAc. The product was separated by column chromatography on a column of silica gel (40% Cyclohexane in EtOAc) and dried in vacuum at 50 °C. The product was isolated as a yellow solid (244 mg, 76% yield). ^{1}H NMR: δ 1.36 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.39 (s, 3H, NCH_3), 3.68 (s, 3H, OCH_3), 4.41 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.74-6.78 (m, 2H, 3'-H, 5'-H), 6.94 (s, 1H, 3-H), 7.25-7.29 (m, 2H, 2'-H, 6'-H), 7.96 (d, J = 2.4, 1H, 5-H), 8.11 (d, J = 2.5, 1H, 7-H). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 55.3 (CH, OCH_3), 63.0 ($\underline{C}H_2CH_3$), 113.3 (C-3', C-5'), 113.8 (C-3), 118.0 (C-6), 125.7 (C-5), 125.8 (C-4a), 127.4 (C-1'), 129.9 (C-2', C-6'), 136.7 (C-7), 149.7 (C-8a), 152.1 (C-2), 159.6 (C-4'), 160.5 (CO_2Et), 169.8 (CON), 176.0 (C-4). LC-MS (m/z): positive mode 460 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. Mp 137-138 °C.

5.2.26 Ethyl 8-(3-(4-hydroxyphenyl)ureido)-4-oxo-4*H*-chromene-2-carboxylate

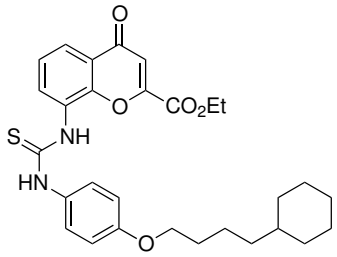
5.2.26.1 Ethyl 8-(3-(4-hydroxyphenyl)ureido)-4-oxo-4*H*-chromene-2-carboxylate 182 (*THB19*)



Triphosgene (1.04 g, 3.5 mmol) was dissolved in 4 mL of DCM and cooled to 0-5 °C in an ice bath. Then a mixture of **19** (234 mg, 1 mmol), DIPEA (0.375 mL) in 8.75 mL DCM was added slowly over a period of 90 min under an argon atmosphere. After stirring for further 5 min at 0-5 °C the reaction mixture was heated to rt and added dropwise to a mixture of p-aminophenol (109 mg, 1 mmol), DIPEA (0.212 mL) and 5 mL DCM under an argon atmosphere and stirred overnight at rt. The solvent was removed under reduced pressure and the crude solid suspended in 30 mL of DCM. After addition of 1 mL of MeOH the product was crystallized by removing half of the DCM and separated by column chromatography on a column of silica gel (0.5% MeOH in DCM). The product was isolated as a white solid (219 mg, 66% yield). ^{1}H NMR: δ 1.36 (t, J = 3.5 Hz, 3H, $CH_2C\underline{H}_3$), 4.41-4.43 (m, 2H, $C\underline{H}_2CH_3$), 6.86 (s, 1H, 3-H), 7.16-7.28 (m, 4H, 2'-H, 3'-H, 5'-H, 6'-H), 7.44 (t, J = 8.0 Hz, 1H, 6-H), 7.66 (dd, J = 1.5, 7.9 Hz, 1H, 5-H), 7.76 (dd, J = 1.5, 7.9 Hz, 1H, 7-H), 8.21 (s, 1H, C1'-NH), 8.87 (s, 1H, C8-NH), 9.11 (s, 1H, OH). ^{13}C NMR: δ 13.6 ($CH_2\underline{C}H_3$), 62.6 ($\underline{C}H_2CH_3$), 110.6 (C-3), 113.5 (C-5), 115.2 (C-3', C-5'), 117.2 (C-7), 120.9 (C-2', C-6'), 124.0 (C-4a), 125.0 (C-6), 129.6 (C-8), 130.6 (C-8a), 146.0 (C-1'), 151.6 (C-4'), 152.4 (NCN), 153.0 (C-2), 159.7 (CO_2Et), 177.0 (C-4). LC-MS (m/z): positive mode 369 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 72.6%. Mp 249-250 °C.

5.2.27 Ethyl 8-(3-(4-(4-cyclohexylbutoxy)phenyl)thioureido)-4-oxo-4*H*-chromene-2-carboxylate

5.2.27.1 Ethyl 8-(3-(4-(4-cyclohexylbutoxy)phenyl)thioureido)-4-oxo-4*H*-chromene-2-carboxylate 185 (*ANM179)*



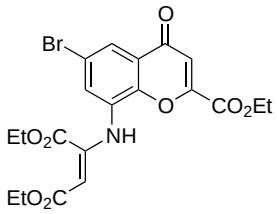
184 (245 mg, 0.9 mmol) was dissolved in 3 mL of dry THF under argon. Then **187** (333 mg, 1.35 mmol) in mL of dry THF was added and the reaction mixture stirred overnight at rt. The product was separated by two column chromatographies on a column of silica gel (1. 25 % cylcohexane in EtOAc, 2. 50 % cylcohexane in EtOAc). The product was isolated as a yellow oil (210 mg, 40% yield). ^{1}H NMR: δ 0.81-0.90 (m, 2H, Cyclohexyl), 1.33 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.37-1.43 (m, 2H, 3''-H), 1.61-1.70 (m, 7H, 2''-H, Cyclohexyl), 3.94 (t, J = 6.5 Hz, 2H, 1''-H), 4.39 (q, J = 7.1 Hz, 2H $C\underline{H}_2CH_3$), 6.89-6.92 (m, 2H, 3'-H, 5'-H), 6.98 (s, 1H, 3-H), 7.32-7.37 (m, 2H, 2'-H, 6'-H), 7.50 (t, J = 7.9 Hz, 1H, 6-H), 7.90 (ddd, J = 1.6, 7.9 Hz, 2H, 5-H, 7-H), 9.41 (s, 1H, C1'-NH), 9.89 (s, 1H, C2'-NH). ^{13}C NMR: δ 14.0 ($CH_2\underline{C}H_3$), 22.9 (C-4''), 26.0 (C-3''', C-5'''), 26.3 (C-4'''), 29.1 (C-3''), 33.0 (C-2''', C-6'''), 36.7 (C-2''), 37.1 (C-1'''), 62.9 ($\underline{C}H_2CH_3$), 67.8 (C-1''), 113.9 (C-3), 114.6 (C-3', C-5'), 122.3 (C-5), 124.6 C-4a), 125.5 (C-7), 126.5 (C-2', C-6'), 129.8 (C-8), 131.7 (C-1'), 133.6 (C-6), 150.7 (C-8a), 151.9 (C-2), 156.5 (C-4'), 160.0 (CO_2Et), 177.4 (C-4), 180.9 (C-5). LC-MS (m/z): positive mode 524 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 71.9%.

5.2.28 Diethyl 2-((2-(ethoxycarbonyl)-4-oxo-4*H*-chromen-8-yl)amino)fumarates

5.2.28.1 General procedure for the synthesis of the diethyl 2-((2-(ethoxycarbonyl)-4-oxo-4*H*-chromen-8-yl)amino)fumarates (GP-11)

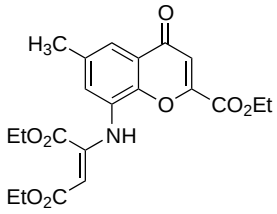
The appropriate amine (4 mmol) was dissolved in MeOH (8 mL) and diethyl acetylendicarboxylate (0.96 mL, 6 mmol) was added slowly over a period of 30 min. Then the reaction mixture was stirred overnight at room temperature and the precipitated solid was filtered, washed with MeOH and dried in vacuum at 50 °C.

5.2.28.2 Diethyl 2-((6-bromo-2-(ethoxycarbonyl)-4-oxo-4*H*-chromen-8-yl)amino)fumarate 270
(*ANM360)*



The compound was synthesized according to GP-11 using **17** (1.98 g, 6.5 mmol). The solvent was removed by reduced pressure and the resulting solid was filtered and washed with cold MeOH. The product was isolated as a yellow solid (1.02 g, 33% yield). ^{1}H NMR: δ 1.14, 1.26 (t, J = 7.0 Hz, 6H, C1'-$CO_2CH_2C\underline{H}_3$, C2'-$CO_2CH_2C\underline{H}_3$), 1.39 (t, J = 7.1 Hz, 3H, C2-$CO_2CH_2C\underline{H}_3$), 4.20 (p, J = 6.9 Hz, 4H, C1'-$CO_2C\underline{H}_2CH_3$, C2'-$CO_2C\underline{H}_2CH_3$), 4.42 (q, J = 7.0 Hz, 2H, C2-$CO_2C\underline{H}_2CH_3$), 5.71 (s, 1H, 2'-H), 6.87-7.07 (m, 1H, 3-H), 7.53 (q, J = 2.9 Hz, 1H, 7-H), 7.79 (q, J = 2.3 Hz, 1H, 5-H), 9.73 (s, 1H, NH). ^{13}C NMR: δ 13.3 (C1'-$CO_2CH_2\underline{C}H_3$, C2'-$CO_2CH_2\underline{C}H_3$), 13.9 (C2-$CO_2CH_2\underline{C}H_3$), 59.9, 62.0 (C1'-$CO_2\underline{C}H_2CH_3$, C2'-$CO_2\underline{C}H_2CH_3$), 62.6 (C2-$CO_2\underline{C}H_2CH_3$), 97.8 (C-2'), 113.6 (C-3), 117.6 (C-6), 120.8 (C-5), 125.2 (C-4a), 127.5 (C-7), 132.5 (C-8), 144.6 (C-1'), 146.6 (C-8a), 151.7 (C-2), 159.2 (C2-$\underline{C}O_2$Et), 162.4 (C1'-$\underline{C}O_2$Et), 167.4 (C2'-$\underline{C}O_2$Et), 175.5 (C-4). LC-MS (m/z): positive mode 482 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 90.2%. Mp 111-112 °C.

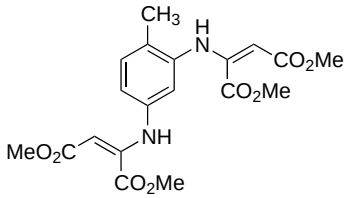
5.2.28.3 Diethyl 2-((2-(ethoxycarbonyl)-6-methyl-4-oxo-4*H*-chromen-8-yl)amino)fumarate 271
(*ANM377)*



The compound was synthesized according to GP-11 using **23** (1.0 g, 4 mmol). The solvent was removed by reduced pressure and the resulting solid was filtered and washed with cold MeOH. The product was isolated as a yellow solid (1.29 g, 77% yield). ^{1}H NMR: δ 1.05, 1.23 (t, J = 7.1 Hz, 6H, C1'-$CO_2CH_2C\underline{H}_3$, C2'-$CO_2CH_2C\underline{H}_3$), 1.36 (t, J = 7.1 Hz, 3H, C2-$CO_2CH_2C\underline{H}_3$), 2.37 (s, 3H, C6-CH_3), 4.14 (dq, emphJ = 7.1 Hz, 4H, C1'-$CO_2C\underline{H}_2CH_3$, C2'-$CO_2C\underline{H}_2CH_3$), 4.37 (q, J = 7.1 Hz, 2H, C2-$CO_2C\underline{H}_2CH_3$), 5.56 (s, 1H, 2'-H), 6.92 (s, 1H, 3-H), 7.16.7.24 (m, J = 2.1 Hz, 1H, 7-H), 7.53 (dd, J = 2.0, 1.0 Hz, 1H, 5-H), 9.82 (s, 1H, NH). ^{13}C NMR: δ 13.6-13.8 (C1'-$CO_2CH_2\underline{C}H_3$, C2'-$CO_2CH_2\underline{C}H_3$), 14.3 (C2-$CO_2CH_2\underline{C}H_3$), 20.7 (C6-CH_3), 60.1, 62.2 (C1'-$CO_2\underline{C}H_2CH_3$, C2'-$CO_2\underline{C}H_2CH_3$), 62.8 (C2-$CO_2\underline{C}H_2CH_3$), 95.6 (C-2'), 113.6 (C-3), 119.0 (C-5), 124.1 (C-6), 126.8 (C-7), 130.7 (C-4a), 135.7 (C-8), 145.9 (C-8a), 146.4 (C-1'), 151.6 (C-2), 159.6 (C2-$\underline{C}O_2$Et), 162.9 (C1'-$\underline{C}O_2$Et), 168.3 (C2'-$\underline{C}O_2$Et),

177.1 (C-4). LC-MS (m/z): positive mode 418 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 84.5%. Mp 100-101 °C.

5.2.28.4 (E)-tetramethyl 2,2'-((4-methyl-1,3-phenylene)bis(azanediyl))dimaleate 276 (*ANM363)*



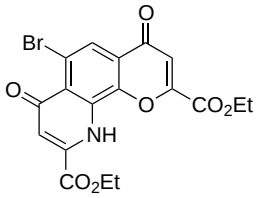
The compound was synthesized according to GP-11 using diaminotoluene (4.88 mg, 40 mmol). The solvent was removed by reduced pressure and the resulting yellow oil was separated by column chromatography on a column of silica gel (gradient: 20% EtOAc in cyclohexane). The product was isolated as a pale white solid (8.58 mg, 63% yield). ^{1}H NMR: δ 2.20 (s, 3H, C1-CH_3), 3.64-3.67 (m, 12H, CO_2CH_3), 5.26-5.33 (m, 2H, 2'-H, 2"-H), 6.37 (d, J = 2.2 Hz, 1H, 3-H), 6.59 (dd, J = 8.1, 2.3 Hz, 1H, 5-H), 7.13 (dd, J = 8.1, 0.9 Hz, 1H, 6-H), 9.45-9.49 (m, 2H, NH). ^{13}C NMR: δ 16.9 (C1-$\underline{C}H_3$), 51.2, 51.3, 53.0, 53.1 ($CO_2\underline{C}H_3$), 91.91, 93.69 (C-2', C-2"), 113.5 (C-3), 116.7 (C-5), 125.5 (C-1), 131.1 (C-6), 139.0, 139.3 (C-2, C-4), 147.2, 147.3 (C-1', C-1"), 163.9, 164.4 (C1'-$\underline{C}O_2CH_3$, C1"-$\underline{C}O_2CH_3$), 168.5, 169.2 (C2'-$\underline{C}O_2CH_3$, C2"-$\underline{C}O_2CH_3$). LC-MS (m/z): positive mode 343 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.4%. Mp 104-106 °C.

5.2.29 Diethyl 4,7-dioxo-7,10-dihydro-4*H*-pyrano[3,2-h]quinoline-2,9-dicarboxylates

5.2.29.1 General procedure for the synthesis of the diethyl 4,7-dioxo-7,10-dihydro-4H-pyrano[3,2-h]quinoline-2,9-dicarboxylates (GP-12)

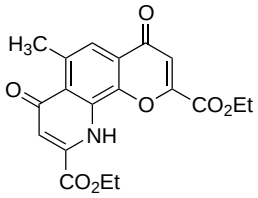
The appropriate dimethylaminomaleate or diethylaminomaleate was added to boiling Dowtherm A® (2 mL/g) and stirred for 2-30 min at 250 °C. After cooling the mixture to room temperature the poduct precipitated, was filtered off, washed with acetone and dried in oven at 60 °C.

5.2.29.2 Diethyl 6-bromo-4,7-dioxo-7,10-dihydro-4*H*-pyrano[3,2-h]quinoline-2,9-dicarboxylate 274 (*ANM366)*



The compound was synthesized according to GP-12 using **270** (964 mg, 2 mmol) and was isolated as a yellow solid (245 mg, 28% yield). ^{1}H NMR: δ 1.40 (td, J = 7.1 Hz, 6H, $CO_2CH_2C\underline{H}_3$), 4.42 (dq, J = 7.1 Hz, 4H, $CO_2C\underline{H}_2CH_3$), 7.11 (s, 2H, 3-H, 8-H), 8.04 (s, 1H, 5H). 13C nicht gelöst! LC-MS (m/z): positive mode 436 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.8%. Mp 236-237 °C.

5.2.29.3 Diethyl 6-methyl-4,7-dioxo-7,10-dihydro-4*H*-pyrano[3,2-h]quinoline-2,9-dicarboxylate 275 (*ANM378)*



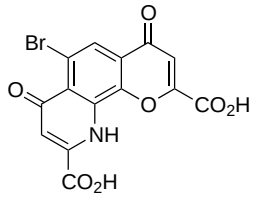
The compound was synthesized according to GP-12 using **271** (1.25 g, 3 mmol) and was isolated as a yellow solid (220 mg, 20% yield). ^{1}H NMR: δ 1.36-1.44 (m, 6H, $CO_2CH_2C\underline{H}_3$), 2.85 (s, 3H, CH_3), 4.40-4.52 (m, 2H, $CO_2\underline{C}H_2CH_3$), 7.08 (s, 1H, 3-H), 7.69 (s, 2H, 5-H, 8-H). ^{13}C NMR: δ 14.0 ($CO_2CH_2\underline{C}H_3$), 24.0 (CH_3), 61.6, 62.8 ($CO_2\underline{C}H_2CH_3$), 108.8 (C-8), 115.1 (C-3), 121.0 (C-5), 123.1-124.0 (C-4a, C-6a), 133.4 (C-9), 141.4 (C-6), 151.3, 152.8 (C-10a, C-10b), 160.1 (C-2), 160.1 ($\underline{C}O_2Et$), 176.9 (C-4, C-7). LC-MS (m/z): positive mode 372 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.9%. Mp 199-200 °C.

5.2.30 4,10-Dioxo-1,4,7,10-tetrahydro-1,7-phenanthroline-2,8-dicarboxylic acids

5.2.30.1 General procedure for the synthesis of the 4,10-dioxo-1,4,7,10-tetrahydro-1,7-phenanthroline-2,8-dicarboxylic acids (GP-13)

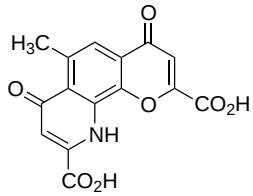
The ester (0.44 mmol) was suspended in THF (12 mL) and EtOH (3 mL). Then 1 N NaOH was added (0.5 mL) and the reaction mixture was stirred for 5 min at rt. After addition of 2 N HCl until a pH of 2 was reached, the precipitated solid was filtered, recrystallized in acetone and dried in vacuum at 50 °C.

5.2.30.2 6-Bromo-4,7-dioxo-7,10-dihydro-4H-pyrano[3,2-h]quinoline-2,9-dicarboxylic acid 272 (*ANM367)*



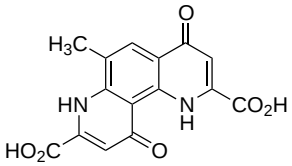
The compound was synthesized according to GP-13 using **274** (191 mg, 0.44 mmol) and was isolated as a yellow solid (87 mg, 52% yield). ^{1}H NMR: δ 7.03, 7.07 (s, 1H, 3-H, 8-H), 7.97 (s, 1H, 5-H). ^{13}C NMR: δ 111.8 (C-8), 114.0 (C-3), 114.3 (C-6), 123.2 (C-5), 123.4, 123.9 (C-4a, C-6a), 135.6 (C-9), 155.2 (C-10a, C-10b), 160.6 (C-2), 163.2 (CO_2H), 175.8 (C-4, C-7). LC-MS (m/z): positive mode 382 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. Mp > 300°C.

5.2.30.3 6-Methyl-4,7-dioxo-7,10-dihydro-4*H*-pyrano[3,2-h]quinoline-2,9-dicarboxylic acid 273 (*ANM379)*



The compound was synthesized according to GP-13 using **275** (163 mg, 0.44 mmol) and was isolated as yellow solid (98 mg, 71% yield). ^{1}H NMR: δ 2.83 (s, 3H, CH_3), 7.04 (s, 1H, 3-H), 7.58 (s, 2H, 5-H, 8-H). 13C nicht gelöst! LC-MS (m/z): positive mode 316 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.1%. Mp > 300°C.

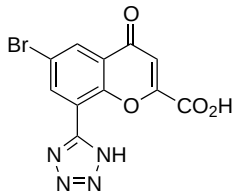
5.2.30.4 6-Methyl-4,10-dioxo-1,4,7,10-tetrahydro-1,7-phenanthroline-2,8-dicarboxylic acid 277 (*ANM365)*[233]



276 (5.16 g, 15 mmol) was added to boiling Dowtherm A® and stirred for 30 min. After cooling the solid was filtered and washed with acetone. Without further purification the solid was dissolved 4 mL of NaOH (1N) and stirred for 5 min at rt. After addition of 2 N HCl until a pH of 2 was reached, the precipitated solid was filtered, recrystallized in acetone and dried in vacuum at 50 °C. The product was isolated as a brown solid (46 mg, 1% yield). ^{1}H NMR: δ 2.69 (s, 3H, C6-CH_3), 7.11, 7.15 (s, 2H, 3-H, 9-H), 8.21 (s, 1H, 5-H). 13C nicht gelöst! LC-MS (m/z): positive mode 315 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.3%. Mp > 300°C.

5.2.31 6-Bromo-4-oxo-8-(1*H*-tetrazol-5-yl)-4*H*-chromene-2-carboxylic acid

5.2.31.1 6-Bromo-4-oxo-8-(1*H*-tetrazol-5-yl)-4*H*-chromene-2-carboxylic acid 268 (*ANM386)*



267 (588 mg, 2 mmol) was dissolved in 12 mL of dry DMF under an argon atmosphere and NH_4Cl (300 mg, 5.6 mmol) was added. Then the reaction mixture was heated to 35 °C and NaN_3 (500 mg, 7.7 mmol) were added. After stirring for 1.5 h at rt the reaction mixture was poured on 30 mL of 2 N HCl and the precipitated solid was filtered off. The solid was recrystallized from concd. HCl and the product was isolated as a yellow-white solid (328 mg, 49% yield). ^{1}H NMR: δ 7.01 (s, 1H, 3-H), 8.31 (d, J = 2.5 Hz, 1H, 5-H), 8.44 (d, J = 2.5 Hz, 1H, 7-H). ^{13}C NMR: δ 113.8 (C-3), 118.4 (C-6), 122.5 (extremely weak signal, C-8), 126.0 (C-4a), 130.0 (C-5), 137.7 (C-7), 151.5 (C-8a), 153.8 (C-2), 158.4 (extremely weak signal, C-tetrazole), 161.1 (CO_2H), 176.0 (C-4). LC-MS (m/z): positive mode 339 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.7%. Mp 265-266 °C.

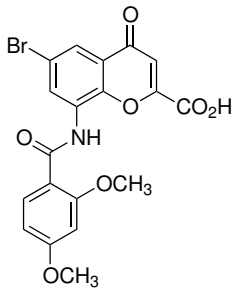
5.2.32 4-Oxo-4*H*-chromene-2-carboxylic acids

5.2.32.1 General procedure for the synthesis of the 4-oxo-4*H*-chromene-2-carboxylic acids (GP-14)

A solution of potassium carbonate (79 mg, 0.57 mmol) in water (4.5 mL) was slowly added to a suspension of the appropriate ethyl 4-oxo-4H-chromene-2-carboxylates (0.44 mmol) in THF (12 mL) and EtOH (3 mL). The reaction mixture was stirred at rt for 16-24 h until a clear solution was obtained. After addition of water (4 mL), the mixture was acidified with diluted aq HCl solution (2 N) until a pH $\leq$ 2 was reached. The solvents THF and EtOH were removed under reduced pressure. The obtained precipitate was filtered off, washed with 15 mL of water, and dried in vacuum at 45 °C. In some cases, further purification was necessary (see individual compounds).

5.2.32.2 6-Bromo-8-(2,4-dimethoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 203

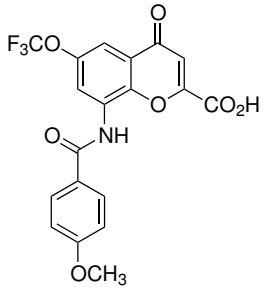
(ANM47)



The compound was synthesized according to GP-14 using **109** (210 mg, 0.44 mmol) and was isolated as a white solid (162 mg, 82% yield). ^{1}H NMR: δ 3.88 (s, 3H, C4'OC$\underline{H}_3$), 4.15 (s, 3H, C2'OC$\underline{H}_3$), 6.73 (dd, J = 8.8, 2.3 Hz, 1H, 5'-H), 6.77 (d, J = 2.3 Hz, 1H, 3'-H), 6.97 (s, 1H, 3-H), 7.78 (d, J = 2.4 Hz, 1H, 5-H), 8.05 (d, J = 8.8 Hz, 1H, 6'-H), 9.09 (d, J = 2.4 Hz, 1H, 7-H), 10.52 (s, 1H, NH). ^{13}C NMR: δ 55.6 (C2'-O$\underline{C}H_3$), 56.7 (C4'-O$\underline{C}H_3$), 98.6 (C-3'), 106.9 (C-5'), 112.8 (C-6), 113.5 (C-3), 118.1 (C-1'), 120.0 (C-7), 124.9 (C-4a), 126.0 (C-5), 130.4 (C-8), 133.3 (C-6'), 144.5 (C-8a), 152.9 (C-2), 159.9 (C-2'), 160.7 (CO_2H); 162.9 (C-4'), 164.3 (CO-NH), 175.8 (C-4). LC-MS (m/z): positive mode 450 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 284-285 °C.

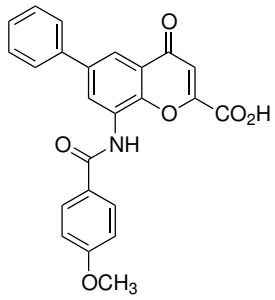
5.2.32.3 8-(4-Methoxybenzamido)-4-oxo-6-(trifluoromethoxy)-4*H*-chromene-2-carboxylic acid 204

(ANM68)



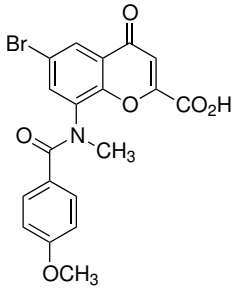
The compound was synthesized according to GP-14 using **110** (40 mg, 0.09 mmol), and was isolated as a white solid (37 mg, 96% yield). ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 6.99 (s, 1H, 3-H), 7.08-7.12 (m, 2H, 3'-H, 5'-H), 7.68-7.74 (m, 1H, 5-H), 7.99-8.02 (m, 2H, 2'-H, 6'-H), 8.21-8.25 (m, 1H, 7-H), 10.18 (s, 1H, NH). ^{13}C NMR: δ 55.7 (O-CH_3), 111.7 (C-5), 113.2 (C-3), 114.1 (C-3', C-5'), 119.2 (O-CF3), 121.2 (C-7), 124.9 (C-4a), 125.7 (C-1'), 129.9 (C-2', C-6'), 131.0 (C-8), 145.0 (C-8a), 147.3 (C-6), 153.4 (C-2), 161.1 (C-4'), 162.7 (CO_2H), 165.0 (CO-NH), 176.8 (C-4). LC-MS (m/z): positive mode 424 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 270-271 °C.

5.2.32.4 8-(4-Methoxybenzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 205 (*ANM77)*



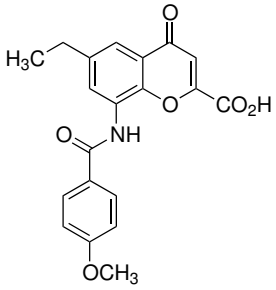
The compound was synthesized according to GP-14 using **111** (195 mg, 0.44 mmol), and was isolated as a white solid (176 mg, 96% yield). ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 6.98 (s, 1H, 3-H), 7.08-7.12 (m, 2H, 3'-H, 5'-H), 7.43-7.45 (m, 1H, 4'-H), 7.50-7.56 (m, 2H, 3'-H, 5'-H), 7.74-7.78 (m, 2H, 2'-H, 6'-H), 8.01-8.08 (m, 2H, 2'-H, 6'-H), 8.08 (d, 1H, J = 2.3, 5-H), 8.42 (d, 1H, J = 2.4, 7-H), 10.15 (s, 1H, NH). ^{13}C NMR: δ 55.6 (OCH_3), 113.5 (C-3), 114.0 (C-3', C-5'), 118.4 (C-5), 124.7 (C-4a), 126.1 (C-1'), 127.0 (C-2", C-6"), 128.4 (C-4"), 129.0 (C-7), 129.3 (C-8), 129.4 (C-3", C-5"), 129.8 (C-2', C-6'), 137.5 (C-6), 138.4 (C-1"), 148.8 (C-8a), 153.1 (C-2), 161.3 (CO_2H), 162.4 (C-4'), 165.1 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 416 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 270-272 °C.

5.2.32.5 6-Bromo-8-(4-methoxy-N-methylbenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 211 *(ANM78)*



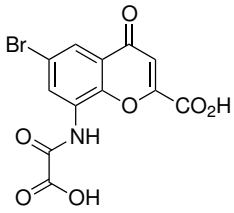
The compound was synthesized according to GP-14 using **181** (202 mg, 0.44 mmol), and was isolated as a white solid (188 mg, 99% yield). ^{1}H NMR: δ 3.38 (s, 3H, NCH), 3.67 (s, 3H, OCH_3), 6.72-6.76 (m, 2H, 3'-H, 5'-H), 6.89 (s, 1H, 3-H); 7.23-7.27 (m, 2H, 2'-H, 6'-H), 7.95 (d, 1H, J = 2.5, 5-H), 8.08 (d, 1H, J = 2.5, 7-H). ^{13}C NMR: δ 55.3 (OCH_3), 113.2 (C-3', C-5'), 113.6 (C-3), 117.8 (C-6), 125.8 (C-5), 125.8 (C-4a), 127.5 (C-1'), 129.8 (C-2', C-6'), 136.7 (C-7), 149.8 (C-8a), 153.3 (C-2), 160.4 (C-4'), 161.0 (CO_2H), 169.8 (CONH), 176.3 (C-4). LC-MS (m/z): positive mode 432 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 146-148 °C.

5.2.32.6 6-Ethyl-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 206 (*ANM172*)



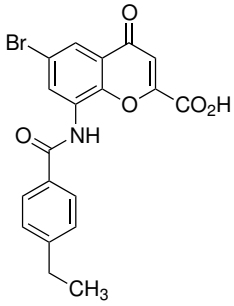
The compound was synthesized according to GP-14 using **112** (173 mg, 0.44 mmol), and was isolated as a white solid (139 mg, 86% yield). ^{1}H NMR: δ 1.24 (t, J = 7.6 Hz, 3H, C6-$CH_2\underline{C}H_3$), 2.76 (q, J = 7.6 Hz, 2H, C6-$C\underline{H}_2CH_3$), 3.85 (s, 3H, O-CH_3), 6.92 (s, 1H, 3-H), 7.07-7.11 (m, 2H, 3'-H, 5'-H), 7.69-7.73 (m, 1H, 5-H), 7.96 (d, J = 2.2 Hz, 1H, 7-H), 7.99-8.03 (m, 2H, 2'-H, 6'-H), 10.01 (s, 1H, NH). ^{13}C NMR: δ 15.4 (C6-$CH_2\underline{C}H_3$), 27.8 (C6-$\underline{C}H_2CH_3$), 55.6 (O-CH_3), 113.4 (C-3), 114.0 (C-3', C-5'), 119.5 (C-5), 124.2 (C-4a), 126.2 (C-1'), 128.4 (C-8), 129.7 (C-2', C-6'), 130.9 (C-7), 141.9 (C-6), 147.9 (C-8a), 152.9 (C-2), 161.4 (CO_2H), 162.4 (C-4'), 165.0 (CONH), 177.5 (C-4). LC-MS (m/z): positive mode 368 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 277-278 °C.

5.2.32.7 6-Bromo-8-(carboxyformamido)-4-oxo-4*H*-chromene-2-carboxylic acid 188 (*ANM183*)



The compound was synthesized according to GP-14 using **120** (181 mg, 0.44 mmol), and was isolated as a white solid (150 mg, 96% yield). ^{1}H NMR: δ 6.94 (s, 1H, 3-H), 7.87 (d, J = 2.4 Hz, 1H, 5-H), 8.58 (d, J = 2.5 Hz, 1H, 7-H), 10.35 (s, 1H, NH). ^{13}C NMR: δ 113.4 (C-3), 117.9 (C-6), 122.2 (C-7), 125.3 (C-4a), 127.6 (C-5), 129.3 (C-8), 145.9 (C-8a), 153.8 (C-2), 158.4 (CONH), 161.0 (CO_2H), 176.3 (C-4). LC-MS (m/z): positive mode 356 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. mp 246-247 °C.

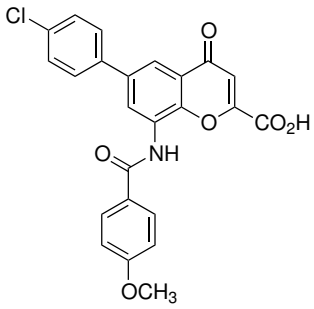
5.2.32.8 6-Bromo-8-(4-ethylbenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 207 (*ANM190*)



The compound was synthesized according to GP-14 using **113** (196 mg, 0.44 mmol), and was isolated as a white solid (173 mg, 94% yield). ^{1}H NMR: δ 1.23 (t, J = 7.5 Hz, 3H, C4?-$CH_2\underline{C}H_3$), 2.70 (q, J = 7.6 Hz, 2H, C4'-$C\underline{H}_2CH_3$), 6.97 (s, 1H, 3-H), 7.40 (d, J = 8.1 Hz, 2H, 3'-H, 5'-H), 7.92-7.96 (m, 3H, 2'-H, 6'-H, 5-H), 8.33 (d, J = 2.5 Hz, 1H, 7-H), 10.19 (s, 1H, NH). ^{13}C NMR: δ 15.4 (C4'-$CH_2\underline{C}H_3$), 28.2 (C4'-$\underline{C}H_2CH_3$), 113.6 (C-3), 117.8 (C-6), 123.1 (C-5),

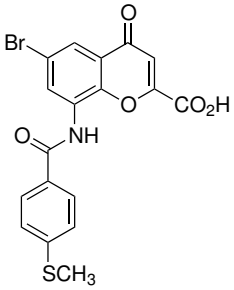
125.5 (C-4a), 128.0 (C-2', C-3', C-5', C-6'), 130.4 (C-8), 131.2 (C-1'), 132.1 (C-7), 148.1 (C-4'),148.7 (C-8a), 153.2 (C-2), 161.1 (CO_2H); 165.5 (CONH), 176.4 (C-4). LC-MS (m/z): positive mode 417 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 297-298 °C.

5.2.32.9 6-(4-Chlorophenyl)-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 208 (*ANM196)*



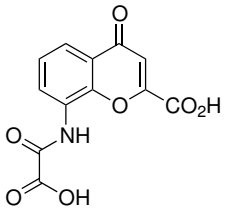
The compound was synthesized according to GP-14 using **114** (156 mg, 0.33 mmol), and was isolated as a white solid (125 mg, 84% yield). ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 6.97 (s, 1H, 3-H), 7.08-7.12 (m, 2H, 3'-H, 5'-H), 7.54-7.57 (m, 2H, 3"-H, 5"-H), 7.76-7.80 (m, 2H, 2"-H, 6"-H), 8.01-8.05 (m, 2H, 2'-H, 6'-H), 8.07 (d, J = 2.3 Hz, 1H, 5-H), 8.41 (d, J = 2.3 Hz, 1H, 7-H), 10.14 (s, 1H, NH). ^{13}C NMR: δ 55.7 (OCH_3), 113.6 (C-3), 114.0 (C-3', C-5'), 118.5 (C-5), 124.7 (C-4a), 126.1 (C-1), 128.8 (C-7), 128.8 (C-2', C-6'), 129.3 (C-3", C-5"), 129.4 (C-8), 129.8 (C-2", C-6"), 133.3 (C-4'), 136.2 (C-6), 137.2 (C-1'), 148.9 (C-8a), 153.1 (C-2), 161.3 (CO_2H), 162.5 (C4"), 165.1 (CONH), 177.5 (C-2). LC-MS (m/z): positive mode 450 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 291-292 °C.

5.2.32.10 6-Bromo-8-(4-(methylthio)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 209 (*THB10)*



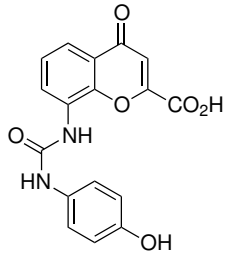
The compound was synthesized according to GP-14 using **115** (187 mg, 0.40 mmol), and was isolated as a white solid (140 mg, 80% yield). ^{1}H NMR: δ 2.55 (s, 3H, S-CH_3), 6.97 (s, 1H, 3-H), 7.39?7.45 (m, 2H, 3'-H, 5'-H), 7.90?7.98 (m, 3H, 5-H, 2'-H, 6'-H), 8.31 (d, J = 2.5 Hz, 1H, 7-H), 10.24 (s, 1H, NH). ^{13}C NMR: δ 14.3 (S-CH_3), 113.7 (C-3', C-5'), 117.8 (C-6), 123.3 (C-3), 125.2 (C-5) (C-5', C-3'), 125.6 (C-4a), 128.4 (C-2', C-6'), 129.6 (C-1'), 130.3 (C-8), 132.4 (C-7), 144.3 (C-8a), 148.3 (C-2), 153.3 (CO_2H), 161.1 (C-4'), 165.1 (CONH), 176.4 (C-4). LC-MS (m/z): positive mode 435 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.7%. mp 285-286 °C.

5.2.32.11 8-(Carboxyformamido)-4-oxo-4*H*-chromene-2-carboxylic acid 189 (*THB12)*



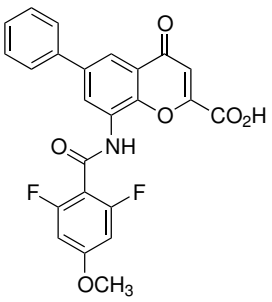
The compound was synthesized according to GP-14 using **121** (281 mg, 0.84 mmol), and was isolated as a red solid (192 mg, 81% yield). ^{1}H NMR: δ 6.94 (s, 1H, 3-H), 7.54 (t, J = 7.9 Hz, 1H, 6-H), 7.86 (dd, J = 1.5, 8.0 Hz, 1H, 5-H), 8.30 (dd, J = 1.3, 7.8 Hz, 1H, 7-H), 10.28 (s, 1H, NH). ^{13}C NMR: δ 113.7 (C-3), 121.3 (C-5), 124.2 (C-4a), 125.8 (C-7), 127.0 (C-8), 127.5 (C-6), 147.5 (C-8a), 152.8 (C-2), 156.6 (CONH), 161.2 (CO_2H), 161.3 (CO_2H), 177.4 (C-4). LC-MS (m/z): positive mode 278 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.9%. mp 245-249 °C.

5.2.32.12 8-(3-(4-Hydroxyphenyl)ureido)-4-oxo-4*H*-chromene-2-carboxylic acid 263 (*THB22)*



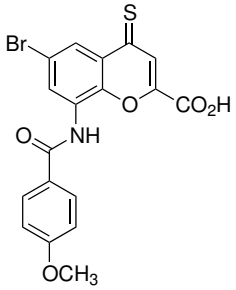
The compound was synthesized accroding to GP-14 using **182** (201 mg, 0.54 mmol). The obtained crude solid was suspended in 5 mL of EtOH and dissolved by addition of 2 mL NaOH (2N). After addition of HCl (6N) the poduct crystallized, was filtrated, dried in vacuum at 50 °C and was isolated as a green solid (14 mg, 8% yield). ^{1}H NMR: δ 6.71 (d, J = 8.8 Hz, 2H, 3'-H, 5'-H), 6.92 (s, 1H, 3-H), 7.26 (d, J = 8.8 Hz, 3H, 5-H, 2'-H, 6'-H), 7.43 (t, J = 8.0 Hz, 1H, 6-H), 7.63 (d, J = 7.9 Hz, 1H, 7-H), 8.36 (s, 1H, C8N-H), 8.43 (d, J = 6.9 Hz, 1H, C1'N-H), 9.34 (s, 1H, OH). ^{13}C NMR: δ 113.5 (C-3), 115.4 (C-3', C-5'), 117.1 (C-5), 120.8 (C-2', C-6'), 124.1 (C-4a), 124.6 (C-7), 125.7 (C-6), 129.8 (C-8), 130.9 (C-8a), 145.9 (C-1), 152.5 (CONH, C-4'), 153.0 (C-2), 161.5 (CO_2H), 177.7 (C-4). LC-MS (m/z): positive mode 341 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. mp 240-241 °C.

5.2.32.13 8-(2,6-Difluoro-4-methoxybenzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 210 (*THB28)*



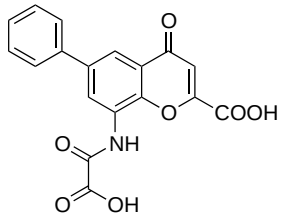
The compound was synthesized according to GP-14 using **116** (216 mg, 0.44 mmol), and was isolated as a yellow solid (189 mg, 95% yield). ^{1}H NMR: δ 3.86 (s, 3H, O-CH_3), 6.90 (d, J = 10.1 Hz, 2H, 3''-H, 5''-H), 6.98 (s, 1H, 3-H), 7.44 (t, J = 7.4 Hz, 1H, 4'-H), 7.53 (t, J = 7.6 Hz, 2H, 3'-H, 5'-H), 7.74 (d, J = 7.5 Hz, 2H, 2'-H, 6'-H), 8.08 (d, J = 2.3 Hz, 1H, 7-H), 8.46 (s, 1H, 5-H), 10.59 (s, 1H, NH). ^{13}C NMR: δ 56.6 (OCH_3), 98.7-98.9 (C-3', C-5'), 107.2 (C-1'), 113.6 (C-3), 118.7 (C-5), 124.8 (C-4a), 127.1 (C-2'', C-6''), 127.8 (C-7), 128.2 (C-8), 128.5 (C-4''), 129.4 (C-3'', C-5''), 137.7 (C-6), 138.3 (C-1''), 147.9 (C-8a), 153.3 (C-2), 159.1 (C-4'), 159.4 (C-2', C-6'), 161.2 (CO_2H), 162.4 (CONH), 177.4 (C-4). LC-MS (m/z): positive mode 452 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 96.4%. mp 267-268 °C.

5.2.32.14 6-Bromo-8-(4-methoxybenzamido)-4-thioxo-4*H*-chromene-2-carboxylic acid 212 (*ANM279)*



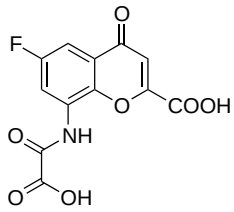
The compound was synthesized according to GP-14 using **119** (166 mg, 0.36 mmol), and was isolated as a green solid (136 mg, 87% yield). ^{1}H NMR: δ 3.87 (s, 3H, OCH_3), 7.06-7.14 (m, 2H, 3'-H, 5'-H), 7.67 (s, 1H, 3-H), 7.96-8.02 (m, 2H, 2'-H, 6'-H), 8.24 (d, 1H, J = 2.4 Hz, 5-H), 8.44 (d, 1H, J = 2.4 Hz, 7-H), 9.88 (s, 1H, NH). ^{13}C NMR: δ 55.4 (OCH_3), 113.9 (C-3', C-5'), 119.2 (C-6), 124.5 (C-3, C-5), 125.7 (C-1'), 129.5 (C-2', C-6'), 130.8 (C-8), 131.0 (C-7), 131.6 (C-4a), 143.2 (C-2, C-8a), 161.1 (CO_2H), 162.5 (C-4'), 164.7 (CONH), 201.4 (C-4). LC-MS (m/z): positive mode 435 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.7%. mp 272-273 °C.

5.2.32.15 8-(Carboxyformamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 190 (*ANM287)*



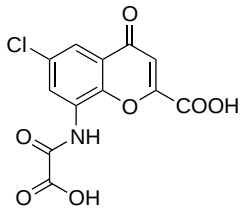
The compound was synthesized according to GP-14 using **122** (180 mg, 0.44 mmol), and was isolated as a white solid (123 mg, 79% yield). ^{1}H NMR: δ 6.98 (s, 1H, 3-H), 7.39-7.46 (m, 1H, 4'-H), 7.48-7.55 (m, 2H, 3'-H, 5'-H), 7.66-7.72 (m, 2H, 2'-H, 6'-H), 7.96 (d, 1H, J = 2.2 Hz, 5-H), 8.84 (d, 1H, J = 2.4 Hz, 7-H), 10.38 (s, 1H, NH). ^{13}C NMR: δ 112.3 (C-3), 116.6 (C-5), 122.6 (C-7), 124.1 (C-4a), 126.6 (C-2', C-6'), 127.9 (C-4'), 128.3 (C-8), 128.9 (C-3', C-5'), 137.3 (C-6), 138.6 (C-1'), 145.6 (C-8a), 155.3 (C-2), 160.3 (CONH), 160.8-161.1 (CO_2H), 177.3 (C-4). LC-MS (m/z): positive mode 354 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97.6%. mp > 300 °C.

5.2.32.16 8-(Carboxyformamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid 191 (*ANM295)*



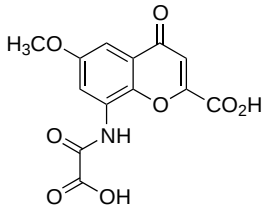
The compound was synthesized according to GP-14 using **123** (154 mg, 0.44 mmol), and was isolated as a white solid (111 mg, 85% yield). ^{1}H NMR: δ 6.95 (s, 1H, 3-H), 7.54 (dd, 1H, J = 3.1, 8.0 Hz, 5-H), 8.25 (dd, 1H, J = 3.1, 9.8 Hz, 7-H), 10.28 (s, 1H, NH). ^{13}C NMR: δ 105.4 (C-5), 113.0 (C-3), 114.4 (C-7), 125.0 (C-4a), 129.1 (C-8), 143.8 (C-8a), 153.0 (C-2), 156.7-157.6 (C-6), 159.6 (CONH), 161.0 (CO_2H), 176.7 (C-4). LC-MS (m/z): positive mode 296 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 254-256 °C.

5.2.32.17 8-(Carboxyformamido)-6-chloro-4-oxo-4*H*-chromene-2-carboxylic acid 192 (*ANM296)*



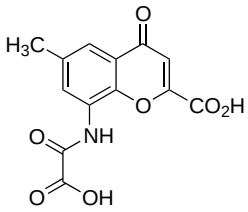
The compound was synthesized according to GP-14 using **124** (63 mg, 0.17 mmol), and was isolated as a white solid (44 mg, 83% yield). ^{1}H NMR: δ 6.97 (s, 1H, 3-H), 7.79 (d, 1H, J = 2.6 Hz, 5-H), 8.38 (d, 1H, J = 2.6 Hz, 7-H), 10.29 (s, 1H, NH). ^{13}C NMR: δ 113.7 (C-3), 119.8 (C-5), 125.1 (C-4a), 126.0 (C-7), 128.9 (C-8), 130.2 (C-6), 146.0 (C-8a), 153.1 (C-2), 156.8 (CONH), 161.0 (CO_2H), 176.3 (C-4). LC-MS (m/z): positive mode 312 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.6%. mp 242-244 °C.

5.2.32.18 8-(Carboxyformamido)-6-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid 193 (*ANM297*)



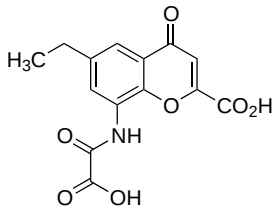
The compound was synthesized according to GP-14 using **125** (60 mg, 0.17 mmol), and was isolated as a white solid (43 mg, 82% yield). ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 6.91 (s, 1H, 3-H), 7.23 (d, 1H, J = 3.1 Hz, 5-H), 7.98 (d, 1H, J = 3.05 Hz, 7-H), 10.19 (s, 1H, NH). ^{13}C NMR: δ 56.08 (OCH_3), 101.0 (C-5), 112.9 (C-3), 115.9 (C-7), 124.8 (C-4a), 128.3 (C-8), 142.2 (C-8a), 152.5 (C-2), 156.6 (CONH, C-6), 161.2 (CO_2H), 177.0 (C-4). LC-MS (m/z): positive mode 308 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 245-247 °C.

5.2.32.19 8-(Carboxyformamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylic acid 194 (*ANM298*)



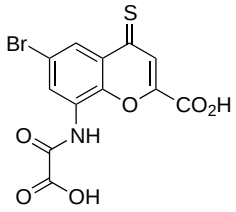
The compound was synthesized according to GP-14 using **126** (100 mg, 0.29 mmol), and was isolated as a white solid (64 mg, 76% yield). ^{1}H NMR: δ 2.43 (s, 3H, CH_3), 6.91 (s, 1H, 3-H), 7.66 (d, 1H, J = 2.2 Hz, 5-H), 8.13 (d, 1H, J = 2.0 Hz, 7-H), 10.22 (s, 1H, NH). ^{13}C NMR: δ 20.9 (CH_3), 113.6 (C-3), 120.7 (C-5), 124.0 (C-4a), 126.7 (C-8), 128.4 (C-7), 135.7 (C-6), 145.9 (C-8a), 152.7 (C-2), 156.7 (CONH), 161.3 (CO_2H), 177.3 (C-4). LC-MS (m/z): positive mode 292 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 250-252 °C.

5.2.32.20 8-(Carboxyformamido)-6-ethyl-4-oxo-4*H*-chromene-2-carboxylic acid 195 (*ANM299*)



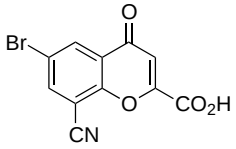
The compound was synthesized according to GP-14 using **127** (108 mg, 0.3 mmol), and was isolated as a white solid (85 mg, 93% yield). ^{1}H NMR: δ 1.22 (t, 3H, J = 7.6 Hz, CH_3), 2.74 (q, 2H, J = 7.6 Hz, CH_2), 6.92 (s, 1H, 3-H), 7.46-7.84 (m, 1H, 5-H), 8.17 (d, 1H, J = 2.16 Hz, 7-H), 10.25 (s, 1H, NH). ^{13}C NMR: δ 15.4 (CH_3), 27.9 (CH_2), 113.6 (C-3), 119.5 (C-5), 124.1 (C-4a), 126.9 (C-8), 127.5 (C-7), 141.7 (C-6), 146.1 (C-8a), 152.7 (C-2), 156.7 (CONH), 161.3 (CO_2H), 177.4 (C-4). LC-MS (m/z): positive mode 306 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 246-248 °C.

5.2.32.21 6-Bromo-8-(carboxyformamido)-4-thioxo-4*H*-chromene-2-carboxylic acid 197 (*ANM300)*



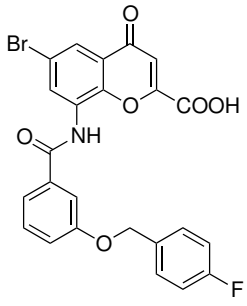
The compound was synthesized according to GP-14 using **128** (88.4 mg, 0.2 mmol), and was isolated as a green solid (36 mg, 48% yield). ^{1}H NMR: δ 7.65 (s, 1H, 3-H), 8.17 (d, 1H, J = 2.4 Hz, 5-H), 8.64 (d, 1H, J = 2.4 Hz, 7-H), 10.41 (s, 1H, NH). ^{13}C NMR: δ 119.6 (C-6), 124.3 (C-7), 124.8 (C-5), 127.6 (C-8), 129.9 (C-3), 131.6 (C-4a), 141.7 (C-8a), 161.0 (CONH), 161.3 (C2), 161.4 (CO_2H), 201.5 (C-4). LC-MS (m/z): positive mode 372 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.6%. mp > 300 °C.

5.2.32.22 6-Bromo-8-cyano-4-oxo-4*H*-chromene-2-carboxylic acid 267 (*ANM384)*



The compound was synthesized according to GP-14 using **266** (143 mg, 0.44 mmol), and was isolated as a white solid (111 mg, 86% yield). ^{1}H NMR: δ 7.02 (s, 1H, 3-H), 8.35 (d, J = 2.5 Hz, 1H, 7-H), 8.69 (d, J = 2.5 Hz, 1H, 5-H). ^{13}C NMR: δ 105.2 (C-8), 113.0 (CN), 114.5 (C-3), 117.9 (C-6), 125.7 (C-4a), 132.6 (C-5), 141.7 (C-7), 153.5 (C-8a), 155.0 (C-2), 160.8 (CO_2H), 175.7 (C-4). LC-MS (m/z): positive mode 294 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.0%. mp 234-236 °C.

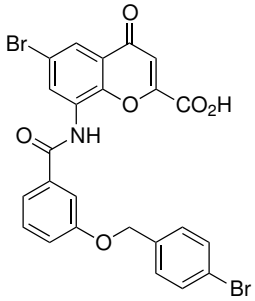
5.2.32.23 6-Bromo-8-(3-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 227 (*ANM20)*



The compound was synthesized accroding to GP-14 using **146** (189 mg, 0.35 mmol) and was isolated as a white solid (128 mg, 71% yield). ^{1}H NMR: δ 5.18 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.19-7.26 (m, 2H, 3''-H, 5''-H), 7.28 (dd, J = 8.2, 2.4 Hz, 1H, 4'-H), 7.49 (t, J = 7.9 Hz, 1H, 5'-H), 7.52-7.58 (m, 2H, 2''-H, 6''-H), 7.58-7.62 (m, 1H, 2'-H), 7.63-7.67 (m, 1H, 6'-H), 7.97 (d, J = 2.4 Hz, 1H, 5-H), 8.33 (d, J = 2.4 Hz, 1H, 7-H), 10.30 (s, 1H, NH). ^{13}C NMR: δ 69.0 (CH_2-O), 113.7 (C-3), 114.1 (C-2'), 115.5 (C-3'', C-5''), 117.8 (C-6), 119.0 (C-6'), 120.4 (C-4'), 123.3 (C-5), 125.6 (C-4a), 130.0 (C-5'), 130.2 (C-2'', C-6''), 130.2 (C-8), 132.3 (C-7), 133.1 (C-1''), 135.2 (C-1'), 148.2 (C-8a), 153.3 (C-2), 158.5

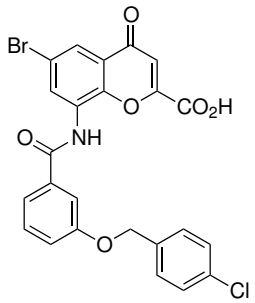
(C-3'), 161.1 (CO_2H), 165.4 (CONH), 176.4 (C-4). LC-MS (m/z): negative mode 566 $[M-CO2H]^-$, positive mode 514 $[M+H]^+$, 531 $[M+NH_4]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 258-259 °C.

5.2.32.24 6-Bromo-8-(3-(4-bromobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 228 (*ANM21)*



The compound was synthesized according to GP-14 using **147** (265 mg, 0.44 mmol) and was isolated as a white solid (74 mg, 30% yield). ^{1}H NMR: δ 5.19 (s, 2H, CH_2-O), 6.96 (s, 1H, 3-H), 7.28 (dd, J = 7.3, 2.2 Hz, 1H, 4'-H), 7.43-7.46 (m, 2H, 3'-H, 5'-H), 7.49 (t, J = 7.9 Hz, 1H, 5'-H), 7.58-7.62 (m, 3H, 2'-H, 2"-H, 6"-H), 7.62-7.66 (m, 1H, 6'-H), 7.96 (d, J = 2.4 Hz, 1H, 5-H), 8.33 (d, J = 2.4 Hz, 1H, 7-H), 10.30 (s, 1H, NH). ^{13}C NMR: δ 68.9 (CH_2-O), 113.5 (C-3), 114.1 (C-2'), 117.8 (C-6), 119.0 (C-6'), 120.5 (C-4'), 121.2 (C-4"), 123.3 (C-5), 125.6 (C-4a), 130.0 (C-2", C-6"), 130.0 (C-5'), 130.18 (C-8), 131.6 (C-3", C-5"), 132.2 (C-7), 135.2 (C-1'), 136.4 (C-1"), 148.2 (C-8a), 153.6 (C-2), 158.4 (C-3'), 161.1 (CO_2H), 165.33 (CONH), 176.4 (C-4). LC-MS (m/z): negative mode 572 $[M-H]^-$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 232-233 °C.

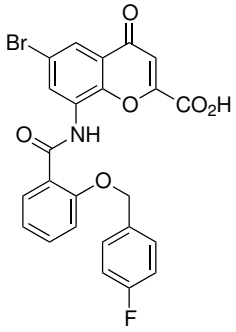
5.2.32.25 6-Bromo-8-(3-(4-chlorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 229 (*ANM22)*



The compound was synthesized according to GP-14 using **148** (116 mg, 0.22 mmol) and was isolated as a white solid (84 mg, 72% yield). ^{1}H NMR: δ 5.20 (s, 2H, CH_2-O), 6.97 (s, 1H, 3-H), 7.28 (dd, J = 7.9, 2.2 Hz, 1H, 4'-H), 7.45-7.53 (m, 5H, 5'-H, 2"-H, 3"-H, 5"-H, 6"-H), 7.59-7.66 (m, 2H, 2'-H, 6'-H), 7.96 (d, J = 2.4 Hz, 1H, 5-H), 8.33 (d, J = 2.4 Hz, 1H, 7-H), 10.30 (s, 1H, CONH). ^{13}C NMR: δ 68.8 (CH_2-O), 113.6 (C-3), 114.1 (C-2'), 117.8 (C-6), 119.0 (C-6'), 120.5 (C-4'), 123.3 (C-5), 125.6 (C-4a), 128.6 (C-3", C5"), 129.7 (C-2", C-6"), 130.0 (C-5'), 130.2 (C-8), 132.3 (C-7), 132.7 (C-1'), 135.2 (C-4"), 135.9 (C-1"), 148.2 (C-8a), 153.4 (C-2), 158.4 (C-3?), 161.1 (CO_2H), 165.3 (CONH), 176.4 (C-4). LC-MS (m/z): negative mode 484 $[M-CO_2H]^-$, 528 $[M-H]^-$, positive mode 530 $[M+H]^+$. Purity

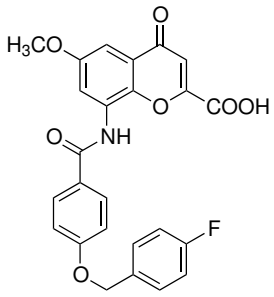
by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 202-203 °C.

5.2.32.26 6-Bromo-8-(2-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 234 (*ANM23)*



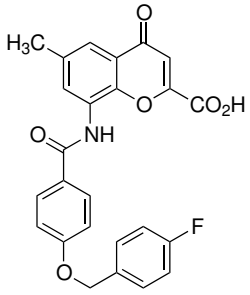
The compound was synthesized according to GP-14 using **153** (238 mg, 0.44 mmol) and was isolated as a white solid (146 mg, 65% yield). ^{1}H NMR: δ 5.62 (s, 2H, CH_2-O), 6.96 (s, 1H, 3-H), 7.04-7.11 (m, 2H, 3″-H, 5″-H), 7.12-7.16 (m, 1H, 5′-H), 7.30 (d, J = 8.2 Hz, 1H, 3′-H), 7.46-7.51 (m, 2H, 2″-H, 6″-H), 7.52-7.57 (m, 1H, 4″-H), 7.84 (d, J = 2.5 Hz, 1H, 5-H), 8.05 (dd, J = 7.8, 1.8 Hz, 1H, 6′-H), 9.01 (s, 1H, 7-H), 10.73 (s, 1H, CONH). ^{13}C NMR: δ 69.8 (CH_2-O), 113.8 (C-3), 114.8 (C-3′), 115.4 (C-3″, C-5″), 118.3 (C-6), 121.0 (C-5′), 121.4 (C-1′), 121.8 (C-5), 125.2 (C-4a), 127.1 (C-7), 129.8 (C-2″, C-6″), 130.4 (C-8), 131.8 (C-6′), 132.7 (C-1″), 134.2 (C-4′), 145.2 (C-8a), 153.0 (C-2), 156.1 (C-2′), 160.9 (CO_2H), 161.1 (C-4″), 163.7 (CONH), 176.3 (C-4). LC-MS (m/z): negative mode 511 $[M-H]^-$, positive mode 513 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.4%. mp 257-258 °C.

5.2.32.27 8-(4-(4-Fluorobenzyloxy)benzamido)-6-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid 213 (*ANM58)*



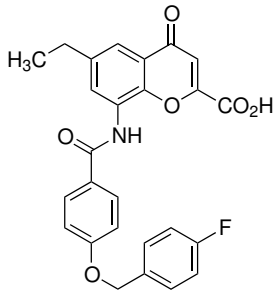
The compound was synthesized according to GP-14 using **133** (108 mg, 0.22 mmol) and was isolated as a white solid (89 mg, 87% yield). ^{1}H NMR: δ 3.89 (s, 3H, O-CH_3), 5.21 (s, 2H, CH_2-O), 6.90 (s, 1H, 3-H), 7.15-7.18 (m, 2H, 3′-H, 5′-H), 7.18-7.23 (m, 2H, 3″-H, 5″-H), 7.27 (d, J = 3.1 Hz, 1H, 5-H), 7.50-7.54 (m, 2H, 2″-H, 6″-H), 7.85 (d, J = 3.1 Hz, 1H, 7-H), 7.97-8.01 (m, 2H, 2′-H, 6′-H), 9.77 (s, 1H, CONH). ^{13}C NMR: δ 55.8 (O-CH_3), 68.9 (CH_2-O), 101.1 (C-5), 112.3 (C-3), 114.7 (C-3′, C-5′), 114.95-115.12 (C-3″, C-5″), 117.9 (C-7), 124.6 (C-4a), 126.4 (C-1′), 129.4 (C-2′, C6′), 129.6-129.7 (C-2″, C-6″), 129.7 (C-8), 132.7 (C-1″), 143.5 (C-8a), 152.5 (C-2), 156.4 (C-6), 160.9 (C-4′), 161.2 (CO_2H), 162.7 (C-4″), 164.7 (CONH), 176.8 (C-4). LC-MS (m/z): negative mode 462 $[M-H]^-$, positive mode 464 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 274-278 °C.

5.2.32.28 8-(4-(4-Fluorobenzyloxy)benzamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylic acid 214 (*ANM59*)



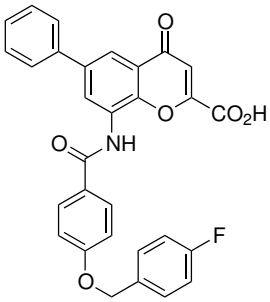
The compound was synthesized according to GP-14 using **134** (105 mg, 0.22 mmol) and was isolated as a white solid (62 mg, 63% yield). ^{1}H NMR: δ 2.48 (s, 3H, CH_3), 5.23 (s, 2H, CH_2-O), 6.91 (s, 1H, 3-H), 7.15-7.20 (m, 2H, 3'-H, 5'-H), 7.20-7.26 (m, 2H, 3"-H, 5"-H), 7.51-7.57 (m, 2H, 2"-H, 6"-H), 7.70 (dd, J = 2.3 Hz, 1H, 5-H), 7.96-8.04 (m, 3H, 7-H, 2'-H, 6'-H), 9.78 (s, 1H, CONH). ^{13}C NMR: δ 20.5 (CH_3), 68.8 (CH_2-O), 113.0 (C-3), 114.7 (C-3', C-5'), 115.0-115.1 (C-3", C-5"), 120.2 (C-5), 123.9 (C-4a), 126.5 (C-1'), 128.1 (C-8), 129.3 (C-2', C6'), 129.6-129.7 (C-2", C6"), 130.7 (C-1"), 135.1 (C-6), 147.1 (C-8a), 152.7 (C-2), 160.9 (CO_2H), 161.1 (C-4'), 162.7 (C-4"), 164.7 (C-4"), 164.7 (CONH), 177.1 (C-4). LC-MS (m/z): negative mode 446 $[M-H]^-$, positive mode 448 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 260-261 °C.

5.2.32.29 6-Ethyl-8-(4-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 215 (*ANM97*)



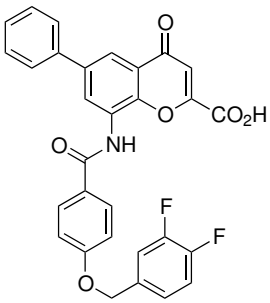
The compound was synthesized according to GP-14 using **135** (215 mg, 0.44 mmol) and was isolated as a white solid (193 mg, 95% yield). ^{1}H NMR: δ 1.24 (t, J = 7.6 Hz, 3H, $CH_2C\underline{H}_3$), 2.76 (q, J = 7.6 Hz, 2H, $C\underline{H}_2CH_3$), 5.20 (s, 2H, CH_2-O), 6.92 (s, 1H, 3-H), 7.14-7.19 (m, 2H, 3'-H, 5'-H), 7.21-7.26 (m, 2H, 3"-H, 5"-H), 7.52-7.58 (m, 2H, 2"-H, 6"-H), 7.71 (d, J = 2.1 Hz, 1H, 5-H), 7.95 (d, J = 2.2 Hz, 1H, 7-H), 7.96-8.02 (m, 2H, 2'-H, 6'-H), 10.04 (s, 1H, CONH). ^{13}C NMR: δ 15.8 ($CH_2\underline{C}H_3$), 28.1 ($\underline{C}H_2CH_3$), 69.2 (CH_2-O), 133.7 (C-3), 115.1 (C-3', C-5'), 115.9 (C-3", C-5"), 119.9 (C-5), 125.5 (C-4a), 126.8 (C-1'), 128.7 (C-8a), 130.1 (C-2', C-6'), 130.1 (C-2", C-6"), 131.3 (C-7), 133.3 (C-1"), 141.9 (C-6), 148.3 (C-8a), 153.2 (C-2), 161.5-161.8 (CO_2H, C-4'), 163.1 (C-4"), 165.3 (CONH), 177.9 (C-4). LC-MS (m/z): negative mode 460 $[M-H]^-$, positive mode 462 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 258-259 °C.

5.2.32.30 8-(4-(4-Fluorobenzyloxy)vbenzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 216 (*ANM98)*



The compound was synthesized according to GP-14 using **136** (161 mg, 0.30 mmol) and was isolated as a white solid (150 mg, 98% yield). ^{1}H NMR: δ 5.20 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.16-7.21 (m, 2H, 3'-H, 5'-H), 7.21-7.26 (m, 2H, 3"-H, 5"-H), 7.41-7.46 (m, 1H, 4"'-H), 7.49-7.56 (m, 4H, 2"-H, 6"-H, 3"-H, 5"-H), 7.74-7.78 (m, 2H, 2"'-H, 6"'-H), 8.01-8.06 (m, 2H, 2'-H, 6'-H), 8.07-8.10 (d, J = 2.3 Hz, 1H, 5-H), 8.41 (d, J = 2.3 Hz, 1H, 7-H), 10.17 (s, 1H, CONH). ^{13}C NMR: δ 69.0 (CH_2-O), 113.5 (C-3), 114.8 (C-3', C-5'), 115.4-115.6 (C-3", C-5"), 118.4 (C-5), 124.7 (C-4a), 126.4 (C-1"'), 127.0 (C-2"', C-6"'), 128.4 (C-7), 129.0 (C-4"'), 129.2 (C-8), 129.4 (C-3"', C-5"'), 129.9 (C-2', C-6'), 130.2-130.3 (C-2", C-6"), 133.0 (C-1"), 137.6 (C-6), 138.4 (C-1'), 148.8 (C-8a), 153.1 (C-2), 161.0-163.0 (C-4"), 161.4 (CO_2H), 161.4 (C-4'), 165.1 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 510 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%. mp 155-156 °C.

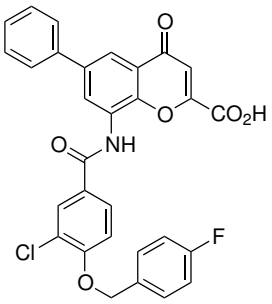
5.2.32.31 8-(4-(3,4-Difluorobenzyloxy)benzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 217 (*ANM130)*



The compound was synthesized according to GP-14 using **137** (144 mg, 026 mmol) and was isolated as a white solid (93 mg, 68% yield). ^{1}H NMR: δ 5.21 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.17-7.21 (m, 2H, 3'-H, 5'-H), 7.33-7.37 (m, 1H, 6"-H), 7.41-7.61 (m, 5H, 2"-H, 5"-H, 3"'-H, 4"'-H, 5"'-H), 7.74-7.78 (m, 2H, 2"'-H, 6"'-H), 8.02-8.06 (m, 2H, 2'-H, 6'-H), 8.09 (d, J = 2.31 Hz, 1H, 5-H), 8.41 (d, J = 2.3 Hz, 1H, 7-H), 10.17 (s, 1H, CONH). ^{13}C NMR: δ 68.3 (CH_2-O), 113.5 (C-3), 114.8 (C-3', C-5'), 117.0-117.1 (C-2"), 117.7-117.8 (C-5"), 118.4 (C-5), 124.7 (C-4a), 124.8-124.9 (C-6"), 126.6 (C-1"'), 127.0 (C-2"', C6"'), 128.4 (C-7), 129.0 (C-4"'), 129.2 (C-8), 129.4 (C-3"', C-5"'), 129.9 (C-2', C-6'), 134.5-134.6 (C-1"), 137.6 (C-6), 138.3 (C-2), 148.2-148.6 (C-4"), 148.8 (C-8a), 150.2-150.5 (C-3"), 153.1 (C-2), 161.2 (C-4'), 161.3 (CO_2H), 165.1 (CONH), 177.6 (C-4). LC-MS (m/z): negative mode 526 $[M-H]^-$, positive mode 528 $[M+H]^+$. Purity by HPLC-UV

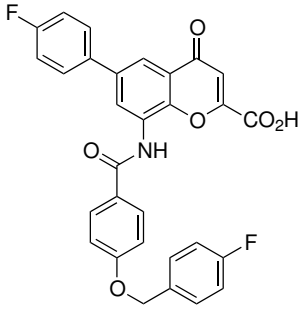
(254 nm)-ESI-MS: 100.0%. mp 281-282 °C.

5.2.32.32 8-(3-Chloro-4-(4-fluorobenzyloxy)benzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 219 (*ANM139)*



The compound was synthesized according to GP-14 using **139** (92 mg, 0.16 mmol) and was isolated as a white solid (77 mg, 89% yield). ^{1}H NMR: δ 5.32 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.23-7.29 (m, 2H, 3''-H, 5''-H), 7.42-7.46 (m, 2H, 4'''-H, 5'-H), 7.50-7.58 (m, 4H, 2''-H, 6''-H, 3'''-H, 5'''-H), 7.74-7.78 (m, 2H, 2'''-H, 6'''-H), 8.02 (dd, J = 2.2, 8.7 Hz, 1H, 6'-H), 8.11 (d, J= 2.3 Hz, 1H, 5-H), 8.16 (d, J = 2.2 Hz, 1H, 2'-H), 8.36 (d, J = 2.3 Hz, 1H, 7-H), 10.36 (s, 1H, CONH). ^{13}C NMR: δ 69.9 (CH_2-O), 113.5 (C-3), 114.1 (C-5'), 115.5-115.6 (C-3'', C-5''), 118.8 (C-5), 121.7 (C-3'), 124.7 (C-4a), 127.0 (C-2''', C-6'''), 127.3 (C-1'''), 128.4 (C-7), 128.9 (C-8), 129.5 (C-2'), 129.7 (C-6'), 130.0-130.1 (C-2'', C-6''), 132.5 (C-1''), 137.6 (C-6), 138.3 (C-1'), 149.1 (C-8a), 153.1 (C-2), 156.5 (C-4'), 161.1 (C-4''), 151.4 (CO_2H), 164.1 (CONH), 177.6 (C-4). LC-MS (m/z): negative mode 542 $[M-H]^-$, positive mode 544 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 259-260 °C.

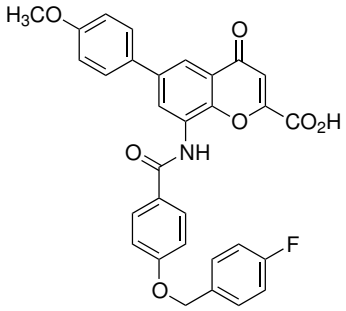
5.2.32.33 8-(4-(4-Fluorobenzyloxy)benzamido)-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylic acid 220 (*ANM141)*



The compound was synthesized according to GP-14 using **140** (206 mg, 0.37 mmol) and was isolated as a white solid (170 mg, 87% yield). ^{1}H NMR: δ 5.20 (s, 2H, CH_2-O), 6.97 (s, 1H, 3-H), 7.16-7.21 (m, 2H, 3'-H, 5'-H), 7.21-7.26 (m, 2H, 3''-H, 5''-H), 7.31-7.37 (m, 2H, 3'''-H, 5'''-H), 7.51-7.56 (m, 2H, 2''-H, 6''-H), 7.78-7.83 (m, 2H, 2'''-H, 6'''-H), 8.01-8.05 (m, 2H, 2'-H, 6'-H), 8.06 (d, J = 2.3 Hz, 1H, 5-H), 8.39 (d, J = 2.3 Hz, 1H, 7-H), 10.16 (s, 1H, CONH). ^{13}C NMR: δ 69.0 (CH_2-O), 113.5 (C-3), 114.8 (C-3', C-5'), 115.4-115.6 (C-3''', C5'''), 116.1-116.3 (C-3'', C-5''), 118.4 (C-5), 124.7 (C-4a), 126.4 (C-1'), 129.0 (C-7), 129.2 (C-2''', C-6'''), 129.3 (C-8), 129.9 (C-2', C-6'), 130.2-130.3 (C-2'', C-6''), 133.0 (C-1''), 134.9 (C-6), 136.6 (C-1'''), 148.8 (C-8a), 153.1 (C-2),

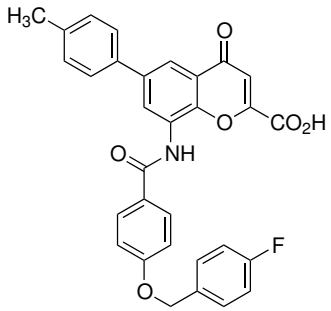
61.0-161.7 (C-4‴, CO_2H), 163.0-163.4 (C-4′, C-4″), 165.1 (CONH), 177.6 (C-4). LC-MS (m/z): negative mode 526 $[M-H]^-$, positive mode 528 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 264-265 °C.

5.2.32.34 8-(4-(4-Fluorobenzyloxy)benzamido)-6-(4-methoxyphenyl)-4-oxo-4*H*-chromene-2-carboxylic acid 221 (*ANM142)*



The compound was synthesized according to GP-14 using **141** (261 mg, 0.46 mmol) and was isolated as a yellow solid (226 mg, 91% yield). ^{1}H NMR: δ 3.81 (s, 3H, O-CH_3), 5.20 (s, 2H, CH_2-O), 6.96 (s, s, 1H, 3-H), 7.05-7.09 (m, 2H, 3‴-H, 5‴-H), 7.16-7.20 (m, 2H, 3′-H, 5′-H), 7.20-7.26 (m, 2H, 3″-H, 5″-H), 7.51-7.55 (m, 2H, 2″-H, 6″-H), 7.68-7.72 (m, 2H, 2‴-H, 6‴-H), 8.01-8.03 (m, 2H, 2′-H, 6′-H), 8.04 (d, 1H, 5-H), 8.36 (d, J = 2.3 Hz, 1H 7-H). ^{13}C NMR: δ 55.4 (O-CH_3), 69.0 (CH_2-O), 113.4 (C-3), 114.8 (C-3′, C5′, C3‴, C5‴), 115.4-115.6 (C-3″, C-5″), 117.6 (C-5), 124.7 (C-4a), 126.5 (C-1‴), 128.2 (C-2‴, C-6‴), 128.6 (C-7), 129.1 (C-8), 129.9 (C-2′, C-6′), 130.2-130.3 (C-2″, C-6″), 130.6 (C-6), 133.3 (C-1″), 137.3 (C-1‴), 148.5 (C-8a), 153.1 (C-2), 159.7 (CO_2H), 161.4 (C-4′), 151.1-163.0 (C-4″), 165.1 (CONH), 177.6 (C-4). LC-MS (m/z): negative mode 538 $[M-H]^-$, positive mode 540 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 264-265 °C.

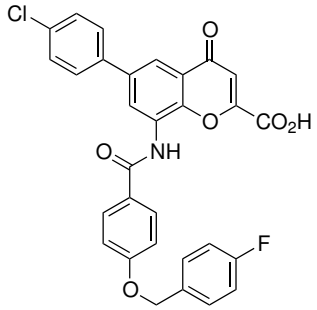
5.2.32.35 8-(4-(4-Fluorobenzyloxy(benzamido)-4-oxo-6-p-tolyl-4*H*-chromene-2-carboxylic acid 222 (*ANM143)*



The compound was synthesized according to GP-14 using **142** (200 mg, 0.36 mmol) and was isolated as a white solid (172 mg, 91% yield). ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 5.20 (s, 2H, CH_2-O), 6.97 (s, 1H, 3-H), 7.18 (d, J = 8.8 Hz, 2H, 3′-H, 5′-H), 7.23 (t, J = 8.8 Hz, 2H, 3″-H, 5″-H), 7.33 (d, J = 8.1 Hz, 2H, 3‴-H, 5‴-H), 7.50-7.57 (m, 2H, 2″-H, 6″-H), 7.65 (d, J = 8.1 Hz, 2H, 2‴-H, 6‴-H), 8.03 (d, J = 8.8 Hz, 2H, 2′-H, 6′-H), 8.06 (d, J = 2.2 Hz, 1H, 5-H), 8.38 (d, J = 2.2 Hz, 1H, 7-H), 10.14 s, 1H, CONH). ^{13}C NMR: δ 20.8 (CH_3), 69.0 (CH_2-O), 113.5 (C-3), 114.8 (C-3′, C-5′),

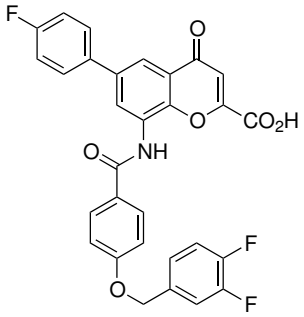
115.4-115.6 (C-3", C-5"), 118.0 (C-5), 124.7 (C-4a), 126.4 (C-1"'), 126.8 (C-2"'. C-6"'), 128.8 (C-7), 129.2 (C-8), 129.9 (C-2', C-6'), 130.0 (C-3"', C-5"'), 130.2-130.3 (C-2", C-6"), 133.0 (C-1"), 135.4 (C-6), 137.5 (C-1"'), 137-9 (C-1'), 148.7 (C-8a), 153.0 (C-2), 161.3 (C-4'), 161.4 (CO_2H), 161.1-163.0 (C-4"), 177.6 (C-4). LC-MS (m/z): negative mode 522 $[M-H]^-$, positive mode 524 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.9%. mp 268-269 °C.

5.2.32.36 6-(4-Chlorophenyl)-8-(4-(4-fluorobenzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 218 (*ANM138)*



The compound was synthesized according to GP-14 using **138** (166 mg, 0.29 mmol) and was isolated as a yellow solid (137 mg, 87% yield). ^{1}H NMR: δ 5.20 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.14-7.28 (m, 4H, 3'-H, 5'-H, 3"-H, 5"-H), 7.50-7.60 (m, 4H, 2"-H, 6"-H, 3"'-H, 5"'-H), 7.75-7.82 (m, 2H, 2"'-H, 6"'-H), 8.00-8.06 (m, 2H, 2'-H, 6'-H), 8.08 (d, J = 2.3 Hz, 1H, 5-H), 8.41 (d, J = 2.3 Hz, 1H, 7-H), 10.16 (s, 1H, CONH). ^{13}C NMR: δ 69.0 (CH_2-O), 113.5 (C-3), 114.8 (C-3', C-5'), 115.4-115.5 (C-3", C-5"), 118.5 (C-5), 124.7 (C-4a), 126.4 (C-8), 128.8-129.8 (C-2"', C-6"', C-2', C-6', C-3"', C-5"', C-7), 130.2-130.3 (C-2", C-6"), 133.0 (C-1"'), 133.3 (C-1"), 136.2 (C-6), 137.1 (C-1'), 149.0 (C-8a), 153.1 (C-2), 161.0 (C-4"), 161.4 (CO_2H), 163.0 (C-4'), 165.1 (CONH), 177.5 (C-4). LC-MS (m/z): positive mode 544 $[M+H^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.3%. mp 264-265 °C.

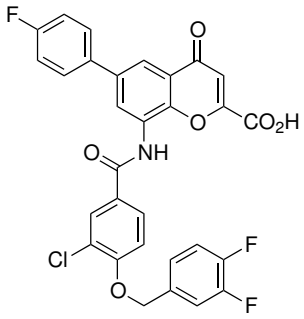
5.2.32.37 8-(4-(3,4-Difluorobenzyloxy)benzamido)-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylic acid 223 (*ANM151)*



The compound was synthesized according to GP-14 using **143** (224 mg, 0.39 mg) and was isolated as a white solid (193 mg, 91% yield). ^{1}H NMR: δ 5.21 (s, 2H, CH_2-O), 6.97 (s, 1H, 3-H), 7.16-7.21 (m, 2H, 3'-H, 5'-H), 7.30-7.38 (m, 3H, 6"-H, 3"'-H, 5"'-H), 7.47 (dt, J = 8.4 Hz, 1H, 5"-H), 7.57 (ddd, J = 7.9, 2.1 Hz, 1H, 2"-H), 7.89 (m, 2H, 2"'-H, 6"'-H), 7.94-8.15 (m, 3H, 5-H, 2'-H, 6'-H), 8.39 (d, J = 2.4 Hz, 1H, 7-H), 10.17

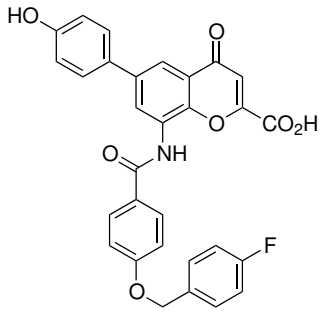
(s, 1H, CONH). ^{13}C NMR: δ 68.3 (CH_2-O), 113.5 (C-3), 114.8 (C-3', C-5'), 116.1-116.3 (C-3''', C5'''), 117.0-117.1 (C-2''), 117.7-117.8 (C-5''), 118.4 (C-7), 125.7 (C-4a), 124.8-124.9 (C-6''), 126.5 (C-1'), 128.9 (C-5), 129.1-129.2 (C-2''', C-6'''), 129.2 (C-8), 129.8 (C-2', C-6'), 134.5 (C-1''), 134.8 (C-6), 136.5 (C-1'''), 148.2-148.6 (C-4''), 148.8 (C-8a), 150.1-150.5 (C-3''), 153.1 (C-2), 161.2-161.3 (C-4'''), 161.4 (CO_2H), 163.4 (C-4'), 165.0 (CONH), 177.5 (C-4). LC-MS (m/z): positive mode 546 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 276-277 °C.

5.2.32.38 8-(3-Chloro-4-(3,4-difluorobenzyloxy)benzamido)-6-(4-fluorophenyl)-4-oxo-4*H*-chromene-2-carboxylic acid 224 (*ANM152)*



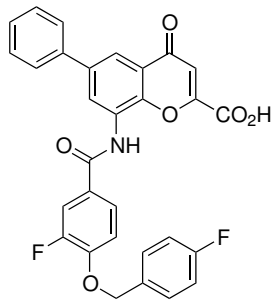
The compound was synthesized according to GP-14 using **144** (386 mg, 0.63 mmol) and was isolated as a yellow solid (310 mg, 85% yield). ^{1}H NMR: δ 5.32 (s, 2H, CH_2-O), 6.97 (s, 1H, 3-H), 7.29-7.40 (m, 3H, 3'''-H, 5'''-H, 6'''-H), 7.42 (d, J = 8.7 Hz, 1H, 5'-H), 7.49 (dt, J = 8.4 Hz, 1H, 5''-H), 7.57 (ddd, J = 7.8, 2.1 Hz, 1H, 2''-H), 7.77-7.83 (m, 2H, 2'''-H, 6'''-H), 8.02 (d, J = 8.7, 2.2 Hz, 1H, 6'-H), 8.07 (d, J = 2.3 Hz, 1H, 5-H), 8.16 (d, J = 2.2 Hz, 1H, 2'-H), 8.34 (d, J = 2.3 Hz, 1H, 7-H), 10.36 (s, NH, CONH). ^{13}C NMR: δ 69.2 (CH_2-O), 113.5 (C-3), 114.0 (C-5'), 116.1-116.3 (C-3''', C-5'''), 116.8-116.9 (C-2''), 177.8-117.9 (C-5''), 118.8 (C-7), 121.7 (C-3'), 124.6 (C-6''), 124.7 (C-4a), 127.4 (C-1'), 128.5-129.7 (C-2', C-6', C-5), 128.9 (C-8), 129.1-129.2 (C-2''', C-6'''), 134.0-134.0 (C-1''), 134.7 (C-6), 136.5 (C-1'''), 148.3-148.6 (C-4''), 149.0 (C-8a), 150.2-150.5 (C-3''), 153.1 (C-2), 161.3 (C-4'''), 161.4 (CO_2H), 163.4 (C-4'), 164.0 (CONH), 177.5 (C-4). LC-MS (m/z): positive mode 580 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.4%. mp 276-277 °C.

5.2.32.39 8-(4-((4-Fluorobenzyl)oxy)benzamido)-6-(4-hydroxyphenyl)-4-oxo-4*H*-chromene-2-carboxylic acid 225 (*ANM203)*



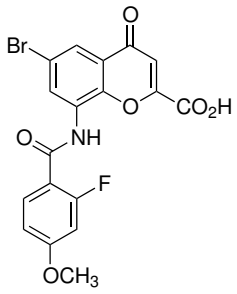
The compound was synthesized according to GP-14 using **131** (221 mg, 0.40 mmol) and was isolated as a yellow solid (178 mg, 85% yield). ^{1}H NMR: δ 5.20 (s, 2H, CH_2-O), 6.87-6.93 (m, 2H, 3‴-H, 5‴-H), 6.95 (s, 1H, 3-H), 7.12-7.20 (m, 2H, 3′-H, 5′-H), 7.20-7.27 (m, 3″-H, 5″-H), 7.48-7.56 (m, 2H, 2″-H, 6″-H), 7.56-7.61 (m, 2H, 2‴-H, 6‴-H), 7.96-8.06 (m, 3H, 2′-H, 6′-H, 5-H), 8.32 (d, J = 2.4 Hz, 1H, 7-H), 9.68 (s, 1H, OH), 10.12 (s, 1H, CONH). ^{13}C NMR: δ 69.0 (CH_2-O), 113.4 (C-3), 114.8 (C-3′, C-5′), 115.4-115.5 (C-3″, C-5″), 116.2 (C-3‴, C-5‴), 117.3 (C-5), 124.7 (C-4a), 126.5 (C-1′), 128.2 (C-2‴, C-6‴), 128.5 (C-7), 129.8 (C-2′, C-6′), 130.2-130.3 (C-2″, C-6″), 133.0 (C-6, C-1″), 137.7 (C-1‴), 148.3 (C-8a), 153.0 (C-2), 158.0 (C-4‴), 161.0 (C-4′), 161.4 (CO_2H), 163.0 (C-4″), 165.1 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 580 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%. mp > 300 °C.

5.2.32.40 8-(3-Fluor-4-(4-fluorbenzyloxy)benzamido)-4-oxo-6-phenyl-4*H*-chromene-2-carboxylic acid 226 (*THB27)*



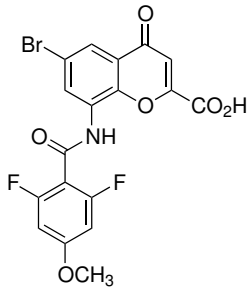
The compound was synthesized according to GP-14 using **145** (343 mg, 0.61 mmol) and was isolated as a yellow solid (301 mg, 94% yield). ^{1}H NMR: δ 5.29 (s, 2H, CH_2-O), 6.98 (s, 1H, 3-H), 7.19-7.31 (m, 2H, 3‴-H, 5‴-H), 7.41-7.50 (m, 3H, 4′-H, 2‴-H, 6‴-H), 7.50-7.57 (m, 4H, 2′-H, 3′-H, 5′-H, 6′-H), 7.70-7.82 (m, 2H, 5″-H, 6″-H), 7.86-7.94 (m, 1H, 2″-H), 8.10 (d, J = 2.3 Hz, 1H, 7-H), 8.37 (d, J = 2.3 Hz, 1H, 5-H), 10.30 (s, 1H, CONH). ^{13}C NMR: δ 69.9 (CH_2-O), 133.5 (C-3), 115.1 (C-5″), 115.4-115.7 (C-3‴, C-5‴), 118.8 (C-5), 124.7 (C-3″), 125.0 (C-7), 126.8 (C-4a), 127.0 (C-2′, C-6′), 128.4 (C-4′), 128.9 (C-8), 129.4 (C-3′, C-5′), 130.4 (C-2″, C-6″), 130.4 (C-2‴, C-6‴), 132.5 (C-1′), 137.5 (C-1‴), 138.2 (C-6), 149.0-149.4 (C-1″), 150.3 (C-8a), 152.3 (C-2), 153.1 (C-4″), 161.3 (C-4‴), 163.1 (C-4‴), 163.1 (CO_2H), 164.1 (CONH), 177.5 (C-4). LC-MS (m/z): positive mode 528 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 97%. mp 262-263 °C.

5.2.32.41 6-Bromo-8-(2-fluoro-4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 198 (*ANM6*)[65]



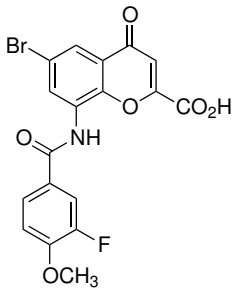
The compound was synthesized according to GP-14 using **104** (204 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ ethanol afforded the product as a white solid (170 mg, 95% yield). ^{1}H NMR: δ 3.87 (s, 3H, OCH_3), 6.96-7.04 (m, 3H, 3-H, 5'-H, 3'-H), 7.88 (d, J = 2.4 Hz, 1H, 5-H), 7.92 (dd, J = 8.9 Hz, 1H, 6'-H), 8.68 (d , J = 2.4 Hz, 1H, 7-H), 9.81 (d, J = 10.2 Hz, 1H, CONH). ^{13}C NMR: δ 56.4 (OCH_3), 102.1 (C-3'), 111.6 (C-5'), 113.4 (C-1'), 113.6 (C-3), 118.1 (C-6), 121.9 (C-5), 125.3 (C-4a), 128.7 (C-7), 130.1 (C-8), 132.5 (C-6'), 146.2 (C-8a), 153.3 (C-2), 161.0 (CO_2H), 161.2 (C-2'), 161.7 (C-4'), 164.1 (CONH), 176.3 (C4). LC-MS (m/z): negative mode 390 $[M\text{-}CO_2H]^-$, 434 $[M\text{-}H]^-$, positive mode 436 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 289-290 °C.

5.2.32.42 6-Bromo-8-(2,6-difluoro-4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 199 (*ANM7*)[65]



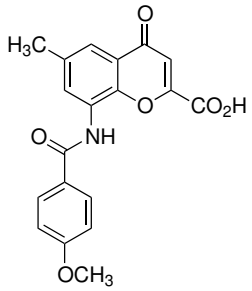
The compound was synthesized according to GP-14 using **105** (97 mg, 0.20 mmol). Recrystallization from 1:1 acetone/ ethanol afforded the product as a white solid (83 mg, 91% yield). ^{1}H NMR: δ 3.85 (s, 3H, OCH_3), 6.89 (d, J = 10.0 Hz, 2H, 3'-H, 5'-H), 6.97 (s, 1H, 3-H), 7.95 (d, J = 2.4 Hz, 1H, 5-H), 8.40 (s 1H, 7-H), 10.62 (s, 1H, CONH). ^{13}C NMR: δ 56.7 (OCH_3), 98.9 (C-3', C-5'), 106.6?106.9 (C-1'), 113.8 (C-3), 117.9 (C-6), 123.3 (C-5), 125.7 (C-4a), 129.4 (C-8), 130.9 (C-7), 147.3 (C-8a), 153.4 (C-2), 159.1 (C-4'), 160.4 (C-2', C-6'), 161.0 (CO_2H), 162.6 (CONH), 176.2 (C4). LC-MS (m/z): negative mode 408 $[M\text{-}CO_2H]^-$, 452 $[M\text{-}H]^-$, positive mode 456 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 296-297 °C.

5.2.32.43 6-Bromo-8-(3-fluoro-4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 200 (*ANM27*)[65]



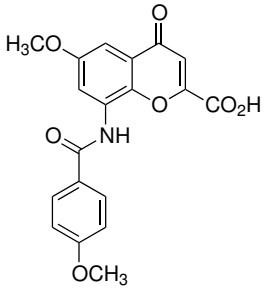
The compound was synthesized according to GP-14 using **106** (204 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ ethanol afforded the product as a white solid (144 mg, 75% yield). ^{1}H NMR: δ 3.94 (s, 3H, OCH_3), 6.97 (s, 1H, 3-H), 7.36 (dd, J = 8.5 Hz, 1H, 5'-H), 7.84?7.89 (m, 2H, 2'-H, 6'-H), 7.96 (d, J = 2.4 Hz, 1H, 5-H), 8.28 (d, J = 2.4 Hz, 1H, 7-H), 10.27 (s, 1H, CONH). ^{13}C NMR: δ 56.5 (OCH_3), 113.6 (C-3 or C-6'), 113.7 (C-3 or C-6'), 115.5 (C-2'), 117.8 (C-6), 123.4 (C-5), 125.3 (C-5'), 125.6 (C-4a), 126.0 (C-1'), 130.2 (C-8), 132.6 (C-7), 148.4 (C-8a), 150.6 (C-4'), 151.0 (C-3'), 153.3 (C-2), 161.1 (CO_2H), 164.1 (CONH), 176.4 (C4). LC-MS (m/z): negative mode 390 $[M\text{-}CO_2H]^-$, 434 $[M\text{-}H]^-$, positive mode 438 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 296-297 °C.

5.2.32.44 8-(4-Methoxybenzamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylic acid 201 (*ANM34*)[65]



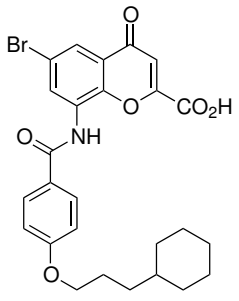
The compound was synthesized according to GP-14 using **107** (168 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (134 mg, 86% yield). ^{1}H NMR: δ 2.45 (s, 3H, $ArCH_3$), 3.85 (s, 3H, OCH_3), 6.91 (s, 1H, 3-H), 7.09 (d, J = 9.1 Hz, 2H, 3'-H, 5'-H), 7.68?7.69 (m, 1H, 5-H), 7.92 (d, J = 2.2 Hz, 1H, 7-H), 8.00 (d, J = 8.8 Hz, 2H, 2'-H, 6'- H), 10.00 (s, 1H, CONH). ^{13}C NMR: δ 20.8 ($ArCH_3$), 55.6 (OCH_3), 113.4 (C-3), 114.0 (C-3', C-5'), 120.7 (C-5), 124.1 (C-4a), 126.2 (C-1'), 128.3 (C-8), 129.7 (C-2', C-6'), 131.9 (C-7), 135.5 (C-6), 147.7 (C-8a), 152.9 (C-2), 161.4 (CO_2H), 162.4 (C-4'), 165.0 (CONH), 177.5 (C4). LC-MS (m/z): negative mode 308 $[M\text{-}CO_2H]^-$, 352 $[M\text{-}H]^-$, positive mode 354 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 283-284 °C.

5.2.32.45 6-Methoxy-8-(4-methoxybenzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 202 (*ANM40*)[65]



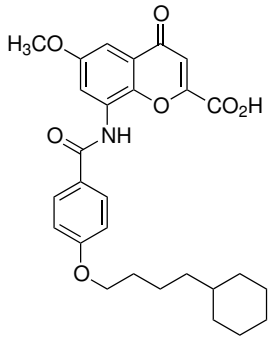
The compound was synthesized according to GP-14 using **108** (175 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (151 mg, 93% yield). ^{1}H NMR: δ 3.85 (s, 3H, 6-OCH_3 or 4'-OCH_3), 3.88 (s, 3H, 6-OCH_3 or 4'-OCH_3), 6.91 (s, 1H, 3-H), 7.09 (d, J = 8.9 Hz, 2H, 3'-H, 5'-H), 7.27 (d, J = 3.1 Hz, 1H, 5-H), 7.77 (d, J = 3.1 Hz, 1H, 7-H), 8.00 (d, J = 8.9 Hz, 2H, 2'-H, 6'-H), 10.01 (s, 1H, CONH). ^{13}C NMR: δ 55.7 (6-OCH_3 or 4'-OCH_3), 56.1 (6-OCH_3 or 4'-OCH_3), 101.2 (C-5), 112.7 (C-3), 114.0 (C-3', C-5'), 119.1 (C-7), 124.9 (C-4a), 126.1 (C-1'), 129.8 (C-2', C-6'), 129.9 (C-8), 144.2 (C-8a), 152.7 (C-2), 156.6 (C-6), 161.4 (CO_2H), 162.5 (C-4'), 165.0 (CONH), 177.2 (C4). LC-MS (m/z): negative mode 324 $[M\text{-}CO_2H]^-$, 368 $[M\text{-}H]^-$, positive mode 370 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 276-277 °C.

5.2.32.46 6-Bromo-8-(4-(3-cyclohexylpropoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 254 (*ANM26*)



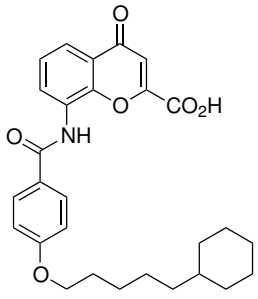
The compound was synthesized according to GP-14 using **172** (1952998221 mg, 0.35 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (167 mg, 90% yield). ^{1}H NMR: δ 0.89-1.76 (m, 15H, Cyclohexyl-H, 2''-H, 3''-H), 4.07 (t, J = 6.5 Hz, 2H, 1''-H), 6.95 (s, 1H, 3-H), 7.07 (m, 2H, 3'-H, 5'-H), 7.93 (d, J = 2.4 Hz, 1H, 5-H), 7.90-7.89 (m, 2H, 2'-H, 6'-H), 8.39 (d, J = 2.4 Hz, 1H, 7-H), 9.86 (s, 1H, CONH). ^{13}C NMR: δ 25.6-26.0 (C-2'', C-3''', C-4''', C-5'''), 32.6 (C-2''', C-6'''), 32.9 (C-3''), 68.3 (CH, O-CH), 113.2 (C-3), 114.3 (C-3', C-5'), 117.5 (C-6), 122.3 (C-5), 125.3 (C-1'), 125.6 (C-4a), 129.5 (C-2', C-6'), 130.4 (C-8), 130.8 (C-7), 147.5 (C-8a), 153.2 (C-2), 160.6 (C-4'), 161.9 (CO_2H), 164.8 (CONH), 175.9 (Cq, C-4). LC-MS (m/z): negative mode 482 $[M\text{-}CO_2H]^-$, 528 $[M\text{-}H]^-$, positive mode 530 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100.0%. mp 246-247 °C.

5.2.32.47 8-(4-(4-Cyclohexylbutoxy)benzamido)-6-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid 255 (*ANM123)*



The compound was synthesized according to GP-14 using **173** (117 mg, 0.22 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (100 mg, 92% yield). ^{1}H NMR: δ 1.29-1.41 (m, 17H, 2"-H, 3"-H, 4"-H, 1"'-H, 2"'-H, 3"'-H, 4"'-H, 5"'-H, 6"'-H), 3.88 (s, 3H, OCH_3), 4.06 (t, J = 6.5 Hz, 2H, 1"-H), 6.91 (s, 1H, 3-H), 7.01-7.15 (m, 2H, 3'-H, 5'-H), 7.26 (d, J = 3.1 Hz, 1H, 5-H), 7.77 (d, J = 3.1 Hz, 1H, 7-H), 7.90-7.99 (m, 2H, 2'-H, 6'-H), 9.99 (s, 1H, CONH). ^{13}C NMR: δ 22.9 (C-3"), 26.0 (C-3"', C-5"'), 26.4 (C-4"'), 29.0 (C-2"), 33.0 (C-2"', C-6"'), 36.7 (C-4"), 37.2 (C-1"'), 56.0 (OCH_3), 68.0 (C-1"), 101.1 (C-5), 112.7 (C-3), 114.4 (C-3', C-5'), 119.0 (C-7), 124.9 (C-4a), 125.9 (C-1'), 129.8 (C-2', C-6'), 129.9 (C-8), 144.1 (C-8a), 152.7 (C-6), 156.6 (C-2), 161.4 (CO_2H), 161.9 (C-4'), 165.0 (CONH), 177.2 (C-4). LC-MS (m/z): negative mode 492 $[M-H]^-$, positive mode 494 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 223-224 °C.

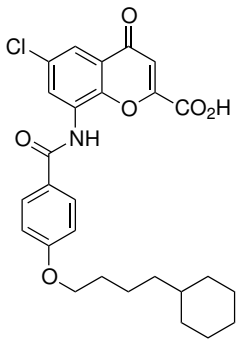
5.2.32.48 8-(4-((5-Cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 260 (*ANM158)*



The compound was synthesized according to GP-14 using **178** (117 mg, 0.35 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (132 mg, 79% yield). ^{1}H NMR: δ 0.86-0.95 (m, 2H, Cyclohexyl), 1.08-1.27 (m, 6H, Cyclohexyl, 5"-H), 1.32-1.50 (m, 4H, 4"-H, 3"-H), 1.60-1.78 (m, 7H, 2"-H, Cyclohexyl), 4.00-4.22 (m, 2H, 1'-H), 6.92 (s, 1H, 3-H), 7.04-7.14 (m, 2H, 3'-H, 5'-H), 7.53 (t, J = 7.9 Hz, 1H, 6-H), 7.88 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 7.90-7.99 (m, 2H, 2'-H, 6'-H), 8.16 (dd, J = 1.58, 7.8 Hz, 1H, 7-H), 9.76 (s, 1H, CONH). ^{13}C NMR: δ 25.6 (C-3"', C-4"'), 25.8-26.0 (C-5", C-4"), 28.4 (C-3"), 32.7 (C-2"', C-6"'), 36.5 (C-2"), 36.8 (C-1"'), 67.9 (C-1"), 113.1 (C-3), 113.2 (C-3', C-5'), 120.6 (C-5), 124.1 (C-4a), 125.2 (C-7), 126.0 (C-1'), 128.4 (C-8), 129.3 (C-2', C-6'), 129.5 (C-6), 148.7 (C-8a), 152.8 (C-2), 160.9 (CO_2H), 161.6 (C-4'), 164.7 (CONH), 177.2 (C-4). LC-MS (m/z): positive mode 478 $[M+H]^+$. Purity by HPLC-UV

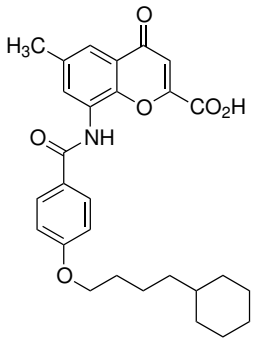
(254 nm)-ESI-MS: 100%. mp 231-232 °C.

5.2.32.49 6-Chloro-8-(4-(4-cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 257 (*ANM170)*



The compound was synthesized according to GP-14 using **175** (200 mg, 0.38 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (177 mg, 94% yield). ^{1}H NMR: δ 0.86-0.95 (m, 2H, Cyclohexyl), 1.10-1.33 (m, 6H, Cyclohexyl, 4''-H), 1.37-1.51 (m, 2H, 3''-H), 1.55-1.82 (m, 7H, 2''-H, Cyclohexyl), 4.08 (t, J = 6.5 Hz, 2H, 1''-H), 6.95 (s, 1H, 3-H), 7.04-7.10 (m, 2H, 3'-H, 5'-H), 7.79 (d, J = 2.6 Hz, 1H, 5-H), 7.94-8.11 (m, 2H, 2'-H, 6'-H), 8.27 (d, J = 2.6 Hz, 1H, 7-H), 9.87 (s, 1H, CONH). ^{13}C NMR: δ 22.5 (C-4''), 25.6 (C-3''', C5'''), 26.0 (C-4'''), 28.7 (C-3''), 32.6 (C-2''', C-6'''), 36.2 (C-2''), 36.8 (C-1'''), 67.9 (C-1''), 113.1 (C-3), 114.3 (C-3', C-5'), 124.9 (C-7), 124.9 (C-4a), 125.5 (C-1'), 128.1 (C-5), 129.4 (C-2', C-6'), 129.8-130.3 (C-8, C-6), 147.1 (C-8a), 153.1 (C-2), 160.6 (CO_2H), 161.9 (C-4'), 164.7 (CONH), 176.1 (C-4). LC-MS (m/z): positive mode 498 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 219-220 °C.

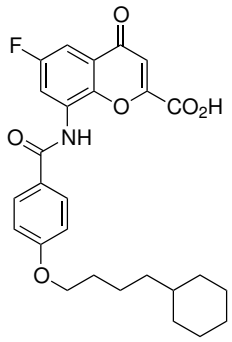
5.2.32.50 8-(4-(4-Cyclohexylbutoxy)benzamido)-6-methyl-4-oxo-4*H*-chromene-2-carboxylic acid 256 (*ANM173)*



The compound was synthesized according to GP-14 using **174** (192 mg, 0.38 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (172 mg, 95% yield). ^{1}H NMR: δ 0.86-0.95 (m, 2H, Cyclohexyl), 1.10-1.33 (m, 6H, Cyclohexyl, 4''-H), 1.36-1.51 (m, 2H, 3''-H), 1.54-1.81 (m, 7H, 2''-H, Cyclohexyl), 2.44 (s, 3H, CH_3), 4.06 (t, J = 6.5 Hz, 2H, 1''-H), 6.91 (s, 1H, 3-H), 7.03-7.10 (m, 2H, 3'-H, 5'-H), 7.69 (dd, J = 2.2 Hz, 1H, 5-H), 7.92 (d, J = 2.2 Hz, 2H, 7-H), 7.93-8.02 (m, 2H, 2'-H, 6'-H), 9.98 (s, 1H, CONH). ^{13}C NMR: δ 20.8 (CH_3), 22.9 (C-4''), 26.0 (C-3''', C5'''), 26.3 (C-4'''), 29.0 (C-3''), 32.0 (C-2''', C-6'''), 36.7 (C-2''), 37.1 (C-1'''), 68.0 (C-1''), 113.4 (C-3), 114.4 (C-3', C-5'), 120.7 (C-5), 124.1 (C-4a), 126.0 (C-1'), 128.3 (C-8), 129.7

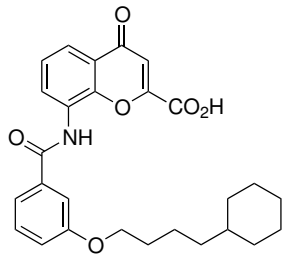
(C-2', C-6'), 131.8 (C-7), 135.4 (C-6), 147.7 (C-8a), 152.9 (C-2), 161.4 (CO_2H), 161.8 (C-4'), 165.0 (CONH), 177.5 (C-4). LC-MS (m/z): positive mode 478 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.9%. mp 223-225 °C.

5.2.32.51 8-(4-(4-Cyclohexylbutoxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid 258 (*ANM177)*



The compound was synthesized according to GP-14 using **176** (178 mg, 0.35 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (138 mg, 82% yield). ^{1}H NMR: δ 0.86-0.96 (m, 2H, Cyclohexyl), 1.11-1.34 (m, 6H, Cyclohexyl, 4"-H), 1.36-1.50 (m, 2H, 3"-H), 1.53-1.80 (m, 7H, 2"-H, Cyclohexyl), 4.05-4.11 (m, 2H, 1"-H), 6.93 (s, 1H, 3-H), 7.03-7.10 (m, 2H, 3'-H, 5'-H), 7.52 (dd, J = 3.1, 8.0 Hz, 1H, 5-H), 7.94-8.02 (m, 2H, 2'-H, 6'-H), 8.15 (dd, J = 3.1, 9.9 Hz, 1H, 7-H), 9.86 (s, 1H, CONH). ^{13}C NMR: δ 22.9 (C-4"), 26.0 (C-3"', C5"'), 26.3 (C-4"'), 29.0 (C-3"), 33.0 (C-2"', C-6"'), 36.7 (C-2"), 37.1 (C-1"'), 68.0 (C-1"), 105.2 (C-5), 112.8 (C-3), 114.5 (C-3', C-5'), 117.4-117.6 (C-7), 125.1 (C-4a), 125.6 (C-1'), 129.9 (C-2', C-6'), 130.8 (C-8), 145.5 (C-8a), 153.3 (C-2), 155.8 (C-6), 159.6 (CO_2H), 161.2 (C-4'), 162.1 (CONH), 177.9 (C-4). LC-MS (m/z): positive mode 482 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. mp 243-244 °C.

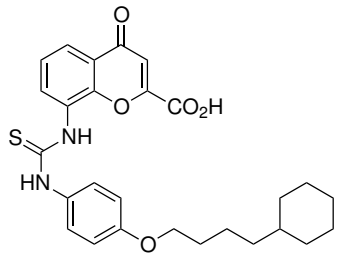
5.2.32.52 8-(3-(4-Cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 240 (*ANM178)*



The compound was synthesized according to GP-14 using **159** (162 mg, 0.33 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (120 mg, 83% yield). ^{1}H NMR: δ 0.82-0.89 (m, 2H, Cyclohexyl), 1.09-1.20 (m, 6H, Cyclohexyl, 4"-H), 1.36-1.50 (m, 2H, 3"-H), 1.51-1.78 (m, 7H, 2"-H, Cyclohexyl), 4.02-4.09 (m, 2H, 1"-H), 6.95 (s, 1H, 3-H), 7.17 (dd, J = 2.5, 8.2 Hz, 1H, 2'-H), 7.45 (t, J = 7.9 Hz, 1H, 6-H), 7.50-7.60 (m, 3H, 4'-H, 5'-H, 6'-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.08 (dd, J = 1.7, 7.8 Hz, 1H, 7-H), 10.19 (s, 1H, CONH). ^{13}C NMR: δ 22.9 (C-4"), 26.0 (C-3"', C5"'), 26.3

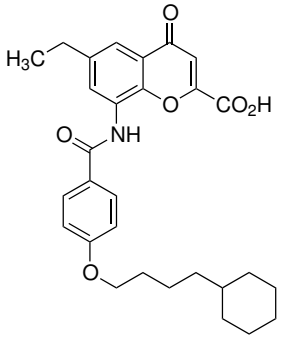
(C-4‴), 29.1 (C-3″), 33.0 (C-2‴, C-6‴), 36.7 (C-2″), 37.1 (C-1‴), 67.9 (C-1″), 113.5 (C-3, C-2′), 118.4-119.9 (C-4′, C-6′), 121.6 (C-5), 124.5 (C-8), 125.7 (C-7), 128.3 (C-4a), 129.9 (C-5′), 131.0 (C-6), 135.5 (C-1′), 149.4 (C-8a), 153.1 (C-2), 158.9 (C-3′), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 464 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 201-202 °C.

5.2.32.53 8-(3-(4-Cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 264 (*ANM182)*



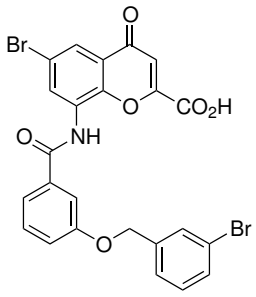
The compound was synthesized according to GP-14 using **185** (173 mg, 0.33 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (89 mg, 56% yield). ^{1}H NMR: δ 0.82-0.89 (m, 2H, Cyclohexyl), 1.09-1.21 (m, 6H, Cyclohexyl, 4″-H), 1.36-1.46 (m, 2H, 3″-H), 1.51-1.78 (m, 7H, 2″-H, Cyclohexyl), 3.94 (t, J = 6.5 Hz, 2H, 1″-H), 6.86-6.92 (s, 2H, 3′-H, 5′-H), 6.93 (s, 1H, 3-H), 7.34-7.40 (m, 2H, 2′-H, 6′-H), 7.49 (t, J = 7.9 Hz, 1H, 6-H), 7.89 (dd, J = 1.6, 7.9 Hz, 1H, 7-H), 7.97 (dd, J = 1.6, 7.8 Hz, 1H, 5-H), 9.41 (s, 1H, 1′-NH), 9.91 (s, 1H, 8-NH). ^{13}C NMR: δ 22.9 (C-4″), 26.0 (C-3‴, C5‴), 26.3 (C-4‴), 29.1 (C-3″), 33.0 (C-2‴, C-6‴), 36.7 (C-2″), 37.1 (C-1‴), 67.8 (C-1″), 113.6 (C-3), 114.6 (C-3′, C-5′), 122.1 (C-5), 124.6 (C-4a), 125.3 (C-7), 126.5 (C-2′, C-6′), 129.7 (C-8), 131.7 (C-1′), 133.4 (C-6), 150.6 (C-8a), 153.1 (C-2), 156.5 (C-4′), 161.5 (CO_2H), 177.7 (C-4), 180.7 (CS). LC-MS (m/z): positive mode 495 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98%. mp 148-150 °C.

5.2.32.54 8-(4-(4-Cyclohexylbutoxy)benzamido)-6-ethyl-4-oxo-4*H*-chromene-2-carboxylic acid 259 (*ANM217)*



The compound was synthesized according to GP-14 using **177** (228 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (208 mg, 96% yield). ^{1}H NMR: δ 0.82-0.89 (m, 2H, Cyclohexyl), 1.10-1.22 (m, 9H, Cyclohexyl, 4''-H, 6-$CH_2C\underline{H}_3$), 1.36-1.46 (m, 2H, 3''-H), 1.53-1.78 (m, 7H, 2''-H, Cyclohexyl), 2.76 (q, J = 7.6 Hz, 2H, 6-$C\underline{H}_2CH_3$), 4.06 (t, J = 6.5 Hz, 2H, 1''-H), 6.92 (s, 1H, 3-H), 7.02-7.11 (m, 2H, 3'-H, 5'-H), 7.71 (d, J = 2.2 Hz, 1H, 5-H), 7.96 (d, J = 2.2 Hz, 1H, 7-H), 7.98 (d, J = 8.7 Hz, 2H, 2'-H, 6'-H), 10.00 (s, 1H, CONH). ^{13}C NMR: δ 15.4 (6-$CH_2\underline{C}H_3$), 22.9 (C-4''), 26.0 (C-3''', C-5'''), 27.0 (C-4'''), 27.8 (6-$\underline{C}H_2CH_3$), 29.0 (C-3''), 36.7 (C-2''), 37.1 (C-1'''), 67.9 (C-1''), 113.3 (C-3), 114.3 (C-3', C-5'), 119.4 (C-5), 124.2 (C-4a), 126.0 (C-1'), 128.4 (C-8), 129.7 (C-2', C-6'), 130.8 (C-7), 141.5 (C-6), 147.9 (C-8a), 152.9 (C-2), 161.4 (CO_2), 161.8 (C-4'), 165.0 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 492 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 220-221 °C.

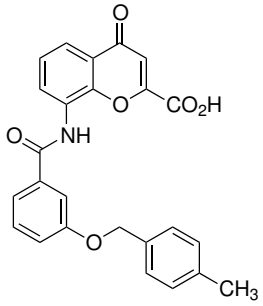
5.2.32.55 6-Bromo-8-(3-((3-bromobenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 233 (*ANMDOT6)*



The compound was synthesized according to GP-14 using **152** (258 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (215 mg, 85% yield). ^{1}H NMR: δ 5.21 (s, 2H, CH_2), 6.98 (s, 1H, 3-H), 7.29 (ddd, J = 0.9, 2.6, 8.2 Hz, 1H, 4'-H), 7.38 (t, J = 7.8 Hz, 1H, 5''-H), 7.45-7.56 (m, 3H, 5'-H, 2''-H, 6''-H), 7.61 (dt, J = 1.1, 7.8 Hz, 1H, 2'-H), 7.65 (dd, J = 1.6, 2.6 Hz, 1H, 6'-H), 7.65-7.72 (m, 1H, 4''-H), 7.97 (d, J = 2.4 Hz, 1H, 5-H), 8.32 (d, J = 2.4 Hz, 1H, 7-H), 10.32 (s, 1H, CONH). ^{13}C NMR: δ 68.7 (CH_2), 113.7 (C-3), 114.1 (C-2'), 117.8 (C-6), 119.0 (C-6'), 120.5 (C-4'), 121.9 (C-3''), 123.4 (C-5), 125.6 (C-4a), 126.8 (C-6''), 130.1 (C-5'), 130.2 (C-8), 130.4 (C-5''), 130.9 (C-4''), 130.9 (C-2''), 132.3 (C-7), 135.2 (C-1'), 139.7 (C-1''), 148.3 (C-8a), 153.3 (C-2), 158.3 (C-3'), 161.1 (CO_2H), 165.3 (CONH), 176.4 (C-4). LC-MS (m/z): positive

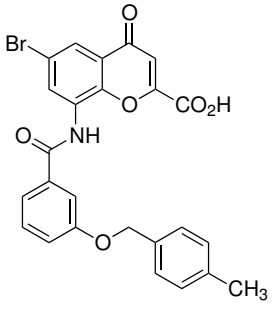
mode 575 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 253-254 °C.

5.2.32.56 8-(3-((4-Methylbenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 230 (*ANM234)*



The compound was synthesized according to GP-14 using **149** (137 mg, 0.30 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (74 mg, 57% yield). ^{1}H NMR: δ 2.30 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 6.94 (s, 1H, 3-H), 7.21 (d, J = 7.7 Hz, 2H, 2″-H, 6″-H), 7.25 (dd, J = 2.6, 8.2 Hz, 1H, 4′-H), 7.36 (d, J = 7.8 Hz, 2H, 3″-H, 5″-H), 7.47 (t, J = 7.9 Hz, 1H, 6-H), 7.55 (t, J = 7.9 Hz, 1H, 5′-H), 7.58-7.63 (m, 1H, 2′-H), 7.65 (t, J = 2.0 Hz, 1H, 6′-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.09 (dd, J = 1.6, 7.9 Hz, 1H, 7-H), 10.20 (s, 1H, CONH). ^{13}C NMR: δ 20.9 (CH_3), 69.6 (CH_2), 113.6 (C-3), 114.0 (C-2′), 118.8 (C-6′), 120.2 (C-4′), 121.6 (C-5), 124.5 (C-8), 125.7 (C-7), 128.0 (C-2″, C-6″), 128.3 (C-4a), 129.2 (C-3″, C-5″), 129.9 (C-5′), 130.9 (C-6), 133.9 (C-4″), 135.5 (C-1′), 137.3 (C-1″), 149.4 (C-8a), 153.1 (C-2), 158.6 (C-3′), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 430 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 229-231 °C.

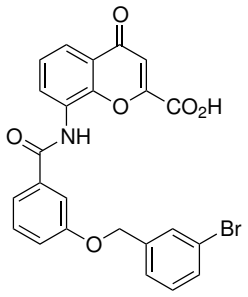
5.2.32.57 6-Bromo-8-(3-((4-methylbenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 231 (*ANM235)*



The compound was synthesized according to GP-14 using **150** (40 mg, 0.075 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (29 mg, 75% yield). ^{1}H NMR: δ 2.0 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 6.97 (s, 1H, 3-H), 7.21 (d, J = 7.7 Hz, 2H, 2″-H, 6″-H), 7.27 (dd, J = 2.5, 8.2 Hz, 1H, 4′-H), 7.33-7.40 (m, 2H, 3″-H, 5″-H), 7.48 (t, J = 7.9 Hz, 1H, 5′-H), 7.56-7.62 (m, 1H, 2′-H), 7.61-7.65 (m, 1H, 6′-H), 7.96 (d, J = 2.5 Hz, 1H, 5-H), 8.33 (d, J = 2.5 Hz, 1H, 7-H), 10.30 (s, 1H, CONH). ^{13}C NMR: δ 20.9 (CH_3), 69.6 (CH_2), 113.6 (C-3), 114.1 (C-2′), 117.8 (C-6), 119.0 (C-6′), 120.3 (C-4′), 123.3 (C-5), 125.6 (C-8), 128.0 (C-2″, C-6″), 129.2 (C-3″, C5″), 130.0 (C-5′), 130.2 (C-4a), 132.3 (C-7), 133.8 (C-4″), 135.2 (C-1′), 137.4 (C-1″), 148.2 (C-8a), 153.3

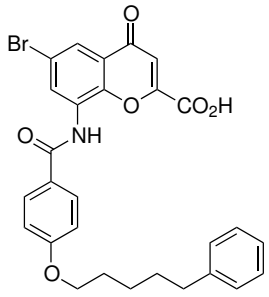
(C-2), 158.6 (C-3'), 161.1 (CO_2H), 165.4 (CONH), 176.4 (C-4). LC-MS (m/z): positive mode 509 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.0%. mp 245-246 °C.

5.2.32.58 8-(3-((3-Bromobenzyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 232 (*ANM236)*



The compound was synthesized according to GP-14 using **151** (157 mg, 0.30 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (148 mg, 99% yield). ^{1}H NMR: δ 5.21 (s, 2H, CH_2), 6.95 (s, 1H, 3-H), 7.28 (dd, J =2.6, 8.3 Hz, 1H, 4'-H), 7.38 (t, J = 7.8 Hz, 1H, 5"-H), 7.46-7.59 (m, 4H, 6-H, 5'-H, 2"-H, 6"-H), 7.60-7.71 (m, 3H, 2'-H, 6'-H, 4"-H), 7.91 (td, J = 1.4, 8.2 Hz, 1H, 5-H), 8.09 (dd, J = 1.7, 7.6 Hz, 1H, 7-H), 10.22 (s, 1H, CONH). ^{13}C NMR: δ 68.7 (CH_2), 113.6 (C-3), 114.1 (C-2'), 118.7 (C-6'), 120.5 (C-4'), 121.7 (C-5), 121.9 (C-3"), 124.5 (C-8), 125.7 (C-7), 126.8 (C-6"), 128.3 (C-4a), 129.9 (C-5'), 130.4 (C-3"), 130.9 (C-4", C-2"), 131.0 (C-6), 135.6 (C-1'), 139.8 (C-1"), 149.5 (C-8a), 153.1 (C-2), 158.4 (C-3'), 161.4 (CO_2H), 165.3 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 495 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.9%. mp 256-257 °C.

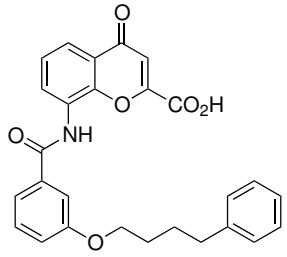
5.2.32.59 6-Bromo-4-oxo-8-(4-((5-phenylpentyl)oxy)benzamido)-4*H*-chromene-2-carboxylic acid 261 (*THB47)*



The compound was synthesized according to GP-14 using **179** (198 mg, 0.34 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (166 mg, 89% yield). ^{1}H NMR: δ 1.38-1.52 (m, 2H, 3"-H), 1.58-1.72 (m, 2H, 4"-H), 1.78 (p, J = 6.8 Hz, 2H, 2"-H), 2.60 (t, J = 7.7 Hz, 2H, 5"-H), 4.06 (t, J = 6.5 Hz, 2H, 1"-H), 6.97 (s, 1H, 3-H), 7.03-7.11 (m, 2H, 3'-H, 5'-H), 7.12-7.1 (m, 5H, 2"'-H, 3"'-H, 4"'-H, 5"'-H, 6"'-H), 7.89-8.01 (m, 3H, 2'-H, 6'-H, 5-H), 8.33 (d, J = 2.5 Hz, 1H, 7-H), 10.10 (s, 1H, CONH). ^{13}C NMR: δ 25.3 (C-2"), 28.5 (C-3"), 30.8 (C-5"), 35.2 (C-4"), 67.9 (C-1"), 113.6 (C-3), 114.4 (C-3', C-5'), 117.8 (C-6), 122.9 (C-5), 125.5 (C-4a, C-1'), 125.7 (C-4"'), 128.3 (C-2', C-6', C-2"', C-6"'), 129.8 (C-3"', C-5"'), 130.5 (C-8), 132.0 (C-7),

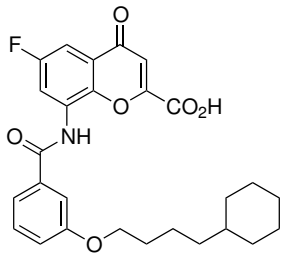
142.3 (C-1‴), 148.1 (C-8a), 153.3 (C-2), 161.1 (C-4′), 162.0 (CO_2H), 165.0 (CONH), 176.4 (C-4). LC-MS (m/z): positive mode 552 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 223-224 °C.

5.2.32.60 4-Oxo-8-(3-(4-phenylbutoxy)benzamido)-4*H*-chromene-2-carboxylic acid 252 (*THB49)*



The compound was synthesized according to GP-14 using **170** (182 mg, 0.37 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (137 mg, 81% yield). ^{1}H NMR: δ 1.76 (dq, J = 3.4, 7.4 Hz, 4H, 2″-H, 3″-H), 2.59-2.70 (m, 2H, 4″-H), 3.88-4.23 (m, 2H, 1″-H), 6.95 (s, 1H, 3-H), 7.13-7.31 (m, 6H, 2′-H, 2‴-H, 3‴-H, 4‴-H, 5‴-H, 6‴-H), 7.45 (t, J = 7.9 Hz, 1H, 6-H), 7.51-7.61 (m, 3H, 4′-H, 5′-H, 6′-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.08 (dd, J = 1.6, 7.7 Hz, 1H, 7-H), 10.17 (s, 1H, CONH). ^{13}C NMR: δ 27.5 (C-2″), 28.4 (C-3″), 34.9 (C-4″), 67.7 (C-1″), 113.5 (C-3), 118.4-119.9 (C-4′, C-6′), 121.6 (C-5), 124.3 (C-8), 125.7 (C-4‴), 125.8 (C-2‴, C-6‴), 128.4 (C-4a), 128.4 (C-7, C-3‴, C-5‴), 129.8 (C-5′), 130.9 (C-6), 135.5 (C-1′), 142.1 (C-1‴), 149.4 (C-8a), 153.01 (C-2), 158.8 (C-3′), 161.3 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 458 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.5%. mp 213-214 °C.

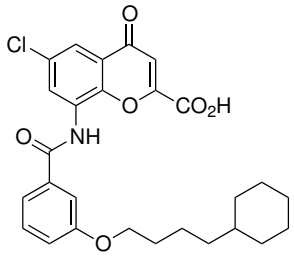
5.2.32.61 8-(3-(4-Cyclohexylbutoxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid 242 (*THB71)*



The compound was synthesized according to GP-14 using **161** (94 mg, 0.18 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (31 mg, 36% yield). ^{1}H NMR: δ 0.78-0.93 (m, 2H, Cyclohexyl), 1.03-1.30 (m, 6H, Cyclohexyl, 4″-H), 1.43 (dt, J = 6.8 Hz, 2H, 3″-H), 1.54-1.77 (m, 7H, 2″-H, Cyclo- hexyl), 4.05 (t, J = 6.4 Hz, 2H, 1″-H), 6.95 (s, 1H, 3-H), 7.09-7.29 (m, 1H, 2′-H), 7.46 (t, J = 7.9 Hz, 1H, 5′-H), 7.51-7.60 (m, 3H, 5-H, 4′-H, 6′-H), 8.10 (dd, J = 3.1, 9.7 Hz, 1H, 7-H), 10.26 (s, 1H, CONH). ^{13}C NMR: δ 22.9 (C-4″), 26.0 (C-3‴, C-5‴), 26.3 (C-4‴), 29.1 (C-3″), 33.0 (C-2‴, C-6‴), 36.7 (C-2″), 37.1 (C-1‴), 67.9 (C-1″), 105.8 (C-5), 112.8 (C-2′), 113.5 (C-3), 117.7 (C-7),

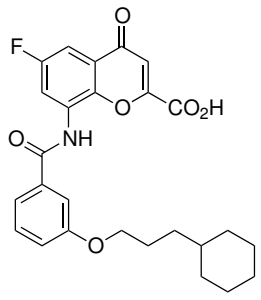
118.7-119.9 (C-4', C-6'), 125.1 (C-4a), 129.9 (C-5'), 130.5 (C-8), 135.1 (C-1'), 145.6 (C-8a), 153.2 (C-2), 153.2 (C-2), 157.6-159.6 (C-6), 158.9 (C-3'), 161.4 (CO_2H), 165.4 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 482 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 218-219 °C.

5.2.32.62 6-Chloro-8-(3-(4-cyclohexylbutoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 241 (*THB72)*



The compound was synthesized according to GP-14 using **160** (206 mg, 0.39 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (155 mg, 80% yield). ^{1}H NMR: δ 0.78-0.93 (m, 2H, Cyclohexyl), 1.02-1.27 (m, 6H, Cyclohexyl, 4"-H), 1.38-1.49 (m, 2H, 3"-H), 1.54-1.77 (m, 7H, 2"-H, Cyclo- hexyl), 4.04 (t, J = 6.4 Hz, 2H, 1"-H), 6.97 (s, 1H, 3-H), 7.18 (ddd, J = 1.0, 2.6, 8.3 Hz, 1H, 2'-H), 7.46 (t, J =7.9 Hz, 1H, 5'-H), 7.51-7.59 (m, 2H, 4'-H, 6'-H), 7.83 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.27 (s, 1H, CONH). ^{13}C NMR: δ 22.9 (C-4"), 26.0 (C-3"', C-5"'), 26.3 (C-4"'), 29.1 (C-3"), 33.0 (C-2"', C-6"'), 36.7 (C-2"), 37.1 (C-1"'), 67.9 (C-1"), 113.5 (C-3, C-2'), 118.7 (C-7), 120.0-120.1 (C-4', C-6'), 125.2 (C-4a), 129.5 (C-5), 130.0 (C-8, C-6), 130.1 (C-5'), 15.1 (C-1'), 147.8 (C-8a), 153.3 (C-2), 158.9 (C-3'), 161.1 (CO_2H), 165.4 (CONH), 176.5 (C-4). LC-MS (m/z): positive mode 498 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 219-220 °C.

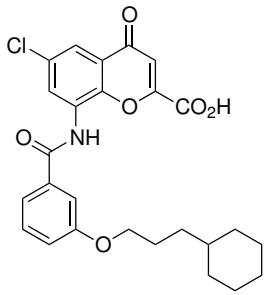
5.2.32.63 8-(3-(3-Cyclohexylpropoxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid 239 (*THB73)*



The compound was synthesized according to GP-14 using **158** (241 mg, 0.49 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (132 mg, 58% yield). ^{1}H NMR: δ 0.80-0.95 (m, 2H, Cyclohexyl), 1.07-1.38 (m, 6H, Cyclohexyl, 3"-H), 1.54-1.81 (m, 7H, 2"-H, Cyclo- hexyl), 4.03 (t, J = 6.4 Hz, 2H, 1"-H), 6.95 (s, 1H, 3-H), 7.19 (ddd, J = 1.0, 2.6, 8.0 Hz, 1H, 2'-H), 7.46 (t, J = 7.9 Hz, 1H, 5'-H), 7.51-7.62 (m, 3H, 5-H, 4'-H, 6'-H), 8.10 (dd, J = 3.1, 9.6 Hz, 1H, 7-H), 10.26 (s, 1H, CONH). ^{13}C NMR: δ 26.3 (C-3"', C-5"',

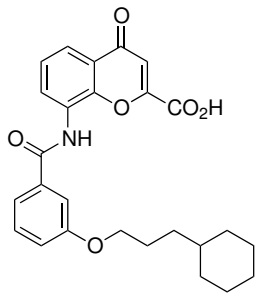
C-4‴. C-3″), 32.9 (C-2‴, C-6‴), 33.3 (C-2″), 36.9 (C-1‴), 68.2 (C-1″), 105.6 (C-5), 112.8 (C-2′), 113.5 (C-3), 117.7 (C-7), 118.7-119.9 (C-4′, C-6′), 125.1 (C-4a), 129.9 (C-5′), 130.5 (C-8), 135.1 (C-1′), 145.6 (C-8a), 153.2 (C-2), 153.2 (C-2), 157.6-159.6 (C-6), 158.9 (C-3′), 161.4 (CO_2H), 165.4 (CONH), 176.9 (C-4). LC-MS (m/z): positive mode 468 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.6%. mp 230-231 °C.

5.2.32.64 6-Chloro-8-(3-(3-cyclohexylpropoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 238 (*THB74)*



The compound was synthesized according to GP-14 using **157** (235 mg, 0.46 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (144 mg, 65% yield). ^{1}H NMR: δ 0.79-0.96 (m, 2H, Cyclohexyl), 1.01-1.39 (m, 6H, Cyclohexyl, 3″-H), 1.54-1.81 (m, 7H, 2″-H, Cyclo- hexyl), 4.03 (t, J = 6.4 Hz, 2H, 1″-H), 6.95 (s, 1H, 3-H), 7.18 (ddd, J = 1.0, 2.6, 8.3 Hz, 1H, 2′-H), 7.46 (t, J =7.9 Hz, 1H, 5′-H), 7.49-7.60 (m, 2H, 4′-H, 6′-H), 7.82 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.26 (s, 1H, CONH). ^{13}C NMR: δ 26.3 (C-3‴, C-5‴, C-4‴. C-3″), 32.9 (C-2‴, C-6‴), 33.3 (C-2″), 37.0 (C-1‴), 68.2 (C-1″), 113.6 (C-3, C-2′), 118.7 (C-7), 120.0-120.1 (C-4′, C-6′), 125.2 (C-4a), 129.5 (C-5), 129.9 (C-8, C-6), 130.2 (C-5′), 15.1 (C-1′), 147.8 (C-8a), 153.2 (C-2), 158.9 (C-3′), 161.1 (CO_2H), 165.4 (CONH), 176.5 (C-4). LC-MS (m/z): positive mode 484 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.5%. mp 239-240 °C.

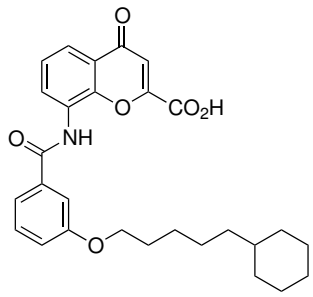
5.2.32.65 8-(3-(3-Cyclohexylpropoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 237 (*THB75)*



The compound was synthesized according to GP-14 using **156** (188 mg, 0.39 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (109 mg, 62% yield). ^{1}H NMR: δ 0.78-0.96 (m, 2H, Cyclohexyl), 1.00-1.39 (m, 6H, Cyclohexyl, 3″-H), 1.52-1.82 (m, 7H, 2″-H, Cyclo- hexyl), 4.03 (t, J = 6.4 Hz, 2H, 1″-H), 6.94 (s, 1H, 3-H), 7.09-7.32 (m, 1H, 2′-H), 7.45 (t, J = 7.9 Hz, 1H, 6-H), 7.51-7.60 (m, 3H, 4′-H, 5′-H, 6′-H), 7.91 (dd, J = 1.6, 8.0 Hz, 1H, 5-H), 8.08 (dd, J = 1.7, 7.8 Hz, 1H, 7-H), 10.19 (s, 1H, CONH). ^{13}C NMR: δ

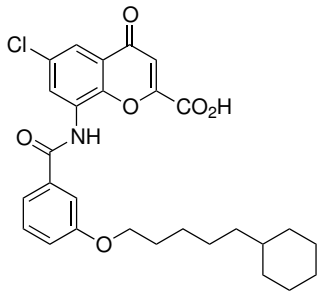
26.0-26.4 (C-3‴, C-5‴, C-4‴. C-3″), 33.0 (C-2‴, C-6‴), 33.4 (C-2″), 37.0 (C-1‴), 68.3 (C-1″), 113.5 (C-3, C-2′), 118.4-119.9 (C-4′, C-6′), 121.6 (C-5), 124.5 (C-8), 125.7 (C-7), 128.3 (C-4a), 129.9 (C-5′), 131.0 (C-6), 135.5 (C-1′), 149.4 (C-8a), 153.1 (C-2), 158.9 (C-3′), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 450 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 98.9%. mp 220-221 °C.

5.2.32.66 8-(3-((5-Cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 243
(*ANM250)*



The compound was synthesized according to GP-14 using **162** (222 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (191 mg, 91% yield). ^{1}H NMR: δ 0.78-0.90 (m, 2H, Cyclohexyl), 1.06-1.23 (m, 6H, 5″-H, Cyclohexyl), 1.55-1.78 (m, 7H, 2″-H, Cyclohexyl), 4.05 (t, J = 6.5 Hz, 2H, 1″-H), 6.95 (s, 1H, 3-H), 7.17 (dd, J = 8.3, 2.5 Hz, 1H, 4′-H), 7.45 (t, J = 7.9 Hz, 1H, 2′-H), 7.52- 7.60 (m, 3H, 5′-H, 6′-H, 6-H), 7.91 (dd, J = 8.1, 2.5 Hz, 1H, 5-H), 8.08 (dd, J = 7.7, 1.6 Hz, 1H, 7-H), 10.17 (s, 1H, CONH). ^{13}C NMR: δ 26.0 (C-5″, C-4″, C-3‴, C-5‴), 26.0 (C-4‴), 28.8 (C-3″), 33.0 (C-2‴, C-6‴), 37.0 (C-2″), 37.1 (C-1‴), 67.9 (C-1‴), 113.5 (C-3, C-2′), 118.5 (C-4′), 119.9 (C-5), 121.6 (C-6′), 124.5 (C-4a), 125.7 (C-7), 128.3 (C-8), 129.9 (C-6), 130.9 (C-5′), 135.5 (C-1′), 149.4 (C-8a), 153.0 (C-2), 158.9 (C-4″), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 478 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%. mp 239-240 °C.

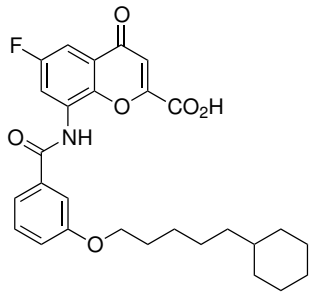
5.2.32.67 6-Chloro-8-(3-((5-cyclohexylpentyl)oxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 244
(*ANM251)*



The compound was synthesized according to GP-14 using **163** (187 mg, 0.35 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (165 mg, 92% yield). ^{1}H NMR: δ 0.81-0.94 (m, 2H, Cyclohexyl), 1.03-1.27 (m, 6H, Cyclohexyl, 5″-H), 1.30-1.49 (m, 4H, 3″-H, 4″-H), 1.54-1.81 (m, 7H, 2″-H, Cyclohexyl), 4.07 (t, J = 6.5 Hz, 2H, 1″-H), 6.95 (s, 1H, 3-H), 7.18 (dd, J = 8.0 Hz, 1H, 4′-H), 7.45

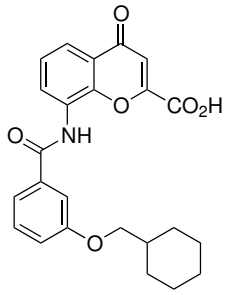
(t, J = 7.9 Hz, 1H, 2'-H), 7.50-7.60 (m, 2H, 5'-H, 6'-H), 7.81 (d, J = 2.5 Hz, 1H, 5-H), 8.26 (d, J = 2.6 Hz, 1H, 7-H), 10.01 (s, 1H, CONH). ^{13}C NMR: δ 25.7 (C-4", C-5", C-3"', C-5"'), 26.1 (C-4"'), 28.6 (C-3"), 32.8 (C-2"', C-6"'), 36.6 (C-2"), 36.9 (C-1"'), 67.9 (C-1"), 113.2 (C-2', C-3), 118.6 (C-4'), 119.7 (C-5, C-6'), 125.0 (C-4a), 128.5 (C-7), 129.7 (C-5'), 130.0 (C-8), 134.0 (C-1'), 147.3 (C-8a), 153.2 (C-2), 158.8 (C-4"), 160.7 (C_2H), 165.3 (CONH), 176.2 (C-4). LC-MS (m/z): positive mode 512 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 230-231 °C.

5.2.32.68 8-(3-((5-Cyclohexylpentyl)oxy)benzamido)-6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid 245 (*ANM252)*



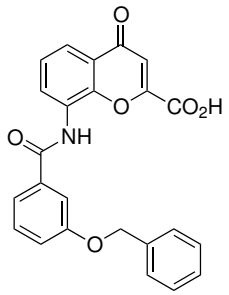
The compound was synthesized according to GP-14 using **164** (172 mg, 0.33 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (142 mg, 87% yield). ^{1}H NMR: δ 0.83-0.90 (m, 2H, Cyclohexyl), 1.01-1.27 (m, 6H, Cyclohexyl, 5"-H), 1.27-1.46 (m, 4H, 3"-H, 4"-H), 1.52-1.80 (m, 7H, 2"-H, Cyclohexyl), 4.05 (t, J = 6.5 Hz, 2H, 1"-H), 6.95 (s, 1H, 3-H), 7.19 (dd, J = 8.3 Hz, 1H, 4'-H), 7.46 (t, J = 8.0 Hz, 1H, 2'-H), 7.50-7.61 (m, 3H, 5'-H, 6'-H, 5-H), 8.10 (d, J = 3.2 Hz, 1H, 7-H), 10.27 (s, 1H, CONH). ^{13}C NMR: δ 26.0 (C-4", C-5", C-3"', C-5"'), 26.0 (C-4"'), 28.8 (C-3"), 33.0 (C-2"', C-6"'), 37.0 (C-2"), 37.1 (C-1"'), 67.9 (C-1"), 105.6-105.8 (C-7), 112.9 (C-2'), 113.5 (C-3), 117.7-117.9 (C-5), 118.8 (C-4'), 120.0 (C-6'), 125.1 (C-4a), 130.0 (C-5'), 130.5 (C-8), 135.1 (C-1'), 145.6 (C-8a), 153.2 (C-2), 157.8-159.4 (C-6), 158.9 (C-4"), 161.2 (CO_2H), 165.4 (CONH). LC-MS (m/z): positive mode 496 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 227-228 °C.

5.2.32.69 8-(3-(Cyclohexylmethoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 235 (*ANM263)*



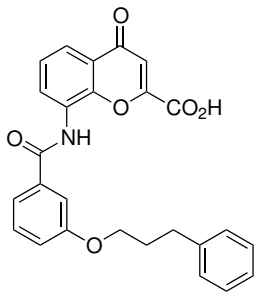
The compound was synthesized according to GP-14 using **154** (198 mg, 0.44 mm32292229ol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (177 mg, 95% yield). ^{1}H NMR: δ 0.99-1.14 (m, 2H, Cyclohexyl), 1.14-1.35 (m, 3H, Cyclohexyl), 1.61-1.89 (m, 7H, Cyclohexyl), 3.87 (d, J = 6.1 Hz, 2H, 1''-H), 6.95 (s, 1H, 3-H), 7.14-7.23 (m, 1H, 4'-H), 7.45 (t, J = 7.8 Hz, 1H, 2'-H), 7.50-7.61 (m, 3H, 5'-H, 6'-H, 6-H), 7.91 (dd, J = 8.0, 1.7 Hz, 1H, 5-H), 8.08 (dd, J = 7.7, 1.7 Hz, 1H, 7-H), 10.18 (s, 1H, CONH). ^{13}C NMR: δ 25.4 (C-3''', C-5'''), 26.2 (C-4'''), 29.4 (C-2''', C-6'''), 37.3 (CH, C-1'''), 73.2 (C-1''), 113.5 (C-3, C-2'), 118.5 (C-4'), 120.0 (C-5), 121.7 (C-6'), 124.5 (C-4a), 125.7 (C-8), 129.9 (C-6), 131.0 (C-5'), 135.5 (C-1'), 149.5 (C-8a), 153.1 (C-2), 159.1 (C-4''), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 422 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.8%. mp 254-255 °C.

5.2.32.70 8-(3-(Benzyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 249 (*ANM264)*



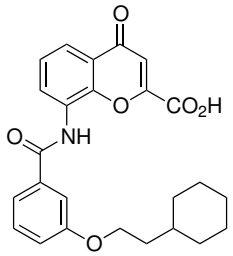
The compound was synthesized according to GP-14 using **167** (195 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (158 mg, 87% yield). ^{1}H NMR: δ 5.20 (s, 2H, OCH_2), 6.95 (s, 1H, 3-H), 7.27 (dd, J = 8.3 Hz, 2H, 4'-H), 7.30-7.37 (m, 1H, 4'''-H), 7.37-7.44 (m, 2H, C-3''', C-5'''), 7.45-7.52 (m, 3H, 2'''-H, 6'''-H, 2'-H), 7.55 (t, J = 7.9 Hz, 1H, 6-H), 7.58-7.64 (m, 1H, 5'-H), 7.68 (dd, J = 2.7 Hz, 1H, 6'-H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.09 (dd, J = 7.8, 1.6 Hz, 1H, 7-H), 10.22 (s, 1H, CONH). ^{13}C NMR: δ 69.7 (OCH_2), 113.5 (C-3), 114.0 (C-2'), 118.8 (C-4'), 120.3 (C-5), 121.6 (C-6'), 123.7 (C-4a), 125.8 (C-7), 127.9 (C-2''', C-6''', C-5'''), 128.1 (C-8), 128.6 (C-3''', C-5'''), 129.9 (C-6), 130.9 (C-5'), 135.6 (C-1'), 136.9 (C-1'''), 149.4 (C-8a), 153.1 (C-2), 158.6 (C-4''), 161.4 (CO_2H), 165.3 (CONH), 177.6 (C4). LC-MS (m/z): positive mode 416 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.9%. mp 243-244 °C.

5.2.32.71 4-Oxo-8-(3-(3-phenylpropoxy)benzamido)-4*H*-chromene-2-carboxylic acid 251 (*ANM265)*



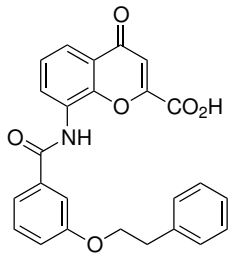
The compound was synthesized according to GP-14 using **169** (132 mg, 0.28 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a pale-yellow solid (99 mg, 80% yield). ^{1}H NMR: δ 1.88-2.20 (m, 2H, 3"-H), 2.63-2.90 (m, 2H, 2"-H), 4.07 (t, J = 6.3 Hz, 2H, 1"-H), 6.95 (s, 1H, 3-H), 7.14-7.33 (m, 6H, 4'-H, 4"'-H, 3"'-H, 5"'-H, 2"'-H, 6"'-H), 7.46 (t, J = 7.9 Hz, 1H, 6-H), 7.51-7.62 (m, 2H, 5'-H, 6'-H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.09 (dd, J = 7.8, 1.6 Hz, 1H, 7-H), 10.20 (s, 1H, CONH). ^{13}C NMR: δ 30.5 (C-2"), 120.1 (C-5), 121.6 (C-6'), 124.4 (C-4a), 125.7 (C-7), 128.3 (C-8), 128.5 (C-2"', C-3", C-4"', C-5"', C-6"'), 129.9 (C-6), 130.9 (C-5'), 135.5 (C-1"'), 141.5 (C-1'), 149.4 (C-8a), 153.0 (C-2), 158.8 (C-4"), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 444 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.9%. mp 222-223 °C.

5.2.32.72 8-(3-(2-Cyclohexylethoxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 236 (*ANM267)*



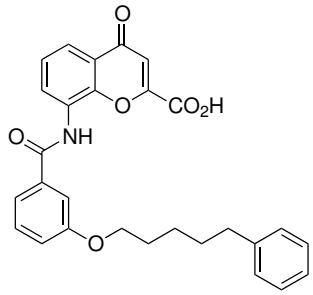
The compound was synthesized according to GP-14 using **155** (203 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (185 mg, 97% yield). ^{1}H NMR: δ 0.88-1.02 (m, 2H, Cyclohexyl), 1.07-1.30 (m, 2H, Cyclohexyl), 1.50-1.60 (m, 2H, Cyclohexyl), 1.56-1.79 (m, 7H, Cyclohexyl, 2"-H), 4.09 (t, J = 6.6 Hz, 2H, 1"-H), 6.95 (s, 1H, 3-H), 7.18 (ddd, J = 8.2, 2.6, 1.0 Hz, 1H, 4'-H), 7.45 (t, J = 7.9 Hz, 1H, 2'-H), 7.50-7.62 (m, 3H, 6-H, 5'-H, 6'-H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H, 5-H), 8.08 (dd, J = 7.7, 1.6 Hz, 1H, 7-H), 10.17 (s, 1H, CONH). ^{13}C NMR: δ 25.9 (C-3"', C-5"'), 26.2 (C-4"'), 32.9 (C-2"', C-6"'), 34.2 (C-1"'), 36.2 (C-2"), 66.0 (C-1"), 113.6 (C-2', C-3),118.4 (C-4'), 120.0 (C-5), 121.7 (C-6'), 124.5 (C-4a), 125.7 (C-7), 128.4 (C-8), 129.9 (C-6), 131.0 (C-5'), 135.5 (C-1'), 149.5 (C-8a), 153.1 (C-2), 158.9 (C-4"), 161.4 (CO_2H), 165.4 (CONH), 176.6 (C-4). LC-MS (m/z): positive mode 436 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 100%. mp 230-231 °C.

5.2.32.73 4-Oxo-8-(3-phenethoxybenzamido)-4*H*-chromene-2-carboxylic acid 250 (*ANM270)*



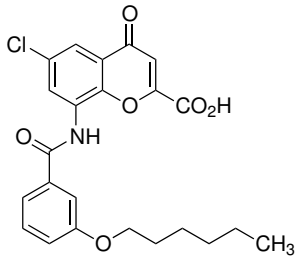
The compound was synthesized according to GP-14 using **168** (174 mg, 0.38 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a pale-yellow solid (142 mg, 87% yield). ^{1}H NMR: δ 3.08 (t, J = 6.8 Hz, 2H, 2″-H), 4.30 (t, *J* = 6.8 Hz, 2H, 1″-H), 6.94 (s, 1H, 3-H), 7.15-7.25 (m, 2H, 4′-H, 4‴-H), 7.28-7.38 (m, 4H, 2‴-H, 3‴-H, 5‴-H, 6‴-H), 7.42-7.49 (m, 1H, 6-H), 7.51-7.61 (m, 3H, 2′-H, 5′-H, 6′-H), 7.91 (dd, *J* = 8.0, 1.6 Hz, 1H, 5-H), 8.07 (dd, *J* = 7.8 Hz, 1.6 Hz, 1H, 7-H), 10.19 (s, 1H, CONH). ^{13}C NMR: δ 35.0 (C-2″), 68.5 (C-1″), 113.5 (C-2′, C-3), 118.4 (C-4′), 120.1 (C-5), 121.6 (C-6′), 124.5 (C-4a), 125.7 (C-7), 126.4 (C-4‴), 128.4 (C-8), 128.4 (C-2‴, C-6‴), 129.1 (C-3‴, C-5‴), 129.9 (C-6), 131.0 (C-5′), 135.5 (C-1′), 138.4 (C-1‴), 149.5 (C-8a), 153.0 (C-2), 158.6 (C-4″), 161.3 (CO_2H), 165.3 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 430 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.2%. mp 230-231 °C.

5.2.32.74 4-Oxo-8-(3-((5-phenylpentyl)oxy)benzamido)-4*H*-chromene-2-carboxylic acid 253 (*ANM280)*



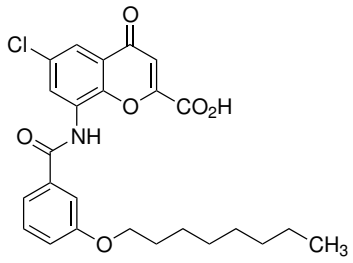
The compound was synthesized according to GP-14 using **171** (138 mg, 0.28 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (123 mg, 93% yield). ^{1}H NMR: δ 1.40-1.52 (m, 2H, 2″-H), 1.59-1.70 (m, 2H, 3″-H), 1.71-1.83 (m, 2H, 5″-H), 2.56-2.64 (m, 2H, 4″-H), 4.05 (t, *J* = 6.4 Hz, 2H, 1″-H), 6.95 (s, 1H, 3-H), 7.11-7.22 (m, 4H, 4′-H, 3‴-H, 4‴-H, 5‴-H), 7.22-7.29 (m, 2H, 2‴-H, 6‴-H), 7.45 (t, *J* = 7.9 Hz, 1H, 6-H), 7.51-7.61 (m, 3H, 5′-H, 6′-H, 2′-H), 7.91 (dd, *J* = 8.0, 1.6 Hz, 1H, 5-H), 8.08 (dd, *J* = 7.7, 1.6 Hz, 1H, 7-H), 10.17 (s, 1H, CONH). ^{13}C NMR: δ 25.4 (C-2″), 28.6 (C-3″), 30.9 (C-5″), 35.2 (C-4″), 67.8 (C-1″), 113.5 (C-3, C-2′), 118.5 (C-4′), 120.0 (C-5), 121.6 (C-6′), 124.5 (C-4a), 125.7 (C-7), 128.3 (C-8), 128.4 (C-2‴, C-3‴, C-4‴, C-5‴, C-6‴), 129.9 (C-6), 130.9 (C-5′), 135.5 (C-1′), 142.3 (C-1‴), 149.4 (C-8a), 153.0 (C-2), 158.9 (C-4″), 161.4 (CO_2H), 165.4 (CONH), 177.6 (C-4). LC-MS (m/z): positive mode 472 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.6%. mp 206-207 °C.

5.2.32.75 6-Chloro-8-(3-(hexyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 246 (*ANM334)*



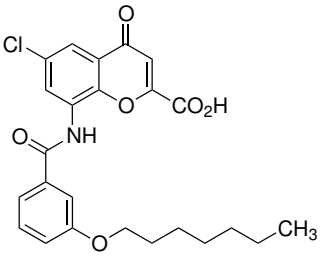
The compound was synthesized according to GP-14 using **165** (40 mg, 0.10 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (34 mg, 95% yield). ^{1}H NMR: δ 0.84-0.91 (m, 3H, 6″-H), 1.19-1.37 (m, 4H, 5″-H, 4″-H), 1.37-1.50 (m, 2H, 3″-H), 1.65-1.80 (m, 2H, 2″-H), 4.05 (t, J = 6.5 Hz, 2H, 1″-H), 6.97 (s, 1H, 3-H), 7.19 (dd, J = 2.5, 8.2Hz, 1H, 4′-H), 7.46 (td, J = 7.9 Hz, 1H, 5′-H), 7.52-7.62 (m, 2H, 2′-H, 6′-H), 7.83 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.28 (s, 1H, CONH). ^{13}C NMR: δ 14.1 (C-6″), 22.2 (C-5″), 25.3 (C-4″), 28.8 (C-3″), 31.2 (C-2″), 67.9 (C-1″), 113.5 (C-2′), 113.6 (C-3), 118.7 (C-4′), 120.0 (C-6′), 120.2 (C-5), 125.2 (C-4a), 130.0 (C-7), 130.0 (C-6, C-1′), 130.2 (C-5′), 135.1 (C-8), 147.9 (C-8a), 153.3 (C-2), 158.9 (C-3′), 161.1 (CONH), 165.4 (C_2H), 176.5 (C-4). LC-MS (m/z): positive mode 444 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.0%. mp 228-230 °C.

5.2.32.76 6-Chloro-8-(3-(octyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 248 (*ANM335)*



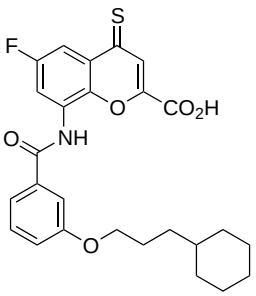
The compound was synthesized according to GP-14 using **294** (219 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (118 mg, 57% yield). ^{1}H NMR: δ 0.80-0.90 (m, 3H, 8″-H), 1.18-1.39 (m, 10H, 7″-H, 6″-H, 5″-H, 4″-H), 1.38-1.50 (m, 2H, 3″-H), 1.72-1.76 (m, 2H, 2″-H), 4.05 (t, J = 6.4 Hz, 2H, 1″-H), 6.97 (s, 1H, 3-H), 7.19 (ddd, J = 8.2, 1.0 Hz, 1H, 4′-H), 7.46 (t, J = 7.9 Hz, 1H, 5′-H), 7.51-7.59 (m, 2H, 2′-H, 6′-H), 7.83 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.27 (s, 1H, CONH). ^{13}C NMR: δ 14.1 (C-8″), 22.2 (C-7″), 25.7 (C-6″), 28.8 (C-3″, C-4″, C-5″), 31.4 (C-2″), 67.9 (C-1″), 113.5 (C-2′, C-3), 118.7 (C-4′), 120.0 (C-6′), 120.2 (C-5), 125.2 (C-4a), 129.6 (C-7), 130.0 (C-5′), 130.2 (C-6, C-1′), 147.9 (C-8a), 153.3 (C-2), 158.9 (C-3′), 161.1 (CONH), 165.4 (CO_2H), 176.5 (C-4). LC-MS (m/z): positive mode 472 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.7%. mp 221-224 °C.

5.2.32.77 6-Chloro-8-(3-(heptyloxy)benzamido)-4-oxo-4*H*-chromene-2-carboxylic acid 247 (*ANM337)*



The compound was synthesized according to GP-14 using **166** (213 mg, 0.44 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (101 mg, 50% yield). ^{1}H NMR: δ 0.86 (t, J = 6.8 Hz, 3H, 7''-H), 1.22-1.38 (m, 8H, 6''-H, 5''-H, 4''-H), 1.39-1.47 (m, 2H, 3''-H), 1.72-1.76 (m, 2H, 2''-H), 4.05 (t, J = 6.4 Hz, 2H, 1''-H), 6.97 (s, 1H, 3-H), 7.19 (dd, J = 8.3, 2.5 Hz, 1H, 4'-H), 7.46 (t, J = 7.9 Hz, 1H, 5'-H), 7.51-7.59 (m, 2H, 2'-H, 6'-H), 7.83 (d, J = 2.6 Hz, 1H, 5-H), 8.21 (d, J = 2.6 Hz, 1H, 7-H), 10.28 (s, 1H, CONH). ^{13}C NMR: δ 140.1 (C-7''), 22.2 (C-6''), 25.6 (C-5''), 28.6 (C-3'', C-4''), 31.4 (C-2''), 67.9 (C-1''), 113.5 (C-2', C-3), 118.7 (C-4'), 120.0 (C-6', C-5), 125.2 (C-4a), 129.9 (C-7), 130.0 (C-3'), 130.1 (C-6, C-1'), 135.1 (C-8), 147.8 (C-8a), 153.2 (C-2), 158.9 (C-3'), 161.1 (CONH), 165.3 (CO_2H), 176.5 (C-4). LC-MS (m/z): positive mode 458 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 99.3%. mp 222-224 °C.

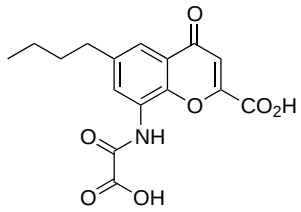
5.2.32.78 8-(3-(3-Cyclohexylpropoxy)benzamido)-6-fluoro-4-thioxo-4*H*-chromene-2-carboxylic acid 262 (*ANM346)*



The compound was synthesized according to GP-14 using **180** (85 mg, 0.16 mmol). Recrystallization from 1:1 acetone/ethanol afforded the product as a white solid (43 mg, 56% yield). ^{1}H NMR: δ 0.80-0.95 (m, 2H, Cyclohexyl), 1.06-1.39 (m, 6H, Cyclohexyl, 3''-H), 1.57-1.82 (m, 7H, 2''-H, Cyclohexyl), 4.04 (t, J = 6.4 Hz, 2H, 1''-H), 7.20 (ddd, J = 8.3, 2.6, 1.0 Hz, 1H, 2'-H), 7.47 (t, J = 7.9 Hz, 1H, 5'-H), 7.52-7.61 (m, 2H, 4'-H, 6'-H), 7.68 (s, 1H, 3-H), 7.86 (dd, J = 9.0, 3.1 Hz, 1H, 5-H), 8.17 (dd, J = 9.4, 3.1 Hz, 1H, 7-H), 10.32 (s, 1H, CONH). ^{13}C NMR: δ 26.0-26.3 (C-3''', C-5''', C-4'''. C-3''), 33.0 (C-2''', C-6''', C-2''), 37.0 (C-1'''), 68.3 (C-1''), 107.8-108.0 (C-5), 113.5 (C-2'), 118.1-118.3 (C-7), 118.7 (C-4'), 120.0 (C-6'), 124.1 (C-3), 130.0 (C-5'), 131.1 (C-8), 131.5 (C-4a),135.0 (C-1'), 141.3 (C-8a), 143.6 (C-2), 158.9 (C-3'), 160.7 (C-6), 161.6 (CO_2H), 165.4 (CONH), 202.0 (C-4). LC-MS (m/z): positive mode 484 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.0%. mp 212-214 °C.

5.2.33 6-Butyl-8-(carboxyformamido)-4-oxo-4*H*-chromene-2-carboxylic acid

5.2.33.1 6-Butyl-8-(carboxyformamido)-4-oxo-4*H*-chromene-2-carboxylic acid 196 (*ANM383)*



33 (20 mg, 0.069 mmol) was dissolved in DCM (2 mL) and one drop of THF under an argon atmosphere. After addition of DIPEA (0.05 mL, 0.069 mmol) and ethyl chlorooxoacetate (0.21 mL, 0.19 mmol) the reaction mixture was stirred at rt for 2 days. After removing the solvent under reduced pressure a solution of potassium carbonate (10 mg, 0.089 mmol) in water (0.7 mL) was slowly added to a suspension of the obtained crude ester in THF (2 mL) and EtOH (0.5 mL). The reaction mixture was stirred at rt overnight until a clear solution was obtained. The mixture was acidified with diluted aq. HCl solution (2 N) until a pH $\leq$ 2 was reached. The solvents THF and EtOH were removed under reduced pressure and the product was filtered and washed with water. The product was isolated as a white solid (18 mg, 78% yield). ^{1}H NMR: δ 0.90 (t, J = 7.3 Hz, 3H, 4'-H), 1.12-1.44 (m, 2H, 4'-H), 1.48-1.74 (m, 2H, 2'-H), 2.71 (t, J = 7.6 Hz, 2H, 1'-H), 6.91 (s, 1H, 3-H), 7.66 (d, J = 2.2 Hz, 1H, 5-H), 8.16 (d, J = 2.2 Hz, 1H, 7-H), 10.23 (s, 1H, NH). ^{13}C NMR: δ 13.8 (C-4'), 21.7 (C-3'), 32.9 (C-2'), 34.4 (C-1'), 113.5 (C-3), 120.0 (C-5), 124.0 (C-4a), 126.8 (C-8), 127.8 (C-7), 140.3 (C-1'), 146.0 (C-8a), 152.7 (C-2), 156.7 (CONH), 161.3 (CO_2H), 177.3 (C-4). LC-MS (m/z): positive mode 334 $[M+H]^+$. Purity by HPLC-UV (254 nm)-ESI-MS: 95.1%. mp 224-225 °C.

5.3 Biological Evaluation

5.3.1 Pharmacological testing of chromenon-4-one-2-carboxylic acid derivatives

5.3.1.1 Calcium assay

The receptor (GPR17) was expressed in 1321N1 astocytoma cells. Cells of two 175 cm^2 culture flasks with a confluence of 70-80% were incubated for 45 min in 30 mL of culture medium in an incubator. Then the cells were sedimented at 200g for 5 min and the supernatant was discarded. The cells were taken up in 994 μL potassium hydrogen phthalate (KHP)-buffer und pipetted into an Eppendorf tube containing 3 μL of Oregon Green®BAPTA(1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*,*N*-tetraacetic acid)-1/AM- and 3 μL of Pluronic® F127 solution. The cells were then incubated for two hours under light exclusion at rt and 200 rpm. Meanwhile 2 μL of the antagonist (different concentrations; in DMSO, final concentration 1%) was pipetted in a 96 well plate. After incubation the cells were sedimented for 1 min at 2000 rpm, the supernatant removed, washed twice with 1 mL of KHP buffer each, and taken up in 1 mL KHP buffer. Then they were isolated and diluted with 19 mL of KHP buffer. 178 μL of cell suspension were added to the antagonist solution per well. After 30 min incubation a validation was performed using the NOVOstar® fluoremeter. Then 20 μL of the agonist solution in DMSO/KHP buffer (1μM MDL29.951[234]) was added automatically by the NOVOstar® fluorometer to the wells. Fluorescence was measured for 36 s (excitation: 485 nm, emission: 520 nm). Concentration-inhibition curves were performed in 3 independent experiments.

These experiments were performed by Dr. Aliaa Abdelrahman.

5.3.1.2 β-Arrestin recruitment assay

The experiments were performed using thePathhunter β-Arrestin recruitment system. For the β-arrestin assays specially modified CHO cells were used. Those cells express fusion proteins, which consist of β-arrestin and a N-terminal deletion mutant of β-galactosidase. Furthermore a receptor was expressed which has an enzyme fragment fused to its C-terminal domain. Cell lines transfected with hGPR18, hGPR35, hGPR55 or hGPR84 were commercially available or prepared in our group. For the generation of cell lines expressing the murine GPR35 receptors,

Pathhunter β-Arrestin parental cell lines were transfected with the corresponding plasmids. For each experiment 20000 cells were plated in 90 μL cell plating 2 reagent or F12 medium supplemented with fetal calf serum (FCS) (10%), Penicillin G (100 U/mL) and Streptomycin (100 μg/mL) on a 96 well plate one day in advance. After 24 h incubation at 37 °C and 5% CO_2 the F12 medium was exchanged for medium without FCS (approximately 3 hours prior to the assay). The assay was started by the addition of 10 μL of the agonist solution to each well (10% DMSO in cell plating 2 reagent). For the antagonist assay 5 μL of the antagonist solution (10% DMSO in cell plating medium 2 each) was added 30 min before 5 μL of the agonist were added (hGPR18: 10 μM THC, hGPR35: 5 μM Zaprinast, hGPR55: 1 μM LPI, hGPR84: 20 μM Embelin). After 90 min incubation 50 μL of detection reagent (hGPR18 and GPR35: Pathhunter, DiscoverX; GPR35 and hGPR55: Galacton-Star® (2mM), Emerald-II™ and lysis buffer 1:5:19) was added followed by further incubation for 60 min. Then luminescence was measured for 1 min (Topcount NXT). Concentration-inhibition curves were performed in 3-4 separate independent experiments.

These experiments were performed by Dr. Dominik Thimm (GPR35), Clara Schoeder (GPR18, GPR55) and Dr. Meryem Köse (GPR84).

5.3.1.3 Membrane preparation for radioligand binding assays

CHO cells were transfected using a retroviral transfection system.[235] GP^+envAM12 packaging cells were transfected with the plasmide pLXSN, in which the human GPR35 was inserted. After pseudotyped retroviruses were formed the virus-containing supernatant was sterile-filtered and added to the CHO cells. Selection of succesfully transfected cells lasted one week (DMEM/F-12 medium, Invitrogen, Carlsbad, CA; 10% FCS; 100 U/mL Penicillin G; 100 μg/mL Streptomycin, 0.8 mg/mL G418). Then the cells were cultured in the same medium containing a reduced concentration of G418 (0.2 mg/mL). After removing the culture medium the cells were washed with phosphate-buffered saline (PBS) buffer and frozen to -20 °C. In a sterile buffer containing 5 mM Tris-HCl and 2 mM ethylenediaminetetraacetic acid (EDTA) (pH = 7.4) the cells were detached followed by mechanical lysis, homogenisation using an Ultra Turrax (11000 UpM, 15 s) and centrifugation (10 min, 1000g, 4 °C). The membranes in the supernatant were sedimented for 1 h at 48000g and 4 °C.

Then the pellet was resuspended in sterile 50 mM Tris-HCl buffer (pH = 7.4). The membrane suspension was homogenized, aliquoted and stored at -80 °C.[236] Protein concentration was determined following the method of Lowry.[237]

These experiments were performed by Dr. Dominik Thimm.

5.3.1.4 Radioligand binding assay

Radioligand binding assays were carried out using [^{3}H]PSB-13253[65] in a final volume of 400 μL (10 μL DMSO or test compound dissolved in 100% DMSO, 190 μL buffer (sterile 50 mM Tris-HCl, 10 mM $MgCl_2$, pH = 7.4), 100 μL radioligand solution (final concentration 5 nM), 100 μL membrane preparation (10 μg protein)). The membrane preparations were diluted with a sterile buffer (50 mM Tris-HCl and 10 mM $MgCl_2$, pH = 7.4). For the competitive studies the assay solution was incubated for 100 min followed by filtration through a GF-B filter using a Brandel Harvester (Brandel, Gaithersburg, MD). The filters were washed three times with 2-3 mL of ice-cold 50 mM Tris-HCl buffer (pH = 7.4) and the radioactivity was measured by scintillation counting using a Tri-Carb 1810 TR (Perkin-Elmer, Waltham, MA) Three to four separate experiment were performed in duplicates.

These experiments were performed by Dr. Dominik Thimm.

5.3.1.5 cAMP accumulation assay

CHO cells expressing hGPR84 were cultured for 2 days on 24 well plates. After removing the culture medium the cells were washed with **HBSS!** (**HBSS!**) (pH = 7.4). Then the cAMP accumulation assay was performed (final assay volume 500 μL). After addition of 300 μL HBSS in each well 50 μL Ro20-1724 as phosphodiesterase inhibitor was pipetted followed by an incubation of 10 min at 37 °C. 50 μL of the antagonist solution (100 % DMSO) (or 50 μL HBSS to the controls with forskolin or decanoic acid) were added and further incubated for 60 min. Then 50 μL decanoic acid as agonist (or 50 μL HBSS containing 10% DMSO as control with forskolin) were added. After an incubation of exactly 5 min 50 μL of forskolin were added for the stimulation of the cellular cAMP production and after further 15 min of incubation the reaction was stopped by removing the reaction buffer and addition of hot lysis buffer (500 μL, 90 °C, 4 mM EDTA, 0.01% Triton^{TM} X-100, pH = 7.4, Sigma, München).

After cooling down for 5 min the 24 well plates were frozen at -20 °C.
Before the assay was continued the cell lysate was thawed up and homogenized with the pipette. To determine the cAMP concentration 50 μL of the cell lysate was incubated with 30 μL [^{3}H]cAMP (3 nM) and 40 μL cAMP binding protein (cAMP dependend protein kinase[238]) diluted in lysis buffer for 1 h at 4 °C followed by filtration through a GF/B filter using a Brandel Harvester (Brandel, Gaithersburg, MD). The radioactivity was measured by scintillation counting using a Tri-Carb 1810 TR (Perkin-Elmer, Waltham, MA). Three to four separate experiment were performed.

These experiments were performed by Dr. Meryem Köse.

5.3.2 Biological testing of ecto-5'-nucleotidase (CD73) inhibitors

5.3.2.1 Radiometric TLC *e*N assay

Human blood was taken from healthy volunteers by venipuncture and was allowed to clot at room temperature for 30 min. Blood samples were centrifuged for 15 min at 2000 g followed by freezing of the serum at -70 °C. The enzyme assays with cultured cell lines (MDA-MB-231 and HUVEC)[222] were carried out in 96 well plates. Serum samples (4-7 μL), or recombinant human *e*N (purified as previously described),[224] or cells, respectively, were incubated for 1 h at 37 °C. The enzyme assays were carried out in a final volume of 80 μL of RPMI-1640 medium containing 5 mM β-glycerophosphate and 40 μM, 40 μM, 400 μM and 200 μM of unlabeled AMP for recombinant eN, human serum eN, MDA-MB-231 and HUVEC, respectively, containing tracer [2-^{3}H]AMP (18.6 Ci/mmol; Amersham, Little Chalfont, UK) as a substrate. The products were separated by TLC on Alugram SIL G/UV$_{254}$ plates (Machery-Nagel, Düren, Germany) (eluent: 1-butanol 1.5 eq.; isoamylalcohol 1 eq.; diethyleneglycol monoethylether 3 eq.; ammonia solution 1.5 eq.; pure water 2.5 eq.) and quantified by scintillation β-counting as previously described.[223] Concentration-inhibition curves were performed in 2-4 separate experiments.

5.3.2.2 Lead-nitrate staining

Frozen human tonsil and mouse spleen sections, thawed to room temperature, were preincubated for 45 min at room temperature with Trizma maleate buffer containing sucrose (40 mM Trizma maleate, 0.25 M sucrose, pH 7.4), alkaline phosphatase

inhibitor tetramisole (3 mM) and in case of inhibitor treatment Ecto-5'-nucleotidase inhibitor (600 nM) or AOPCP (20 μM). After addition of $Pb(NO)_3$ (2 mM), AMP (1mM) and $CaCl_2$ (0.5 mM) the enzyme reaction was performed for 90 min at 37 °C. After incubation of the tissue sections for 30 sec in 0.5% NH_4S and mounting with Vecta Mount (Vector Laboratories, Burlingame, CA) images were taken using an Olympus BX60 microscope.[225]

5.3.2.3 Haematoxylin-Eosin staining

Frozen human tonsil and mouse spleen sections, thawed to room temperature, were preincubated for 10 min with Mayers Hematoxylin solution. After washing with water the sections were washed for 30 sec with Milli Q water followed by incubation for 40 sec in eosin solution. Then the sections were washed twice in 70%, 96%, and 99% EtOH for 30 sec each. After fixing twice in xylene for 5 min each, the sections were mounted with Aquatex (Merck).

6 List of abbreviations

GPCR G protein-coupled receptor

7TM-receptor 7-transmembrane receptor

CALCR calcitonin receptor

CRHR corticotropin-releasing hormone receptor

GCGR glucagon receptor

GRM metabotropic glutamate receptor

GABA γ-aminobutyric acid

CASR calcium-sensing receptor

MECA melanocortin, endothelial differentiation sphingolipid, cannabinoid, adenosine

SOG somatostatin, chemokine, galanin

MCH melanin-concentrating hormone

GEF guanosine triphosphate exchange factor

GDP guanosine diphosphate

GTP guanosine triphosphate

AC adenylate cyclase

ATP adenosine triphosphate

cAMP cyclic adenosine monophosphate

PKA protein kinase A

CREB responsive element binding protein

PKB phosphorylase kinase B

PLC phospholipase C

PIP_2 phosphatidylinositol-4,5-bisphosphate

IP_3 inositol trisphosphate

DAG diacylglycerol

CAMK Ca^{2+}/calmodulin-dependent protein kinase

PKC protein kinase C

RhoGEF Rho guanine nucleotide exchange factors

GRK G protein-coupled receptor-kinase

AP-2 β2-adaptin

IUPHAR International Union of Basic and Clinical Pharmacology

$5HT_{1A}$ receptor 5-hydroxytryptamine receptor

AMP adenosine 5'-monophosphate

ADP adenosine 5'-diphosphate

UTP uridine triphosphate

UDP uridine diphosphate

MS multiple sclerosis

CNS central nervous system

cDNA complementary DNA

RT-PCR reverse transcription polymerase chain reaction

$CysLT_1$ cysteinyl-leukotriene receptor 1

$CysLT_2$ cysteinyl-leukotriene receptor 2

CysLT cysteinyl-leukotriene receptor

LTD_4 leukotriene D4

LTC_4 leukotriene C4

LTE_4 leukotriene E4

ERK extracellular-signal regulated kinase

SCI spinal cord injury

bHLH basic helix-loop-helix

OPC oligodentrocyte precursor cell

NGF neuronal growth factor

siRNA small interfering RNA

SAR structure-activity relationship

SNP single nucleotide polymorphism

IBD inflammatory bowel disease

iNKT invariant natural killer T cell

TRPV1 transient receptor potential vanilloid 1

LPA lysophosphatidic acid

PDE phosphodiesterase

AEA anandamide

2-AG 2-arachidonylglycerol

CB cannabinoid

EST expressed sequence tag

PCR polymerase chain reaction

mRNA messenger RNA

LPI lysophosphatidylinositol

BMI body mass index

DSS dextran sulfate sodium

TNBS trinitrobenzene sulfonic acid

THC tetrahydrocannabinol

MEA methanandamide

DRG dorsal root ganglion

MCFFA medium-chain free fatty acid

SCFFA short-chain free fatty acid

LCFFA long-chain free fatty acid

DIM diindolylmethane

LPS lipopolysaccharide

IL interleukin

TNF-α tumor necrosis factor-α

EAE experimental autoimmune encephalomyelitis

HFC high-fat chow

ADSC adipose-derived stem cell

6-OAU 6-*n*-octylaminouracil

PMN polymorphonuclear neutrophil

LSC leukemic stem cell

AML acute myeloid leukemia

MLL mixed lineage leukemia

AD Alzheimer's disease

GERD gastroesophageal reflux disease

eNPP ecto-nucleotide pyrophosphatases/phosphodiesterase

eNTPDase ecto-nucleoside triphosphate diphosphohydrolase

AP alkaline phosphatase

eN ecto-5'-nucleotidase

ADA ecto-adenosine deaminase

PNP purine nucleoside phosphorylase

NTP nucleoside triphosphate

NMP nucleoside monophosphate

NDP nucleoside diphosphate

GPI glycosylphosphatidylinositol

AOPCP α,β-methylene-ADP

rt room temperature

mp melting point

DMF dimethylformamide

TBDMS *tert*-butyldimethylsilyl

aq. aquaous

NBS *N*-bromosuccinimide

AIBN azobisisobutyronitrile

THF tetrahydrofurane

DMF dichlormethane

DIPEA *N*,*N*-diisopropylethylamine

Boc *tert*-butyloxycarbonyl

EtOAc ethyl acetate

TLC thin-layer chromatography

EA enzyme acceptor

HUVEC human umbilical vein endothelial cell

DMSO dimethylsulfoxide

ESI electrospray ionization

LC-MS liquid chromatography-mass spectrometry

HPLC high-performance liquid chromatography

UV ultraviolet

mp melting point

KHP potassium hydrogen phthalate

FCS fetal calf serum

PBS phosphate-buffered saline

EDTA ethylenediaminetetraacetic acid

PNL partial sciatic nerve ligation

DCM dichloromethane

7 Literature

[1] Fredriksson, R.; Lagerström, M. C.; Lundin, L.-G.; Schiöth, H. B. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol. Pharmacol.* **2003**, *63*, 1256–72.

[2] Hopkins, A. L.; Groom, C. R. The druggable genome. *Nat. Rev. Drug Discov.* **2002**, *1*, 727–30.

[3] Neubig, R. R.; Spedding, M.; Kenakin, T.; Christopoulos, A. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. *Pharmacol. Rev.* **2003**, *55*, 597–606.

[4] May, L. T.; Leach, K.; Sexton, P. M.; Christopoulos, A. Allosteric modulation of G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 1–51.

[5] Katritch, V.; Cherezov, V.; Stevens, R. C. Diversity and modularity of G protein-coupled receptor structures. *Trends Pharmacol. Sci.* **2012**, *33*, 17–27.

[6] Kolakowski, L. F. J. GCRDb: a G-protein-coupled receptor database. *Receptors Channels* **1994**, *2*, 1–7.

[7] Stevens, R. C.; Cherezov, V.; Katritch, V.; Abagyan, R.; Kuhn, P.; Rosen, H.; Wüthrich, K. The GPCR Network: a large-scale collaboration to determine human GPCR structure and function. *Nat. Rev. Drug Discov.* **2013**, *12*, 25–34.

[8] Rodbell, M.; Krans, H. M.; Pohl, S. L.; Birnbaumer, L. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. IV. Effects of guanyl-nucleotides on binding of 125I-glucagon. *J. Biol. Chem.* **1971**, *246*, 1872–1876.

[9] Pierce, K. L.; Premont, R. T.; Lefkowitz, R. J. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 639–50.

[10] Milligan, G.; Kostenis, E. Heterotrimeric G-proteins: a short history. *Br. J.*

Pharmacol. **2006**, *147 Suppl*, S46–S55.

[11] Siehler, S. Regulation of RhoGEF proteins by G12/13-coupled receptors. *Br. J. Pharmacol.* **2009**, *158*, 41–49.

[12] Stadel, R.; Ahn, K. H.; Kendall, D. A. The cannabinoid type-1 receptor carboxyl-terminus, more than just a tail. *J. Neurochem.* **2011**, *117*, 1–18.

[13] Kelly, E.; Bailey, C. P.; Henderson, G. Agonist-selective mechanisms of GPCR desensitization. *Br. J. Pharmacol.* **2009**, *153*, S379–S388.

[14] Alexander, S. P. H.; Davenport, A. P.; Kelly, E.; Marrion, N.; Peters, J. A.; Benson, H. E.; Faccenda, E.; Pawson, A. J.; Sharman, J. L.; Southan, C.; Davies, J. A. The Concise Guide to Pharmacology 2015/16: G protein-coupled receptors. *Br. J. Pharmacol.* **2015**, *172*, 5744–5869.

[15] Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature* **1988**, *335*, 358–360.

[16] Civelli, O.; Reinscheid, R. K.; Zhang, Y.; Wang, Z.; Fredriksson, R.; Schiöth, H. B. G protein-coupled receptor deorphanizations. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 127–46.

[17] Lohmann, K. Über die Pyrophosphatfraktion im Muskel. *Naturwissenschaften* **1929**, *17*, 624–625.

[18] Burnstock, G. Purinergic nerves. *Pharmacol. Rev.* **1972**, *24*, 509–581.

[19] Burnstock, G. A basis for distinguishing two types of purinergic receptor. *Cell Membr. Recept. Drugs Horm. A Multidiscip. Approach* **1978**, 107–118.

[20] Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* **2011**, *53*, 527–552.

[21] Burnstock, G. Introduction: P2 receptors. *Curr. Top. Med. Chem.* **2004**, *4*, 793–803.

[22] von Kügelgen, I.; Schiedel, A. C.; Hoffmann, K.; Alsdorf, B. B. A.; Abdelrahman, A.; Müller, C. E. Cloning and functional expression of a novel Gi protein-coupled receptor for adenine from mouse brain. *Mol. Pharmacol.* **2008**, *73*, 469–477.

[23] Bender, E.; Buist, A.; Jurzak, M.; Langlois, X.; Baggerman, G.; Verhasselt, P.; Ercken, M.; Guo, H.-Q.; Wintmolders, C.; Van den Wyngaert, I.; Van Oers, I.; Schoofs, L.; Luyten, W. Characterization of an orphan G protein-coupled receptor localized in the dorsal root ganglia reveals adenine as a signaling molecule. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 8573–8578.

[24] Jacobson, K. A.; Müller, C. E. Medicinal chemistry of adenosine, P2Y and P2X receptors. *Neuropharmacology* **2016**, *104*, 31–49.

[25] von Kügelgen, I.; Hoffmann, K. Pharmacology and structure of P2Y receptors. *Neuropharmacology* **2016**, *104*, 50–61.

[26] Woods, L. T.; Ajit, D.; Camden, J. M.; Erb, L.; Weisman, G. A. Purinergic receptors as potential therapeutic targets in Alzheimer's disease. *Neuropharmacology* **2015**, *104*, 169–179.

[27] Grigoriadis, N.; van Pesch, V. A basic overview of multiple sclerosis immunopathology. *Eur. J. Neurol.* **2015**, *22*, 3–13.

[28] Goldenberg, M. M. Multiple sclerosis review. *P. T.* **2012**, *37*, 175–84.

[29] Fumagalli, M.; Lecca, D.; Abbracchio, M. P. CNS remyelination as a novel reparative approach to neurodegenerative diseases: The roles of purinergic signaling and the P2Y-like receptor GPR17. *Neuropharmacology* **2016**, *104*, 82–93.

[30] Raport, C. J.; Schweickart, V. L.; Chantry, D.; Eddy, R. L.; Shows, T. B.; Godiska, R.; Gray, P. W. New members of the chemokine receptor gene family. *J. Leukoc. Biol.* **1996**, *59*, 18–23.

[31] Bläsius, R.; Weber, R. G.; Lichter, P.; Ogilvie, A. A Novel Orphan G Protein-Coupled Receptor Primarily Expressed in the Brain Is Localized on Human Chromosomal Band 2q21. *J. Neurochem.* **1998**, *70*, 1357–1365.

[32] Nakai, H.; Konno, M.; Kosuge, S.; Sakuyama, S.; Toda, M.; Arai, Y.; Obata, T.; Katsube, N.; Miyamoto, T.; Okegawa, T. New potent antagonists of leukotrienes C4 and D4. 1. Synthesis and structure-activity relationships. *J. Med. Chem.* **1988**, *31*, 84–91.

[33] von Kügelgen, I.; Harden, T. K. Molecular Pharmacology, Physiology, and Structure of the P2Y Receptors. *Adv. Pharmacol.* **2011**, *61*, 373–415.

[34] Kanaoka, Y.; Boyce, J. a. Cysteinyl leukotrienes and their receptors: cellular

distribution and function in immune and inflammatory responses. *J. Immunol.* **2004**, *173*, 1503–1510.

[35] Ciana, P.; Fumagalli, M.; Trincavelli, M. L.; Verderio, C.; Rosa, P.; Lecca, D.; Ferrario, S.; Parravicini, C.; Capra, V.; Gelosa, P.; Guerrini, U.; Belcredito, S.; Cimino, M.; Sironi, L.; Tremoli, E.; Rovati, G. E.; Martini, C.; Abbracchio, M. P. The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *EMBO J.* **2006**, *25*, 4615–4627.

[36] Pugliese, A. M.; Trincavelli, M. L.; Lecca, D.; Coppi, E.; Fumagalli, M.; Ferrario, S.; Failli, P.; Daniele, S.; Martini, C.; Pedata, F.; Abbracchio, M. P. Functional characterization of two isoforms of the P2Y-like receptor GPR17: [35S]GTPgammaS binding and electrophysiological studies in 1321N1 cells. *Am. J. Physiol. Cell Physiol.* **2009**, *297*, C1028–40.

[37] Maekawa, A.; Balestrieri, B.; Austen, K. F.; Kanaoka, Y. GPR17 is a negative regulator of the cysteinyl leukotriene 1 receptor response to leukotriene D4. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 11685–11690.

[38] Benned-Jensen, T.; Rosenkilde, M. M. Distinct expression and ligand-binding profiles of two constitutively active GPR17 splice variants. *Br. J. Pharmacol.* **2010**, *159*, 1092–1105.

[39] Qi, A.-D.; Harden, T. K.; Nicholas, R. A. Is GPR17 a P2Y/leukotriene receptor? examination of uracil nucleotides, nucleotide sugars, and cysteinyl leukotrienes as agonists of GPR17. *J. Pharmacol. Exp. Ther.* **2013**, *347*, 38–46.

[40] Hennen, S.; Wang, H.; Peters, L.; Merten, N.; Simon, K.; Spinrath, A.; Blättermann, S.; Akkari, R.; Schrage, R.; Schröder, R.; Schulz, D.; Vermeiren, C.; Zimmermann, K.; Kehraus, S.; Drewke, C.; Pfeifer, A.; König, G. M.; Mohr, K.; Gillard, M.; Müller, C. E.; Lu, Q. R.; Gomeza, J.; Kostenis, E. Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. *Sci. Signal.* **2013**, *6*, ra93.

[41] Maisel, M.; Herr, A.; Milosevic, J.; Hermann, A.; Habisch, H.; Schwarz, S.; Kirsch, M.; Antoniadis, G.; Brenner, R.; Hallmeyer-Elgner, S.; Lerche, H.; Schwarz, J.; Storch, A. Transcription profiling of adult and fetal human neuroprogenitors identifies divergent paths to maintain the neuroprogenitor cell state. *Stem Cells* **2007**, *25*, 1231–1240.

[42] Lecca, D.; Trincavelli, M. L.; Gelosa, P.; Sironi, L.; Ciana, P.; Fumagalli, M.; Villa, G.; Verderio, C.; Grumelli, C.; Guerrini, U.; Tremoli, E.; Rosa, P.; Cuboni, S.; Martini, C.; Buffo, A.; Cimino, M.; Abbracchio, M. P. The recently identified P2Y-like receptor GPR17 is a sensor of brain damage and a new target for brain repair. *PLoS One* **2008**, *3*.

[43] Zhao, B.; Zhao, C. Z.; Zhang, X. Y.; Huang, X. Q.; Shi, W. Z.; Fang, S. H.; Lu, Y. B.; Zhang, W. P.; Xia, Q.; Wei, E. Q. The new P2Y-like receptor G protein-coupled receptor 17 mediates acute neuronal injury and late microgliosis after focal cerebral ischemia in rats. *Neuroscience* **2012**, *202*, 42–57.

[44] Ceruti, S.; Villa, G.; Genovese, T.; Mazzon, E.; Longhi, R.; Rosa, P.; Bramanti, P.; Cuzzocrea, S.; Abbracchio, M. P. The P2Y-like receptor GPR17 as a sensor of damage and a new potential target in spinal cord injury. *Brain* **2009**, *132*, 2206–2218.

[45] Arnett, H. a.; Alberta, J. a.; Fancy, S. P. J.; Zhao, C.; Plant, S. R.; Kaing, S.; Raine, C. S.; Rowitch, D. H.; Franklin, R. J. M.; Stiles, C. D. bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS. *Science* **2004**, *306*, 2111–5.

[46] Chen, Y.; Wu, H.; Wang, S.; Koito, H.; Li, J.; Ye, F.; Hoang, J.; Escobar, S. S.; Gow, A.; Arnett, H. A.; Trapp, B. D.; Karandikar, N. J.; Hsieh, J.; Lu, Q. R. The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. *Nat. Neurosci.* **2009**, *12*, 1398–1406.

[47] Daniele, S.; Lecca, D.; Trincavelli, M. L.; Ciampi, O.; Abbracchio, M. P.; Martini, C. Regulation of PC12 cell survival and differentiation by the new P2Y-like receptor GPR17. *Cell. Signal.* **2010**, *22*, 697–706.

[48] Huber, C.; Marschallinger, J.; Tempfer, H.; Furtner, T.; Couillard-Despres, S.; Bauer, H. C.; Rivera, F. J.; Aigner, L. Inhibition of leukotriene receptors boosts neural progenitor proliferation. *Cell. Physiol. Biochem.* **2011**, *28*, 793–804.

[49] Fumagalli, M.; Daniele, S.; Lecca, D.; Lee, P. R.; Parravicini, C.; Douglas Fields, R.; Rosa, P.; Antonucci, F.; Verderio, C.; Letizia Trincavelli, M.; Bramanti, P.; Martini, C.; Abbracchio, M. P. Phenotypic changes, signaling pathway, and functional correlates of GPR17-expressing neural precursor cells during oligodendrocyte differentiation. *J. Biol. Chem.* **2011**, *286*, 10593–10604.

[50] Coppi, E.; Maraula, G.; Fumagalli, M.; Failli, P.; Cellai, L.; Bonfanti, E.; Mazzoni, L.; Coppini, R.; Abbracchio, M. P.; Pedata, F.; Pugliese, A. M. UDP-glucose enhances outward K+ currents necessary for cell differentiation and stimulates cell migration by activating the GPR17 receptor in oligodendrocyte precursors. *Glia* **2013**, *61*, 1155–1171.

[51] Hennen, S.; Wang, H.; Peters, L.; Merten, N.; Simon, K.; Spinrath, A.; Blättermann, S.; Akkari, R.; Schrage, R.; Schröder, R.; Schulz, D.; Vermeiren, C.; Zimmermann, K.; Kehraus, S.; Drewke, C.; Pfeifer, A.; König, G. M.; Mohr, K.; Gillard, M.; Müller, C. E.; Lu, Q. R.; Gomeza, J.; Kostenis, E. Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. *Sci. Signal.* **2013**, *6*, ra93.

[52] Marschallinger, J.; Schäffner, I.; Klein, B.; Gelfert, R.; Rivera, F. J.; Illes, S.; Grassner, L.; Janssen, M.; Rotheneichner, P.; Schmuckermair, C.; Coras, R.; Boccazzi, M.; Chishty, M.; Lagler, F. B.; Renic, M.; Bauer, H.-C.; Singewald, N.; Blümcke, I.; Bogdahn, U.; Couillard-Despres, S.; Lie, D. C.; Abbracchio, M. P.; Aigner, L. Structural and functional rejuvenation of the aged brain by an approved anti-asthmatic drug. *Nat. Commun.* **2015**, *6*, 8466.

[53] Maekawa, A.; Xing, W.; Austen, K. F.; Kanaoka, Y. GPR17 regulates immune pulmonary inflammation induced by house dust mites. *J. Immunol.* **2010**, *185*, 1846–54.

[54] Ren, H.; Orozco, I. J.; Su, Y.; Suyama, S.; Gutiérrez-Juárez, R.; Horvath, T. L.; Wardlaw, S. L.; Plum, L.; Arancio, O.; Accili, D. FoxO1 target Gpr17 activates AgRP neurons to regulate food intake. *Cell* **2012**, *149*, 1314–1326.

[55] Ren, H.; Cook, J. R.; Kon, N.; Accili, D. Gpr17 in AgRP Neurons Regulates Feeding And Sensitivity To Insulin And Leptin. *Diabetes* **2015**, *64*, 3670–3679.

[56] Mastaitis, J.; Min, S.; Elvert, R.; Kannt, A.; Xin, Y.; Ochoa, F.; Gale, N. W.; Valenzuela, D. M.; Murphy, A. J.; Yancopoulos, G. D.; Gromada, J. GPR17 gene disruption does not alter food intake or glucose homeostasis in mice. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 1845–9.

[57] Ingall, A. H.; Dixon, J.; Bailey, A.; Coombs, M. E.; Cox, D.; McInally, J. I.; Hunt, S. F.; Kindon, N. D.; Teobald, B. J.; Willis, P. A.; Humphries, R. G.; Leff, P.; Clegg, J. A.; Smith, J. A.; Tomlinson, W. Antagonists of the platelet P(2t) receptor: A novel approach to antithrombotic therapy. *J. Med. Chem.* **1999**, *42*,

213–220.

[58] Jacobson, K. A.; Jarvis, M. F.; Williams, M. Purine and pyrimidine (P2) receptors as drug targets. *J Med Chem* **2002**, *45*, 4057–4093.

[59] Brink, C.; Dahlén, S.-E.; Drazen, J.; Evans, J. F.; Hay, D. W. P.; Nicosia, S.; Serhan, C. N.; Shimizu, T.; Yokomizo, T. International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. *Pharmacol. Rev.* **2003**, *55*, 195–227.

[60] Capra, V.; Thompson, M. D.; Sala, A.; Cole, D. E.; Folco, G.; Rovati, G. E. Cysteinyl-leukotrienes and their receptors in asthma and other inflammatory diseases: Critical update and emerging trends. *Med. Res. Rev.* **2007**, *27*, 469–527.

[61] Köse, M.; Ritter, K.; Thiemke, K.; Gillard, M.; Kostenis, E.; Müller, C. E. Development of [(3)H]2-Carboxy-4,6-dichloro-1H-indole-3-propionic Acid ([(3)H]PSB-12150): A Useful Tool for Studying GPR17. *ACS Med. Chem. Lett.* **2014**, *5*, 326–30.

[62] O'Dowd, B. F.; Nguyen, T.; Marchese, A.; Cheng, R.; Lynch, K. R.; Heng, H. H.; Kolakowski, L. F.; George, S. R. Discovery of three novel G-protein-coupled receptor genes. *Genomics* **1998**, *47*, 310–3.

[63] Okumura, S. I.; Baba, H.; Kumada, T.; Nanmoku, K.; Nakajima, H.; Nakane, Y.; Hioki, K.; Ikenaka, K. Cloning of a G-protein-coupled receptor that shows an activity to transform NIH3T3 cells and is expressed in gastric cancer cells. *Cancer Sci.* **2004**, *95*, 131–135.

[64] Sawzdargo, M.; Nguyen, T.; Lee, D. K.; Lynch, K. R.; Cheng, R.; Heng, H. H.; George, S. R.; O'Dowd, B. F. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain Res. Mol. Brain Res.* **1999**, *64*, 193–198.

[65] Thimm, D.; Funke, M.; Meyer, A.; Müller, C. E. 6-Bromo-8-(4-[3H]methoxybenzamido)-4-oxo-4H-chromene-2-carboxylic acid: A powerful tool for studying orphan G protein-coupled receptor GPR35. *J. Med. Chem.* **2013**, *56*, 7084–7099.

[66] Thimm, D. Medizinische Chemie und molekulare Pharmakologie G-Protein-

gekoppelter Purin- und verwandter Waisen-Rezeptoren. Ph.D. thesis, University of Bonn, 2014.

[67] Wang, J.; Wu, X.; Simonavicius, N.; Tian, H.; Ling, L. Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. *J. Biol. Chem.* **2006**, *281*, 34457–64.

[68] Guo, J.; Williams, D. J.; Puhl, H. L.; Ikeda, S. R. Inhibition of N-type calcium channels by activation of GPR35, an orphan receptor, heterologously expressed in rat sympathetic neurons. *J. Pharmacol. Exp. Ther.* **2008**, *324*, 342–51.

[69] Jenkins, L.; Brea, J.; Smith, N. J.; Hudson, B. D.; Reilly, G.; Bryant, N. J.; Castro, M.; Loza, M.-I.; Milligan, G. Identification of novel species-selective agonists of the G-protein-coupled receptor GPR35 that promote recruitment of β-arrestin-2 and activate Gα13. *Biochem. J.* **2010**, *432*, 451–459.

[70] Zhao, P.; Sharir, H.; Kapur, A.; Cowan, A.; Geller, E. B.; Adler, M. W.; Seltzman, H. H.; Reggio, P. H.; Heynen-Genel, S.; Sauer, M.; Chung, T. D. Y.; Bai, Y.; Chen, W.; Caron, M. G.; Barak, L. S.; Abood, M. E. Targeting of the orphan receptor GPR35 by pamoic acid: a potent activator of extracellular signal-regulated kinase and β-arrestin2 with antinociceptive activity. *Mol. Pharmacol.* **2010**, *78*, 560–568.

[71] Taniguchi, Y.; Tonai-Kachi, H.; Shinjo, K. Zaprinast, a well-known cyclic guanosine monophosphate-specific phosphodiesterase inhibitor, is an agonist for GPR35. *FEBS Lett.* **2006**, *580*, 5003–5008.

[72] Imielinski, M.; Baldassano, R. N.; Griffiths, A.; Russell, R. K.; Annese, V.; Dubinsky, M.; Kugathasan, S.; Bradfield, J. P.; Walters, T. D.; Sleiman, P.; Kim, C. E.; Muise, A.; Wang, K.; Glessner, J. T.; Saeed, S.; Zhang, H.; Frackelton, E. C.; Hou, C.; Flory, J. H.; Otieno, G.; Chiavacci, R. M.; Grundmeier, R.; Castro, M.; Latiano, A.; Dallapiccola, B.; Stempak, J.; Abrams, D. J.; Taylor, K.; McGovern, D.; Western Regional Alliance for Pediatric IBD,; Silber, G.; Wrobel, I.; Quiros, A.; International IBD Genetics Consortium,; Barrett, J. C.; Hansoul, S.; Nicolae, D. L.; Cho, J. H.; Duerr, R. H.; Rioux, J. D.; Brant, S. R.; Silverberg, M. S.; Taylor, K. D.; Barmuda, M. M.; Bitton, A.; Dassopoulos, T.; Datta, L. W.; Green, T.; Griffiths, A. M.; Kistner, E. O.; Murtha, M. T.; Regueiro, M. D.; Rotter, J. I.; Schumm, L. P.; Steinhart, A. H.; Targan, S. R.; Xavier, R. J.; NIDDK

IBD Genetics Consortium,; Libioulle, C.; Sandor, C.; Lathrop, M.; Belaiche, J.; Dewit, O.; Gut, I.; Heath, S.; Laukens, D.; Mni, M.; Rutgeerts, P.; Van Gossum, A.; Zelenika, D.; Franchimont, D.; Hugot, J. P.; de Vos, M.; Vermeire, S.; Louis, E.; Belgian-French IBD Consortium,; Wellcome Trust Case Control Consortium,; Cardon, L. R.; Anderson, C. A.; Drummond, H.; Nimmo, E.; Ahmad, T.; Prescott, N. J.; Onnie, C. M.; Fisher, S. A.; Marchini, J.; Ghori, J.; Bumpstead, S.; Gwillam, R.; Tremelling, M.; Delukas, P.; Mansfield, J.; Jewell, D.; Satsangi, J.; Mathew, C. G.; Parkes, M.; Georges, M.; Daly, M. J.; Heyman, M. B.; Ferry, G. D.; Kirschner, B.; Lee, J.; Essers, J.; Grand, R.; Stephens, M.; Levine, A.; Piccoli, D.; Van Limbergen, J.; Cucchiara, S.; Monos, D. S.; Guthery, S. L.; Denson, L.; Wilson, D. C.; Grant, S. F. A.; Daly, M.; Silverberg, M. S.; Satsangi, J.; Hakonarson, H. Common variants at five new loci associated with early-onset inflammatory bowel disease. *Nat. Genet.* **2009**, *41*, 1335–40.

[73] Min, K.-D.; Asakura, M.; Liao, Y.; Nakamaru, K.; Okazaki, H.; Takahashi, T.; Fujimoto, K.; Ito, S.; Takahashi, A.; Asanuma, H.; Yamazaki, S.; Minamino, T.; Sanada, S.; Seguchi, O.; Nakano, A.; Ando, Y.; Otsuka, T.; Furukawa, H.; Isomura, T.; Takashima, S.; Mochizuki, N.; Kitakaze, M. Identification of genes related to heart failure using global gene expression profiling of human failing myocardium. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 55–60.

[74] Sun, Y.; Bielak, L.; Peyser, P.; Turner, S. Application of machine learning algorithms to predict coronary artery calcification with a sibship-based design. *Genet Epidemiol.* **2008**, *32*, 350–360.

[75] Ronkainen, V. P.; Tuomainen, T.; Huusko, J.; Laidinen, S.; Malinen, M.; Palvimo, J. J.; Ylä-Herttuala, S.; Vuolteenaho, O.; Tavi, P. Hypoxia-inducible factor 1-induced G protein-coupled receptor 35 expression is an early marker of progressive cardiac remodelling. *Cardiovasc. Res.* **2014**, *101*, 69–77.

[76] Wang, J.; Simonavicius, N.; Wu, X.; Swaminath, G.; Reagan, J.; Tian, H.; Ling, L. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J. Biol. Chem.* **2006**, *281*, 22021–22028.

[77] Barth, M. C.; Ahluwalia, N.; Anderson, T. J. T.; Hardy, G. J.; Sinha, S.; Alvarez-Cardona, J. A.; Pruitt, I. E.; Rhee, E. P.; Colvin, R. A.; Gerszten, R. E. Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. *J. Biol. Chem.* **2009**, *284*, 19189–19195.

[78] Yang, Y.; Lu, J. Y. L.; Wu, X.; Summer, S.; Whoriskey, J.; Saris, C.; Reagan, J. D. G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. *Pharmacology* **2010**, *86*, 1–5.

[79] Fallarini, S.; Magliulo, L.; Paoletti, T.; de Lalla, C.; Lombardi, G. Expression of functional GPR35 in human iNKT cells. *Biochem. Biophys. Res. Commun.* **2010**, *398*, 420–425.

[80] Horikawa, Y.; Oda, N.; Cox, N. J.; Li, X.; Orho-Melander, M.; Hara, M.; Hinokio, Y.; Lindner, T. H.; Mashima, H.; Schwarz, P. E.; del Bosque-Plata, L.; Horikawa, Y.; Oda, Y.; Yoshiuchi, I.; Colilla, S.; Polonsky, K. S.; Wei, S.; Concannon, P.; Iwasaki, N.; Schulze, J.; Baier, L. J.; Bogardus, C.; Groop, L.; Boerwinkle, E.; Hanis, C. L.; Bell, G. I. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat. Genet.* **2000**, *26*, 163–175.

[81] Ohshiro, H.; Tonai-Kachi, H.; Ichikawa, K. GPR35 is a functional receptor in rat dorsal root ganglion neurons. *Biochem. Biophys. Res. Commun.* **2008**, *365*, 344–348.

[82] Cosi, C.; Mannaioni, G.; Cozzi, A.; Carl, V.; Sili, M.; Cavone, L.; Maratea, D.; Moroni, F. G-protein coupled receptor 35 (GPR35) activation and inflammatory pain: Studies on the antinociceptive effects of kynurenic acid and zaprinast. *Neuropharmacology* **2011**, *60*, 1227–1231.

[83] Oka, S.; Ota, R.; Shima, M.; Yamashita, A.; Sugiura, T. GPR35 is a novel lysophosphatidic acid receptor. *Biochem. Biophys. Res. Commun.* **2010**, *395*, 232–237.

[84] Maravillas-Montero, J. L.; Burkhardt, A. M.; Hevezi, P. A.; Carnevale, C. D.; Smit, M. J.; Zlotnik, A. Cutting edge: GPR35/CXCR8 is the receptor of the mucosal chemokine CXCL17. *J. Immunol.* **2015**, *194*, 29–33.

[85] MacKenzie, A. E.; Caltabiano, G.; Kent, T. C.; Jenkins, L.; McCallum, J. E.; Hudson, B. D.; Nicklin, S. a.; Fawcett, L.; Markwick, R.; Charlton, S. J.; Milligan, G. The antiallergic mast cell stabilizers lodoxamide and bufrolin as the first high and equipotent agonists of human and rat GPR35. *Mol. Pharmacol.* **2014**, *85*, 91–104.

[86] Deng, H.; Fang, Y. Aspirin metabolites are GPR35 agonists. *Naunyn.*

Schmiedebergs. Arch. Pharmacol. **2012**, *385*, 729–737.

[87] Deng, H.; Hu, H.; Fang, Y. Tyrphostin analogs are GPR35 agonists. *FEBS Lett.* **2011**, *585*, 1957–1962.

[88] Deng, H.; Fang, Y. The Three Catecholics Benserazide, Catechol and Pyrogallol are GPR35 Agonists. *Pharmaceuticals* **2013**, *6*, 500–509.

[89] Deng, H.; Hu, H.; Ling, S.; Ferrie, A. M.; Fang, Y. Discovery of natural phenols as G protein-coupled receptor-35 (GPR35) agonists. *ACS Med. Chem. Lett.* **2012**, *3*, 165–169.

[90] Deng, H.; Fang, Y. Discovery of nitrophenols as GPR35 agonists. *Med. Chem. Commun.* **2012**, *3*, 1270–1274.

[91] Hu, H.; Deng, H.; Fang, Y. Label-Free Phenotypic Profiling Identified D-Luciferin as a GPR35 Agonist. *PLoS One* **2012**, *7*, e34934.

[92] Deng, H.; Hu, H.; He, M.; Hu, J.; Niu, W.; Ferrie, A. M.; Fang, Y. Discovery of 2-(4-Methylfuran-2(5H)-ylidene)malononitrile and Thieno[3,2-b]thiophene-2-carboxylic Acid Derivatives as G Protein-Coupled Receptor 35 (GPR35) Agonists. *J. Med. Chem.* **2011**, *2*, 7385–7396.

[93] Deng, H.; Fang, Y. Anti-inflammatory gallic acid and wedelolactone are G protein-coupled receptor-35 agonists. *Pharmacology* **2012**, *89*, 211–219.

[94] Yang, Y.; Fu, A.; Wu, X.; Reagan, J. D. GPR35 is a target of the loop diuretic drugs bumetanide and furosemide. *Pharmacology* **2012**, *89*, 13–17.

[95] Funke, M.; Thimm, D.; Schiedel, A. C.; 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*, 5182–5197.

[96] Jenkins, L.; Harries, N.; Lappin, J. E. J.; a.E. MacKenzie, A. E.; Neetoo-Isseljee, Z.; Southern, C.; McIver, E. E. G.; Nicklin, S. A. S.; Taylor, D. L. D.; Milligan, G. Antagonists of GPR35 display high species ortholog selectivity and varying modes of action. *J. Pharmacol. Exp. Ther.* **2013**, *343*, 1–3.

[97] Console-Bram, L.; Marcu, J.; Abood, M. E. Cannabinoid receptors: Nomenclature and pharmacological principles. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **2012**, *38*, 4–15.

[98] Ryberg, E.; Larsson, N.; Sjögren, S.; Hjorth, S.; Hermansson, N.-O.; Leonova, J.;

Elebring, T.; Nilsson, K.; Drmota, T.; Greasley, P. J. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharmacol.* **2007**, *152*, 1092–101.

[99] Baker, D.; Pryce, G.; Davies, W. L.; Hiley, C. R. In silico patent searching reveals a new cannabinoid receptor. 2006.

[100] Lauckner, J. E.; Jensen, J. B.; Chen, H.-Y.; Lu, H.-C.; Hille, B.; Mackie, K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 2699–704.

[101] Henstridge, C. M.; Balenga, N. a. B.; Ford, L. a.; Ross, R. a.; Waldhoer, M.; Irving, A. J. The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca2+ signaling and NFAT activation. *FASEB J.* **2009**, *23*, 183–93.

[102] Moreno-Navarrete, J. M.; Catalan, V.; Whyte, L.; Diaz-Arteaga, A.; Vazquez-Martinez, R.; Rotellar, F.; Guzman, R.; Gomez-Ambrosi, J.; Pulido, M. R.; Russell, W. R.; Imbernon, M.; Ross, R. A.; Malagon, M. M.; Dieguez, C.; Fernandez-Real, J. M.; Fruhbeck, G.; Nogueiras, R. The L-alpha-lysophosphatidylinositol/GPR55 system and its potential role in human obesity. *Diabetes* **2012**, *61*, 281–291.

[103] Whyte, L. S.; Ryberg, E.; Sims, N. A.; Ridge, S. A.; Mackie, K.; Greasley, P. J.; Ross, R. A.; Rogers, M. J. The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 16511–16516.

[104] Henstridge, C. M.; Brown, A. J.; Waldhoer, M. GPR55: Metabolic Help or Hindrance? *Trends Endocrinol Metab.* **2016**, *27*, 606–608.

[105] Meadows, A.; Lee, J.; Wu, C.-S.; Wei, Q.; Pradhan, G.; Yafi, M.; Lu, H.-C. Deletion of G-protein-coupled receptor 55 promotes obesity by reducing physical activity. *Int. J. Obes.* **2016**, *40*, 417–424.

[106] Liu, Y.; Zhang, Q.; Chen, L.-H.; Yang, H.; Lu, W.; Xie, X.; Nan, F.-J. Design and Synthesis of 2-Alkylpyrimidine-4,6-diol and 6-Alkylpyridine-2,4-diol as Potent GPR84 Agonists. *ACS Med. Chem. Lett.* **2016**, *7*, 579–583.

[107] Xiao, Y.; Chen, Y.; Kennedy, a. W.; Belinson, J.; Xu, Y. Evaluation of plasma lysophospholipids for diagnostic significance using electrospray ionization mass spectrometry (ESI-MS) analyses. *Ann. N. Y. Acad. Sci.* **2000**, *905*, 242–259.

[108] Sutphen, R.; Xu, Y.; Wilbanks, G. D.; Fiorica, J.; Grendys, E. C.; LaPolla, J. P.; Arango, H.; Hoffman, M. S.; Martino, M.; Wakeley, K.; Griffin, D.; Blanco, R. W.; Cantor, A. B.; Xiao, Y. J.; Krischer, J. P. Lysophospholipids are potential biomarkers of ovarian cancer. *Cancer Epidemiol. Biomarkers Prev.* **2004**, *13*, 1185–1191.

[109] Piñeiro, R.; Maffucci, T.; Falasca, M. The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation. *Oncogene* **2011**, *30*, 142–152.

[110] Andradas, C.; Caffarel, M. M.; Pérez-Gómez, E.; Salazar, M.; Lorente, M.; Velasco, G.; Guzmán, M.; Sánchez, C. The orphan G protein-coupled receptor GPR55 promotes cancer cell proliferation via ERK. *Oncogene* **2011**, *30*, 245–252.

[111] Pérez-Gómez, E.; Andradas, C.; Flores, J. M.; Quintanilla, M.; Paramio, J. M.; Guzmán, M.; Sánchez, C. The orphan receptor GPR55 drives skin carcinogenesis and is upregulated in human squamous cell carcinomas. *Oncogene* **2012**, 2534–2542.

[112] Moreno, E.; Andradas, C.; Medrano, M.; Caffarel, M. M.; Pérez-Gómez, E.; Blasco-Benito, S.; Gómez-Cañas, M.; Pazos, M. R.; Irving, A. J.; Lluís, C.; Canela, E. I.; Fernández-Ruiz, J.; Guzmán, M.; McCormick, P. J.; Sánchez, C. Targeting CB2-GPR55 receptor heteromers modulates cancer cell signaling. *J. Biol. Chem.* **2014**, *289*, 21960–21972.

[113] Andradas, C.; Blasco-Benito, S.; Castillo-Lluva, S.; Dillenburg-Pilla, P.; Diez-Alarcia, R.; Juanes-García, A.; García-Taboada, E.; Hernando-Llorente, R.; Soriano, J.; Hamann, S.; Wenners, A.; Alkatout, I.; Klapper, W.; Rocken, C.; Bauer, M.; Arnold, N.; Quintanilla, M.; Megías, D.; Vicente-Manzanares, M.; Urigüen, L.; Gutkind, J.; Guzmán, M.; Pérez-Gómez, E.; Sánchez, C. Activation of the orphan receptor GPR55 by lysophosphatidylinositol promotes metastasis in triple-negative breast cancer. *Oncotarget.* **2016**, Epub ahead of print.

[114] Kargl, J.; Andersen, L.; Hasenöhrl, C.; Feuersinger, D.; Stančic, A.; Fauland, A.; Magnes, C.; El-Heliebi, A.; Lax, S.; Uranitsch, S.; Haybaeck, J.; Heinemann, A.; Schicho, R. GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis. *Br. J. Pharmacol.* **2016**, *173*, 142–154.

[115] Heynen-Genel, S.; Dahl, R.; Shi, S.; Milan, L.; Hariharan, S.; Bravo, Y.; Sergienko, E.; Hedrick, M.; Dad, S.; Stonich, D.; Su, Y.; Vicchiarelli, M.;

Mangravita-Novo, A.; Smith, L. H.; Chung, T. D. Y.; Sharir, H.; Barak, L. S.; Abood, M. E.; Caron, M. G. Screening for Selective Ligands for GPR55 - Antagonists. *Probe Reports from NIH Mol. Libr. Progr.* **2010**,

[116] Stancic, A.; Jandl, K.; Hasenöhrl, C.; Reichmann, F.; Marsche, G.; Schuligoi, R.; Heinemann, A.; Storr, M.; Schicho, R. The GPR55 antagonist CID16020046 protects against intestinal inflammation. *Neurogastroenterol. Motil.* **2015**, *27*, 1432–1445.

[117] Petitet, F.; Donlan, M.; Michel, A. GPR55 as a New Cannabinoid Receptor: Still a Long Way to Prove It. *Chem. Biol. Drug Des.* **2006**, *67*, 252–253.

[118] Johns, D. G.; Behm, D. J.; Walker, D. J.; Ao, Z.; Shapland, E. M.; Daniels, D. A.; Riddick, M.; Dowell, S.; Staton, P. C.; Green, P.; Shabon, U.; Bao, W.; Aiyar, N.; Yue, T.-L.; Brown, A. J.; Morrison, A. D.; Douglas, S. A. The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. *Br. J. Pharmacol.* **2007**, *152*, 825–31.

[119] Rempel, V.; Fuchs, A.; Hinz, S.; Karcz, T.; Lehr, M.; Koetter, U.; Müller, C. E. Magnolia extract, magnolol, and metabolites: Activation of cannabinoid CB2 receptors and blockade of the related GPR55. *ACS Med. Chem. Lett.* **2013**, *4*, 41–45.

[120] 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 beta-arrestin PathHunter assay. *J. Biol. Chem.* **2009**, *284*, 12328–12338.

[121] Oka, S.; Toshida, T.; Maruyama, K.; Nakajima, K.; Yamashita, A.; Sugiura, T. 2-Arachidonoyl-sn-glycero-3-phosphoinositol: A possible natural ligand for GPR55. *J. Biochem.* **2009**, *145*, 13–20.

[122] Rempel, V.; Volz, N.; Gläser, F.; Nieger, M.; Bräse, S.; Müller, C. E. Antagonists for the orphan G-protein-coupled receptor GPR55 based on a coumarin scaffold. *J. Med. Chem.* **2013**, *56*, 4798–4810.

[123] Oka, S.; Nakajima, K.; Yamashita, A.; Kishimoto, S.; Sugiura, T. Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem. Biophys. Res. Commun.* **2007**, *362*, 928–934.

[124] Kapur, A.; Zhao, P.; Sharir, H.; Bai, Y.; Caron, M. G.; Barak, L. S.; Abood, M. E. Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands.

J. Biol. Chem. **2009**, *284*, 29817–29827.

[125] Heynen-Genel, S.; Dahl, R.; Shi, S.; Milan, L.; Hariharan, S.; Bravo, Y.; Sergienko, E.; Hedrick, M.; Dad, S.; Stonich, D.; Su, Y.; Vicchiarelli, M.; Mangravita-Novo, A.; Smith, L.; Chung, T.; Sharir, H.; Barak, L.; Abood, M. Screening for Selective Ligands for GPR55 - Agonists. *Probe Reports from NIH Mol. Libr. Progr.* **2011**,

[126] Hess, C.; Schoeder, C. T.; Pillaiyar, T.; Madea, B.; Müller, C. E. Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. *Forensic Toxicol.* **2016**, *34*, 329–343.

[127] Yrjölä, S.; Parkkari, T.; Navia-Paldanius, D.; Laitinen, T.; Kaczor, A. A.; Kokkola, T.; Adusei-Mensah, F.; Savinainen, J. R.; Laitinen, J. T.; Poso, A.; Alexander, A.; Penman, J.; Stott, L.; Anskat, M.; Irving, A. J.; Nevalainen, T. J. Potent and selective N-(4-sulfamoylphenyl)thiourea-based GPR55 agonists. *Eur. J. Med. Chem.* **2016**, *107*, 119–132.

[128] Meza-Aviña, M.; Lingerfelt, M.; Console-Bram, L.; Gamage, T.; Sharir, H.; Gettys, K.; Hurst, D.; Kotsikorou, E.; Shore, D.; Caron, M.; Rao, N.; Barak, L.; Abood, M.; Reggio, P.; Croatt, M. Design, synthesis, and analysis of antagonists of GPR55: Piperidine-substituted 1,3,4-oxadiazol-2-ones. *Bioorg Med Chem Lett.* **2016**, *26*, 1827–1830.

[129] Wittenberger, T.; Schaller, H. C.; Hellebrand, S. An expressed sequence tag (EST) data mining strategy succeeding in the discovery of new G-protein coupled receptors. *J. Mol. Biol.* **2001**, *307*, 799–813.

[130] Yousefi, S.; Cooper, P. P. R.; Potter, S. L.; Mueck, B.; Jarai, G. Cloning and expression analysis of a novel G-protein-coupled receptor selectively expressed on granulocytes. *J. Leukoc. Biol.* **2001**, *69*, 1045–1052.

[131] Venkataraman, C.; Kuo, F. The G-protein coupled receptor, GPR84 regulates IL-4 production by T lymphocytes in response to CD3 crosslinking. *Immunol. Lett.* **2005**, *101*, 144–53.

[132] Brown, A. J.; Goldsworthy, S. M.; Barnes, A. A.; Eilert, M. M.; Tcheang, L.; Daniels, D.; Muir, A. I.; Wigglesworth, M. J.; Kinghorn, I.; Fraser, N. J.; Pike, N. B.; Strum, J. C.; Steplewski, K. M.; Murdock, P. R.; Holder, J. C.; Marshall, F. H.; Szekeres, P. G.; Wilson, S.; Ignar, D. M.; Foord, S. M.; Wise, A.;

Dowell, S. J. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* **2003**, *278*, 11312–11319.

[133] Briscoe, C. P.; Tadayyon, M.; Andrews, J. L.; Benson, W. G.; Chambers, J. K.; Eilert, M. M.; Ellis, C.; Elshourbagy, N. A.; Goetz, A. S.; Minnick, D. T.; Murdock, P. R.; Sauls, H. R.; Shabon, U.; Spinage, L. D.; Strum, J. C.; Szekeres, P. G.; Tan, K. B.; Way, J. M.; Ignar, D. M.; Wilson, S.; Muir, A. I. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.* **2003**, *278*, 11303–11311.

[134] Lattin, J. E.; Schroder, K.; Su, A. I.; Walker, J. R.; Zhang, J.; Wiltshire, T.; Saijo, K.; Glass, C. K.; Hume, D. a.; Kellie, S.; Sweet, M. J. Expression analysis of G Protein-Coupled Receptors in mouse macrophages. *Immunome Res.* **2008**, *4*, 5.

[135] Bouchard, C.; Pagé, J.; Bédard, A.; Tremblay, P.; Vallières, L. G protein-coupled receptor 84, a microglia-associated protein expressed in neuroinflammatory conditions. *Glia* **2007**, *55*, 790–800.

[136] Perry, K. J.; Johnson, V. R.; Malloch, E. L.; Fukui, L.; Wever, J.; Thomas, A. G.; Hamilton, P. W.; Henry, J. J. The G-protein-coupled receptor, GPR84, is important for eye development in Xenopus laevis. *Dev. Dyn.* **2010**, *239*, 3024–37.

[137] Suzuki, M.; Takaishi, S.; Nagasaki, M.; Onozawa, Y.; Iino, I.; Maeda, H.; Komai, T.; Oda, T. Medium-chain fatty acid-sensing receptor, GPR84, is a proinflammatory receptor. *J. Biol. Chem.* **2013**, *288*, 10684–10691.

[138] Zhang, Q.; Yang, H.; Li, J.; Xie, X. Discovery and Characterization of a Novel Small-Molecule Agonist for Medium-Chain Free Fatty Acid Receptor G Protein-Coupled Receptor 84. *J Pharmacol Exp Ther.* **2016**, *357*, 337–344.

[139] Nagasaki, H.; Kondo, T.; Fuchigami, M.; Hashimoto, H.; Sugimura, Y.; Ozaki, N.; Arima, H.; Ota, A.; Oiso, Y.; Hamada, Y. Inflammatory changes in adipose tissue enhance expression of GPR84, a medium-chain fatty acid receptor: TNFα enhances GPR84 expression in adipocytes. *FEBS Lett.* **2012**, *586*, 368–72.

[140] Hakak, Y.; Unett, D. J.; Gatlin, J.; Liaw, C. W. Human g protein-coupled receptor and modulators thereof for the treatment of atherosclerosis and atherosclerotic disease and for the treatment of conditions related to mcp-1 expression.

WO2007027661 A2. 2007.

[141] Kimachi, T.; Tanaka, K.; Yoneda, F. Synthesis of a proposed isomer of F420 having a-glutamyl bonding. *J. Heterocyc. Chem.* **1991**, *28*, 439–443.

[142] Huang, Q.; Feng, D.; Liu, K.; Wang, P.; Xiao, H.; Wang, Y.; Zhang, S.; Liu, Z. A medium-chain fatty acid receptor Gpr84 in zebrafish: expression pattern and roles in immune regulation. *Dev. Comp. Immunol.* **2014**, *45*, 252–8.

[143] Dietrich, P. A.; Yang, C.; Leung, H. H. L.; Lynch, J. R.; Gonzales, E.; Liu, B.; Haber, M.; Norris, M. D.; Wang, J.; Wang, J. Y. GPR84 sustains aberrant b -catenin signaling in leukemic stem cells for maintenance of MLL leukemogenesis. **2015**, *124*, 3284–3295.

[144] Madeddu, S.; Woods, T. A.; Mukherjee, P.; Sturdevant, D.; Butchi, N. B.; Peterson, K. E. Identification of glial activation markers by comparison of transcriptome changes between astrocytes and microglia following innate immune stimulation. *PLoS One* **2015**, *10*, e0127336.

[145] Nikaido, Y.; Koyama, Y.; Yoshikawa, Y.; Furuya, T.; Takeda, S. Mutation analysis and molecular modeling for the investigation of ligand-binding modes of GPR84. *J. Biochem.* **2014**, 1–10.

[146] Audoy-Rémus, J.; Bozoyan, L.; Dumas, A.; Filali, M.; Lecours, C.; Lacroix, S.; Rivest, S.; Tremblay, M.-E.; Vallières, L. GPR84 deficiency reduces microgliosis, but accelerates dendritic degeneration and cognitive decline in a mouse model of Alzheimer's disease. *Brain. Behav. Immun.* **2015**, 1–9.

[147] Nicol, L. S. C.; Dawes, J. M.; La Russa, F.; Didangelos, A.; Clark, A. K.; Gentry, C.; Grist, J.; Davies, J. B.; Malcangio, M.; McMahon, S. B. The role of G-protein receptor 84 in experimental neuropathic pain. *J. Neurosci.* **2015**, *35*, 8959–69.

[148] Abdel-Aziz, H.; Schneider, M.; Neuhuber, W.; Meguid Kassem, A.; Khailah, S.; Müller, J.; Gamaleldeen, H.; Khairy, A.; Khayyal, M. T.; Shcherbakova, A.; Efferth, T.; Ulrich-Merzenich, G. GPR84 and TREM-1 signaling contribute to the pathogenesis of reflux esophagitis Running Head: GPR84 and TREM-1 signaling in GERD. *Mol. Med.* **2015**,

[149] Labéguère, F.; Alvey, L.; Newsome, G.; Sanière, L.; Fletcher, S. Novel dihydropyrimidinoisoquinolinones and pharmaceutical compositions thereof for the

treatment of inflammatory disorders (gpr84 antagonists) WO2014095798 A1, EP2935262A1. 2014.

[150] Yegutkin, G. G. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. *Biochim. Biophys. Acta* **2008**, *1783*, 673–94.

[151] Zimmermann, H.; Zebisch, M.; Sträter, N. Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signal.* **2012**, *8*, 437–502.

[152] Zimmermann, H. Extracellular metabolism of ATP and other nucleotides. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **2000**, *362*, 299–309.

[153] Grondal, E. J.; Zimmermann, H. Purification, characterization and cellular localization of 5'-nucleotidase from Torpedo electric organ. *Biochem J* **1987**, *245*, 805–810.

[154] Knapp, K.; Zebisch, M.; Pippel, J.; El-Tayeb, A.; Müller, C. E.; Sträter, N. Crystal structure of the human ecto-5'-nucleotidase (CD73): Insights into the regulation of purinergic signaling. *Structure* **2012**, *20*, 2161–2173.

[155] Robson, S. C.; Schuppan, D. Adenosine: Tipping the balance towards hepatic steatosis and fibrosis. *J. Hepatol.* **2010**, *52*, 941–943.

[156] Kriz, W. Adenosine and ATP: Traffic regulators in the kidney. *J. Clin. Invest.* **2004**, *114*, 611–613.

[157] Eltzschig, H. K. Adenosine: an old drug newly discovered. *Anesthesiology* **2009**, *111*, 904–15.

[158] Antonioli, L.; Pacher, P.; Vizi, E. S.; Haskó, G. CD39 and CD73 in immunity and inflammation. *Trends Mol. Med.* **2013**, *19*, 355–367.

[159] Jin, D.; Fan, J.; Wang, L.; Thompson, L. F.; Liu, A.; Daniel, B. J.; Shin, T.; Curiel, T. J.; Zhang, B. CD73 on tumor cells impairs antitumor T-cell responses: A novel mechanism of tumor-induced immune suppression. *Cancer Res.* **2010**, *70*, 2245–2255.

[160] Mikhailov, A.; Sokolovskaya, A.; Yegutkin, G. G.; Amdahl, H.; West, A.; Yagita, H.; Lahesmaa, R.; Thompson, L. F.; Jalkanen, S.; Blokhin, D.; Eriksson, J. E. CD73 participates in cellular multiresistance program and protects against TRAIL-induced apoptosis. *J. Immunol.* **2008**, *181*, 464–475.

[161] Stagg, J.; Divisekera, U.; Duret, H.; Sparwasser, T.; Teng, M. W. L.; Darcy, P. K.; Smyth, M. J. CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. *Cancer Res.* **2011**, *71*, 2892–2900.

[162] Stagg, J.; Divisekera, U.; McLaughlin, N.; Sharkey, J.; Pommey, S.; Denoyer, D.; Dwyer, K. M.; Smyth, M. J. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 1547–1552.

[163] Yegutkin, G. G.; Marttila-Ichihara, F.; Karikoski, M.; Niemelä, J.; Laurila, J. P.; Elima, K.; Jalkanen, S.; Salmi, M. Altered purinergic signaling in CD73-deficient mice inhibits tumor progression. *Eur. J. Immunol.* **2011**, *41*, 1231–1241.

[164] Ohta, A.; Gorelik, E.; Prasad, S. J.; Ronchese, F.; Lukashev, D.; Wong, M. K. K.; Huang, X.; Caldwell, S.; Liu, K.; Smith, P.; Chen, J.-F.; Jackson, E. K.; Apasov, S.; Abrams, S.; Sitkovsky, M. A2A adenosine receptor protects tumors from anti-tumor T cells. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 13132–7.

[165] Iqbal, J.; Jirovsky, D.; Lee, S. Y.; Zimmermann, H.; Müller, C. E. Capillary electrophoresis-based nanoscale assays for monitoring ecto-5'-nucleotidase activity and inhibition in preparations of recombinant enzyme and melanoma cell membranes. *Anal. Biochem.* **2008**, *373*, 129–140.

[166] Freundlieb, M.; Zimmermann, H.; Müller, C. E. A new, sensitive ecto-5'-nucleotidase assay for compound screening. *Anal. Biochem.* **2014**, *446*, 53–58.

[167] Baqi, Y.; Lee, S.-Y.; Iqbal, J.; Ripphausen, P.; Lehr, A.; Scheiff, A. B.; Zimmermann, H.; Bajorath, J.; Müller, C. E. Development of Potent and Selective Inhibitors of ecto-5'-Nucleotidase Based on an Anthraquinone Scaffold. *J. Med. Chem.* **2010**, *53*, 2076–2086.

[168] Ripphausen, P.; Freundlieb, M.; Brunschweiger, A.; Zimmermann, H.; Müller, C. E.; Bajorath, J. Virtual screening identifies novel sulfonamide inhibitors of ecto-5'-nucleotidase. *J. Med. Chem.* **2012**, *55*, 6576–81.

[169] Braganhol, E.; Tamajusuku, A. S. K.; Bernardi, A.; Wink, M. R.; Battastini, A. M. O. Ecto-5'-nucleotidase/CD73 inhibition by quercetin in the human U138MG glioma cell line. *Biochim. Biophys. Acta - Gen. Subj.* **2007**, *1770*, 1352–1359.

[170] Lee, S. Y.; Fiene, A.; Li, W.; Hanck, T.; Brylev, K. A.; Fedorov, V. E.; Lecka, J.; Haider, A.; Pietzsch, H. J.; Zimmermann, H.; Sevigny, J.; Kortz, U.; Stephan, H.;

Müller, C. E. Polyoxometalates - Potent and selective ecto-nucleotidase inhibitors. *Biochem. Pharmacol.* **2015**, *93*, 171–181.

[171] Bhattarai, S.; Freundlieb, M.; Pippel, J.; Meyer, A.; Abdelrahman, A.; Fiene, A.; Lee, S. Y.; Zimmermann, H.; Yegutkin, G. G.; Sträter, N.; El-Tayeb, A.; Müller, C. E. α,β-Methylene-ADP (AOPCP) Derivatives and Analogues: Development of Potent and Selective ecto-5'-Nucleotidase (CD73) Inhibitors. *J. Med. Chem.* **2015**, *58*, 6248–6263.

[172] Funke, M. Medizinische Chemie G-Protein-gekoppelter P2Y- und verwandter Waisen-Rezeptoren: Synthese, Optimierung und Charakterisierung selektiver Liganden als pharmakologische Tools. Ph.D. thesis, University of Bonn, 2014.

[173] Keri, R. S.; Budagumpi, S.; Pai, R. K.; Balakrishna, R. G. Chromones as a privileged scaffold in drug discovery: A review. *Eur. J. Med. Chem.* **2014**, *78*, 340–374.

[174] Burdeska, K. 4,6-Disubstituted 2-aminophenols. *Synthesis (Stuttg).* **1982**, *11*, 940–942.

[175] Gowan, J. E.; MacGiolla Riogh, S. P.; MacMahon, G. J.; O'Cleirigh, S.; Philbin, E. M.; Wheeler, T. S. Synthesis of 6,8-dihydroxyflavone. *Tetrahedron* **1958**, *2*, 116–121.

[176] Ramsden, C. A.; Knowles, P.; Lewis, E. J. L. E.; Wright, D. E. Tetrazole derivatives. DE 2846931 A1, 19790510. 1979.

[177] Baker, W.; McOmie, J. F. W.; Miles, D. 168. Synthesis of aurantiogliocladin. *J. Chem. Soc.* **1953**, 820–822.

[178] Raposo, C.; Luengo, A.; Almaraz, M.; M., M.; Mussons, M. L.; Caballero, M. C.; Mor??n, J. R. Malonic acid receptors with decarboxylative activity. *Tetrahedron* **1996**, *52*, 12323–12332.

[179] Carganico, G.; Mauleon Casellas, D.; Pascual Avellana, J.; Garcia Perez, M. L.; Palomer Benet, A. Preparation of aryloxobenzopyrancarboxylate derivatives as leukotriene antagonists. WO 9734885 A1, 19970925. 1997.

[180] Raposo, C.; Crego, M.; Mussons, M. L.; Caballero, M. C.; Moran, J. R. Readily available chromenone receptors for carboxylates. *Tetrahedron Lett.* **1994**, *35*, 3409–3410.

[181] Toda, M.; Arai, Y.; Miyamoto, T. (Acylamino))benzodioxanes and -chromenones.

EP 173516 A2 19860305. 1986.

[182] Yu, C.; Chen, Y.; Yang, J. A process for preparing 8-amino-2-(2H-tetrazol-5-yl)-4H-1-benzopyran-4-one useful as intermediate for manufacturing pranlukast. CN 101781288 A, 20100721. 2010.

[183] Anderluh, M.; Marc, G.; Dolenc, M. Convenient synthesis and reactivity of ethyl 8-amino-6-methyl-2,3-dihydro-4H-1-benzopyran-2-carboxylate. *Synth. Commun.* **2005**, *35*, 1461–1470.

[184] Cox, D.; Cairns, H.; Chadwick, N.; Suschitzky, J. L. Heterocyclic nitrogen derivatives. DE 2943658 A1, 19800514. 1980.

[185] Witiak, D. T.; Heilman, W. P.; Sankarappa, S. K.; Cavestri, R. C.; Newman, H. A. I. Synthesis of ethyl 6-substituted-chroman- and -chromone-2-carboxylates. Comparative structure-activity study employing the 6-phenyl and phenoxy analogs in the triton hyperlipidemic rat model. *J. Med. Chem.* **1975**, *18*, 934–942.

[186] Alonso, D. A.; Nájera, C.; Pacheco, M. C. Highly active oxime-derived palladacycle complexes for Suzuki-Miyaura and Ullmann-type coupling reactions. *J. Org. Chem.* **2002**, *67*, 5588–5594.

[187] Huff, B. E.; Koenig, T. M.; Mitchell, D.; Staszak, M. A. Synthesis of unsymmetrical biaryls using a modified Suzuki cross-coupling: 4-Biphenylcarboxaldehyde. *Org. Synth.* **1998**, *75*, 53–60.

[188] Cava, M. P.; Levinson, M. I. Thionation reactions of lawesson's reagents. *Tetrahedron* **1985**, *41*, 5061–5087.

[189] Higashii, T.; Ushio, H.; Fujimoto, Y.; Matsumoto, T.; Minai, M.; Yasunaga, K.; Sogabe, H.; Kotera, T. Process of producing 2-cyano-4-oxo-4H-benzopyran compounds. EP 634409 A1. 1995.

[190] Thanigaimalai, P.; Le Hoang, T. A.; Lee, K. C.; Sharma, V. K.; Bang, S. C.; Yun, J. H.; Roh, E.; Kim, Y.; Jung, S. H. Synthesis and evaluation of novel chromone analogs for their inhibitory activity against interleukin-5. *Eur. J. Med. Chem.* **2010**, *45*, 2531–2536.

[191] Jiang, X.; London, E. K.; Morris, D. J.; Clarkson, G. J.; Wills, M. Gold-catalysed cyclic ether formation from diols. *Tetrahedron* **2010**, *66*, 9828–9834.

[192] Lin, C. H.; Aristoff, P. A.; Johnson, P. D.; Mcgrath, J. P.; Timko, J. M.; Robert, A.

Benzidene prostaglandins - Synthesis of optically pure 15-deoxy-U-68,215 and ist enantiomer via a modified intramolecular Wadsworth-Emmons-Wittig reaction. *J. Org. Chem.* **1987**, *52*, 5594–5601.

[193] Hiers, G. S.; Adams, R. w-Cyclohexyl derivatives of various normal alipathic acids. IV. *J. Am. Chem. Soc.* **1926**, *48*, 2385–2393.

[194] Hibino, H.; Yoshida, T. Method for producing carboxylic acid compound. EP1739071, 20070103. 2007.

[195] Hodogaya Chemical Co., p-Benzyloxybenzoic acid derivatives. JP 57145838 A, 19820909. 1981.

[196] Jones, B. Halogenation of phenolic ethers and anilides. XIV. m-Substituted phenyl ethers. *J. Chem. Soc.* **1943**, 430–432.

[197] Schnatterer, S.; Maier, M.; Lochhaas, F.; Knauf, W.; Seeger, K. Preparation of pesticidal phenyl ether derivatives. WO 2006119876 A1 20061116. 2006.

[198] Montgomery, J. I.; Toogood, P. L.; Hutchings, K. M.; Liu, J.; Narasimhan, L.; Braden, T.; Dermyer, M. R.; Kulynych, A. D.; Smith, Y. D.; Warmus, J. S.; Taylor, C. Discovery and SAR of benzyl phenyl ethers as inhibitors of bacterial phenylalanyl-tRNA synthetase. *Bioorganic Med. Chem. Lett.* **2009**, *19*, 665–669.

[199] Brown, A. D.; Rawson, D. J.; Storer, R. I.; Swain, N. A. Preparation of sulfonamide derivatives as Nav1.7 inhibitors. WO 2012007868 A2, 20120119. 2012.

[200] Jolidon, S.; Rodriguez Sarmiento, R. M.; Thomas, A. W.; Wyler, R. Preparation of fluorobenzamides as monoamine oxidase B inhibitors for the treatment of treatment of Alzheimer's disease or senile dementia. WO 2003106380 A2, 20031224. 2003.

[201] Yanagita, H.; Yamamoto, N.; Fuji, H.; Liu, X.; Ogata, M.; Yokota, M.; Takaku, H.; Hasegawa, H.; Odagiri, T.; Tashiro, M.; Hoshino, T. Mechanism of drug resistance of hemagglutinin of influenza virus and potent scaffolds inhibiting its function. *ACS Chem. Biol.* **2012**, *7*, 552–562.

[202] Walker, M. A.; Johnson, T. D.; Meanwell, N. A.; Banville, J. Preparation of carbamoyl keto acid tautomers as HIV integrase inhibitors for treatment of AIDS or ARC. WO 2001096283 A2, 20011220. 2001.

[203] Rakowitz, D.; Muigg, P.; Schroeder, N.; Matuszczak, B. On the synthesis of

bioisosteres of O-benzothiazolyl-oxybenzoic acids and evaluation as aldose reductase inhibitors. *Arch. Pharm. (Weinheim).* **2005**, *338*, 419–426.

[204] Andriani, G.; Amata, E.; Beatty, J.; Clements, Z.; Coffey, B. J.; Courtemanche, G.; Devine, W.; Erath, J.; Juda, C. E.; Wawrzak, Z.; Wood, J. T.; Lepesheva, G. I.; Rodriguez, A.; Pollastri, M. P. Antitrypanosomal lead discovery: Identification of a ligand-efficient inhibitor of Trypanosoma cruzi CYP51 and parasite growth. *J. Med. Chem.* **2013**, *56*, 2556–2567.

[205] Shiraishi, M.; Baba, M.; Seto, M.; Kanzaki, N.; Nishimura, O. Preparation of cyclic compounds having antagonism against ß-beta chemokine receptor (CCR5). WO 2000068203 A1, 20001116. 2000.

[206] Forrest, J.; Tucker, S. H.; Whalley, M. Oxidation of diphenylamine and of its N-derivatives. II. Oxidation of the benzyl group of some benzylamines and benzyl ethers to benzoyl. *J. Chem. Soc.* **1951**, 303–305.

[207] Zarghi, A.; Tabatabai, S. a.; Faizi, M.; Ahadian, A.; Navabi, P.; Zanganeh, V.; Shafiee, A. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1863–1865.

[208] Hara, H.; Igarashi, S.; Kimura, T.; Isaka, M.; Naito, R.; Nagaoka, H.; Koutoku, Hiroshi; Tomioka, K.; Mase, T. Medicine containing benzoic acid derivative as testosterone 5a-reductase inhibitor and novel benzoic acid derivative. WO 9324442 A1 19931209. **1993**,

[209] Mase, T.; Murase, K.; Hara, H.; Tomioka, K. Preparation of phenylene derivatives as allergy inhibitors. WO 8605779 A1, 19861009. 1986.

[210] Coates, L. V.; Drain, D. J.; Macrae, F. J.; Tattersall, K. Preparation and evaluation of some phenolic ethers as antifungal agents. *J. Pharm. Pharmacol.* **1959**, *11*, 240T–249T.

[211] Ishii, T.; Sugane, T.; Kakefuda, A.; Takahashi, T.; Kanayama, T.; Sato, K.; Kuriwaki, I.; Kitada, C.; Suzuki, J. Preparation of piperazine-1-carboxamide and piperidine-1-carboxamide derivatives as inhibitors of fatty acid amide hydrolase (FAAH).WO 2008023720 A1, 20080228. 2008.

[212] Ueno, H.; Morioka, M.; Hatanaka, F. Preparation of hydronaphthalenecarboxylates as 5a-reductase inhibitors. EP 579223 A1, 19940119. 1994.

[213] Hall, C. M.; Wright, J. B.; Johnson, H. G.; Taylor, A. J. Quinoline derivatives as antiallergy agents. 2. Fused-ring quinaldic acids. *J. Med. Chem.* **1977**, *20*, 1337–1343.

[214] Milligan, G. Orthologue selectivity and ligand bias: Translating the pharmacology of GPR35. *Trends Pharmacol. Sci.* **2011**, *32*, 317–325.

[215] MacKenzie, A. E.; Caltabiano, G.; Kent, T. C.; Jenkins, L.; McCallum, J. E.; Hudson, B. D.; Nicklin, S. a.; Fawcett, L.; Markwick, R.; Charlton, S. J.; Milligan, G. The antiallergic mast cell stabilizers lodoxamide and bufrolin as the first high and equipotent agonists of human and rat GPR35. *Mol. Pharmacol.* **2014**, *85*, 91–104.

[216] Meanwell, N. A. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* **2011**, *54*, 2529–2591.

[217] Lipinski, C.; Lombardo, F.; Dominy, B.; Feeney, P. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Develop ment Settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25.

[218] Spinrath, A. Development of pharmacological modulators for the orphan G protein-coupled receptor GPR17 and their application for the investigation of molecular signal transduction pathways. Ph.D. thesis, Universität Bonn, 2011.

[219] Dosa, P. I.; Amin, E. A. Tactical Approaches to Interconverting GPCR Agonists and Antagonists. *J. Med. Chem.* **2016**, *59*, 810–840.

[220] Sawzdargo, M.; George, S. R.; Nguyen, T.; Xu, S.; Kolakowski, L. F.; O'Dowd, B. F. A cluster of four novel human G protein-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 543–547.

[221] Hirasawa, A.; Tsumaya, K.; Awaji, T.; Katsuma, S.; Adachi, T.; Yamada, M.; Sugimoto, Y.; Miyazaki, S.; Tsujimoto, G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* **2005**, *11*, 90–94.

[222] Helenius, M.; Jalkanen, S.; Yegutkin, G. G. Enzyme-coupled assays for simultaneous detection of nanomolar ATP, ADP, AMP, adenosine, inosine and pyrophosphate concentrations in extracellular fluids. *Biochim. Biophys. Acta - Mol. Cell Res.* **2012**, *1823*, 1967–1975.

[223] Yegutkin, G. G.; Samburski, S. S.; Jalkanen, S. Soluble purine-converting en-

zymes circulate in human blood and regulate extracellular ATP level via counteracting pyrophosphatase and phosphotransfer reactions. *FASEB J.* **2003**, *17*, 1328–1330.

[224] Yegutkin, G. G.; Auvinen, K.; Karikoski, M.; Rantakari, P.; Gerke, H.; Elima, K.; Maksimow, M.; Quintero, I. B.; Vihko, P.; Salmi, M.; Jalkanen, S. Consequences of the Lack of CD73 and Prostatic Acid Phosphatase in the Lymphoid Organs. *Mediators Inflamm.* **2014**, *2014*.

[225] Mercier, N.; Kiviniemi, T. O.; Saraste, A.; Miiluniemi, M.; Silvola, J.; Jalkanen, S.; Yegutkin, G. G. Impaired ATP-induced coronary blood flow and diminished aortic NTPDase activity precede lesion formation in apolipoprotein E-deficient mice. *Am. J. Pathol.* **2012**, *180*, 419–28.

[226] Sharma, H.; Patil, S.; Sanchez, T. W.; Neamati, N.; Schinazi, R. F.; Buolamwini, J. K. Synthesis, biological evaluation and 3D-QSAR studies of 3-keto salicylic acid chalcones and related amides as novel HIV-1 integrase inhibitors. *Bioorganic Med. Chem.* **2011**, *19*, 2030–2045.

[227] Aggarwal, R.; Giles, R. G. F.; Green, I. R.; Oosthuizen, F. J.; Taylor, C. P. Syntheses in enantiopure form of four diastereoisomeric naphthopyranquinones derived from aphid insect pigments. *Org. Biomol. Chem.* **2005**, *3*, 263–273.

[228] China Raju, B.; Nageswara Rao, R.; Suman, P.; Yogeeswari, P.; Sriram, D.; Shaik, T. B.; Kalivendi, S. V. Synthesis, structure-activity relationship of novel substituted 4H-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates as potential anti-mycobacterial and anticancer agents. *Bioorganic Med. Chem. Lett.* **2011**, *21*, 2855–2859.

[229] Pfannl, M. Exchange of Alkyls in Organic Esters. *Monatshefte fuer Chemie* **1911**, *31*, 301–317.

[230] Saha, A. K.; Kavarana, M. J.; Evindar, G.; Satz, A. L.; Morgan, B. Preparation of aromatic compounds as modulators of sphingosine-1-phosphate (S1P1) receptor activity. WO 2006020951 A1, 20060223. 2006.

[231] Moeller, H.; Banduhn, N. Antiseborrheic topical compositions containing aryl ethers. EP 315913 A1, 19890517. 1989.

[232] Sakakibara, K.; Yoneshima, N.; Osawa, T. Preparation of N-pyridyl-4-(benzyloxy)benzamides as cardiotonics. JP 62158253 A, 19870714. 1987.

[233] Molock, F. F.; Boykin, D. W. The synthesis of pyridoquinolines with dialkylaminopropylamine side chains. *J. Heterocycl. Chem.* **1983**, *20*, 681–686.

[234] Hennen, S.; Wang, H.; Peters, L.; Merten, N.; Simon, K.; Spinrath, A.; Blättermann, S.; Akkari, R.; Schrage, R.; Schröder, R.; Schulz, D.; Vermeiren, C.; Zimmermann, K.; Kehraus, S.; Drewke, C.; Pfeifer, A.; König, G. M.; Mohr, K.; Gillard, M.; Müller, C. E.; Lu, Q. R.; Gomeza, J.; Kostenis, E. Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. *Sci. Signal.* **2013**, *6*, ra93.

[235] Thimm, D.; Knospe, M.; Abdelrahman, A.; Moutinho, M.; Alsdorf, B. B. A.; von Kügelgen, I.; Schiedel, A. C.; Müller, C. E. Characterization of new G protein-coupled adenine receptors in mouse and hamster. *Purinergic Signal.* **2013**, *9*, 415–426.

[236] Thimm, D.; Schiedel, A. C.; Sherbiny, F. F.; Hinz, S.; Hochheiser, K.; Bertarelli, D. C. G.; Maass, A.; Müller, C. E. Ligand-specific binding and activation of the human adenosine A(2B) receptor. *Biochemistry* **2013**, *52*, 726–40.

[237] Lowry, O. H.; Roseborough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

[238] Nordstedt, C.; Fredholm, B. B. A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. *Anal. Biochem.* **1990**, *189*, 231–234.