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From Propafenone to Fumitremorgin C – Probing
Pgp/BCRP Inhibitor Selectivity

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Abstract

ABC transporters are membrane proteins that facilitate the transport of various substances over cellular membranes. Among the most well-known members of this superfamily are P-glycoprotein (Pgp, ABCB1) and Breast Cancer Resistance Protein (BCRP, ABCG2). By transporting chemotherapeutic agents out of cancer cells, they are both responsible for treatment failure in multidrug-resistant cancers. Additionally, they are expressed in barrier tissues in the body (liver, blood-brain barrier, blood-testis barrier, placenta, ...) and thus limit the absorption of xenobiotics and drugs to these tissues, making them both targets and antitargets.

A multitude of substrates and inhibitors of Pgp and BCRP are known, however, it has been shown that while some compounds only interact selectively with one transporter, many modulate both of them. Still, the structural requirements for the design of selective or general inhibitors are not fully known and, if possible at all, predictions can only be made for limited structural scaffolds.

In order to learn more about modulators of Pgp and/or BCRP, our approach was to pick a known, selective inhibitor of Pgp (propafenone analogue GPV005) and one of BCRP (fumitremorgin C) and explore the chemical space in between. 7 target structures were defined which carried more and more features of fumitremorgin C, while still retaining some structural elements of GPV005. By adding some structural variety, 28 compounds were synthesized. Structures ranged from compounds very similar to propafenones where only the nitrogen substituent was replaced by proline or by a small cyclic dipeptide consisting of proline and glycine, over shortening the 2-hydroxypropylene linker to an ethylene linker to chromone and chromanone compounds in order to study the effect of the rigidity increase by ring closure. New synthetic routes were developed for all compounds, and their inhibitory activity on both Pgp and BCRP was determined by a cellular uptake assay of a fluorescent substrate. In general, it could be shown that compounds most similar to propafenones inhibit Pgp most, and fewer active or moderately active compounds can be found among the compounds more similar to fumitremorgin C. Interestingly, also the most active inhibitors for BCRP are among the propafenone analogues but carry the dipeptide moiety. Chromone and chromanone compounds showed higher activity on BCRP than on Pgp, although mostly acting as dual inhibitors.

This work shows the influence of functional groups and molecular patterns of known inhibitors on the inhibition of Pgp and BCRP. This knowledge could help the development of new selective or general inhibitors.

Kurzfassung

ABC-Transporter sind Membranproteine, die durch Hydrolyse von ATP den Transport einer Vielzahl von Substanzen über Membranen bewerkstelligen. Unter den menschlichen ABC-Transportern zählen P-Glykoprotein (Pgp, ABCB1) und Breast Cancer Resistance Protein (BCRP, ABCG2) zu den bekanntesten. Ihre Entdeckung beruhte auf der Tatsache, dass sie Krebszellen resistent gegenüber Gruppen von Zytostatika machen, was durch den Transport dieser Arzneimittel aus der Zelle vor sich geht. Außerdem werden Pgp und BCRP im Körper an verschiedenen Barrieren wie der Blut-Hirn-Schranke, der Blut-Plazenta-Schranke oder in der Leber exprimiert, wo sie die Absorption bzw. die Ausscheidung von Xenobiotika und Medikamenten beeinflussen. Durch diese Aufgaben stellen diese ABC-Transporter Targets und Antitargets dar und es ist wichtig zu wissen, ob neue Arzneimittel Wechselwirkungen mit ihnen zeigen.

Innerhalb der letzten Jahrzehnte wurden viele Substanzen als Substrate oder Inhibitoren von Pgp und BCRP identifiziert. Dabei wurde bemerkt, dass sowohl selektive Interaktion mit nur einem, als auch generelle Interaktion mit beiden Transportern vorkommt. Die genauen strukturellen Anforderungen um einen selektiven oder einen generellen Inhibitor zu erhalten sind aber noch unklar. Falls möglich, können Vorhersagen meist nur für begrenzte Substanzserien gemacht werden.

Um das Verständnis von Inhibitoren von BCRP und/oder Pgp zu verbessern, wählten wir den Ansatz, einen selektiven Inhibitor Pgps (Propafenon-Analog GPV005) schrittweise einem selektiven Inhibitor von BCRP (Fumitremorgin C) ähnlicher zu machen. 7 Zielstrukturen, die den chemischen Raum zwischen diesen beiden Molekülen bestmöglich abdecken sollen, wurden definiert. Diese beinhalten immer mehr Teilstrukturen von Fumitremorgin C, es werden aber auch strukturelle Elemente von GPV005 beibehalten. Im Detail beinhaltet dies erst ein Ersetzen des Stickstoff-Substituenten in GPV005 durch Prolin oder ein cyclisches Dipeptid aus Prolin und Glycin, dann die Verkürzung des Linkers zwischen dem zentralen aromatischen Ring und Prolin-Komponente von einem 2-Hydroxypropylen- zu einem Ethylen-Linker, gefolgt von einem Ringschluss zu Chromon- und Chromanon-Verbindungen. Dadurch können die Einflüsse von Flexibilität und Rigidität durch Fixierung dieser Strukturen untersucht werden. Für alle Zielsubstanzen wurden neue Synthesewege entwickelt und die inhibitorische Wirkung auf Pgp und BCRP wurde in einem zellulären Aufnahme-Assay getestet.

Generell zeigten die Substanzen, die GPV005 am ähnlichsten waren, die höchste Aktivität für Pgp- und BCRP-Inhibition, wobei BCRP-Inhibitoren die Dipeptid-Einheit aufweisen. Chromone und Chromanone inhibierten BCRP etwas stärker als Pgp, wobei Chromanone auf beiden Transportern aktiver waren als ihre oxidierten Äquivalente. Es zeigte sich, dass nur wenige selektive Inhibitoren erhalten wurden, und dass die meisten Verbindungen beide Transporter hemmen.

Durch diese Arbeit konnten Einblicke in den Einfluss verschiedener funktioneller Gruppen und Strukturelemente zweier bekannter Inhibitoren auf die Inhibition zweier ABC-Transporter gewonnen werden. In Zukunft können die Erkenntnisse für die Entwicklung selektiver oder Breitband-wirksamer Inhibitoren genutzt werden.

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Abbreviations

ABC	ATP binding cassette
Ac	acetyl
ACN	acetonitrile
ADMETox	absorption, distribution, metabolism, excretion and toxicity
ALL	acute lymphoblastic leukaemia
AML	acute myelogenous leukaemia
ATP	adenosine triphosphate
BBB	blood-brain barrier
BCRP	Breast Cancer Resistance Protein (ABCG2)
Bn	benzyl
Boc	tert-butyloxycarbonyl
Bu	butyl
CDK	cyclin-dependent kinase
CML	chronic lymphocytic leukaemia
CNS	central nervous system
conc.	concentrated
d	day/days
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-dicyclohexylcarbodiimide
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
Dik, diketopiperazine	(S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (11)
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
EtOAc	ethyl acetate
ESI	electrospray ionisation
FTC	fumitremorgin C
h	hour/hours
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HPLC	high pressure liquid chromatography
L-selectride	lithium tri-sec-butylborohydride
MDR	multidrug resistance
Me	methyl
Mes	methanesulfonate
min	minutes
MPLC	medium pressure liquid chromatography

MRP2	multidrug-resistance associated protein (ABCC2)
MTBE	methyl tert-butyl ether
NMR	nuclear magnetic resonance
oN	overnight
Pgp	P-glycoprotein (ABCB1)
PE	petrol ether (Benzin ÖAB)
Ph	phenyl
quant.	quantitative
ref	reference
Red-Al	sodium bis(2-methoxyethoxy)aluminumhydride
RNA	ribonucleic acid
RT	room temperature
SAR	structure-activity relationship
sat.	saturated
start. mat.	starting material
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TOF	time of flight
Tos	p-toluenesulfonyl

General

All compounds prepared or used as starting materials in this thesis are numbered in bold Arabic numbers. Compounds unknown to the literature are additionally underlined. Compounds of which the synthesis failed are named with lowercase bold letters. To compound numbers, which were already used in a publication, a p is added and they are otherwise left unchanged. Literature citations are indicated by superscript Arabic numbers. The nomenclature of compounds not described in the chemical literature is carried out according to the regulations of the Chemical Abstracts. Any compounds or reagents which are exempted or are known to literature are also described by means of simplified names, trivial or trade names.

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H. LITERATURE I

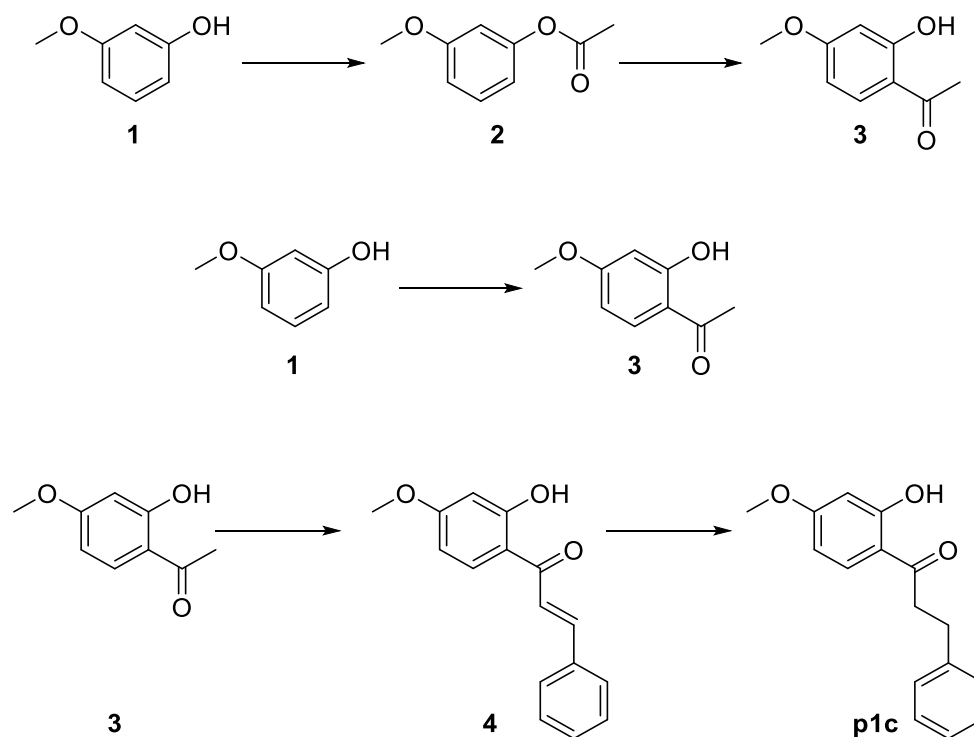
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PUBLICATION: "SUBTLE STRUCTURAL DIFFERENCES TRIGGER INHIBITORY ACTIVITY OF PROPAFENONE ANALOGUES AT THE TWO POLYSPECIFIC ABC TRANSPORTERS: P-GLYCOPROTEIN (P-GP) AND BREAST CANCER RESISTANCE PROTEIN (BCRP)", PROLINE AND DIKETOPIPERAZINE DERIVATIVES OF PROPAFENONES AND 2-ETHOXYACETOPHENONES XIV

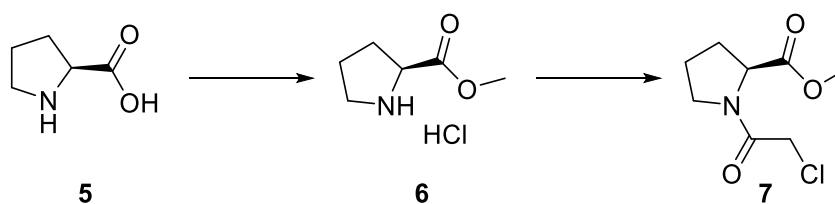
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A.1. Synthesis of starting materials, reagents and reference compounds

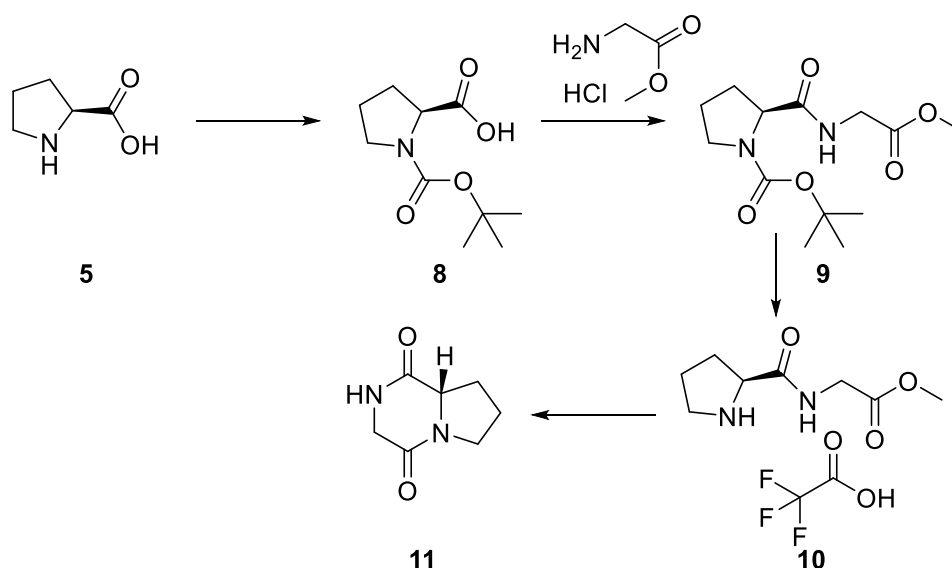
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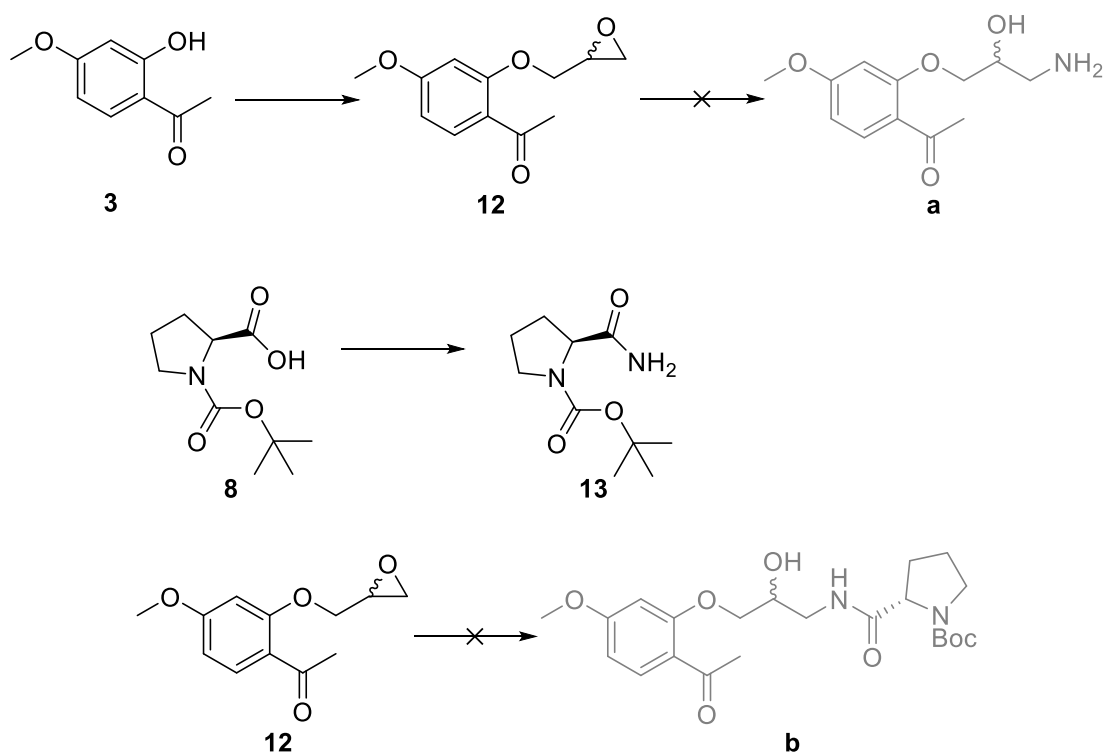


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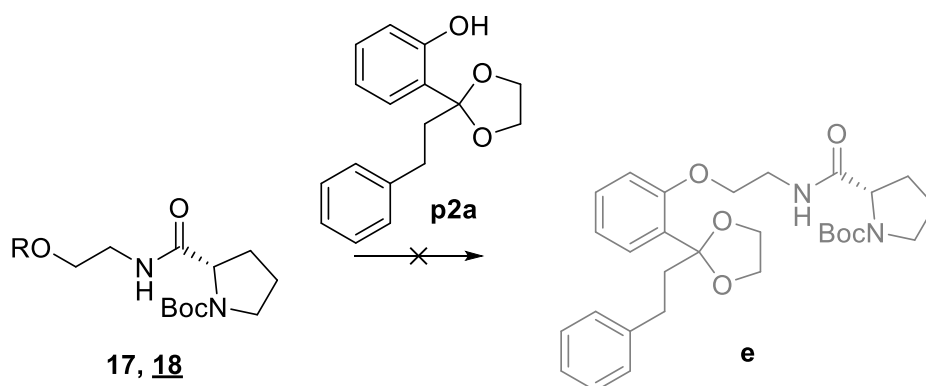
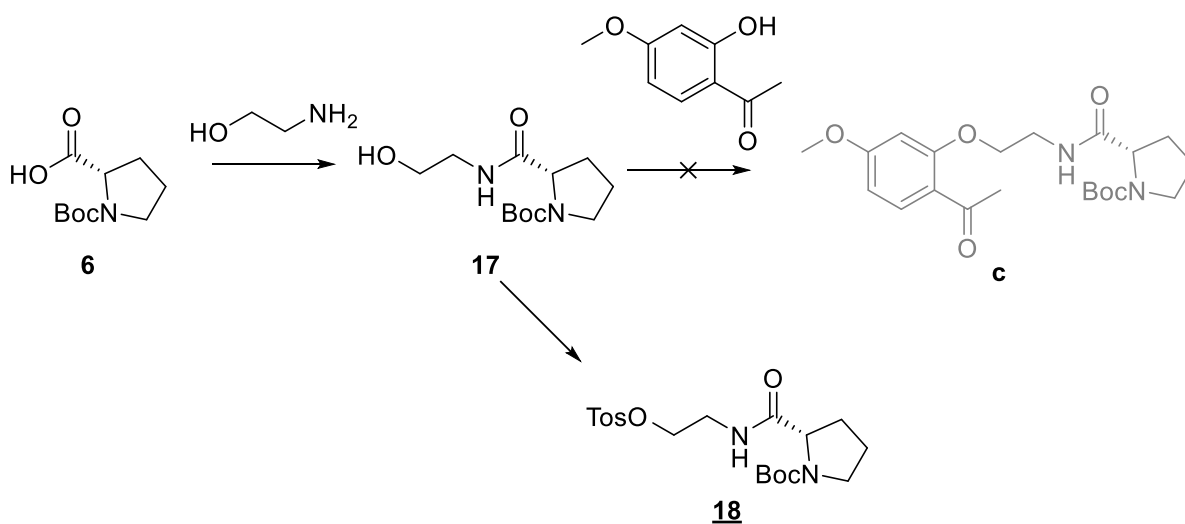
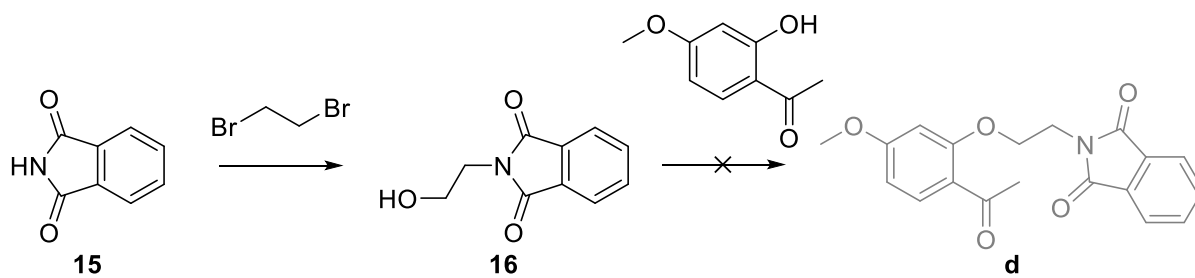
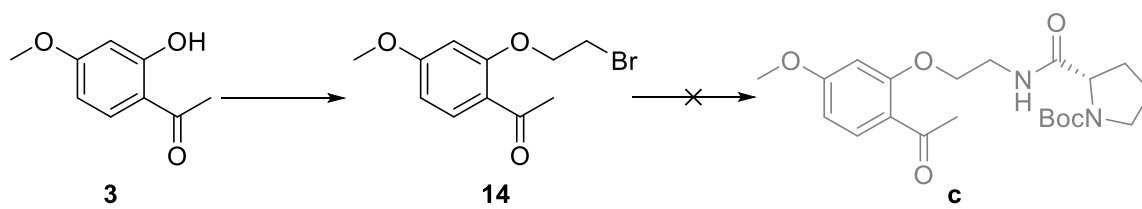


A.2. Synthesis of proline and diketopiperazine derivatives of propafenones and 2-ethoxyacetophenones

A.2.1. Attempts at the synthesis of proline derivatives of propafenone derivatives and 2-ethoxyacetophenones



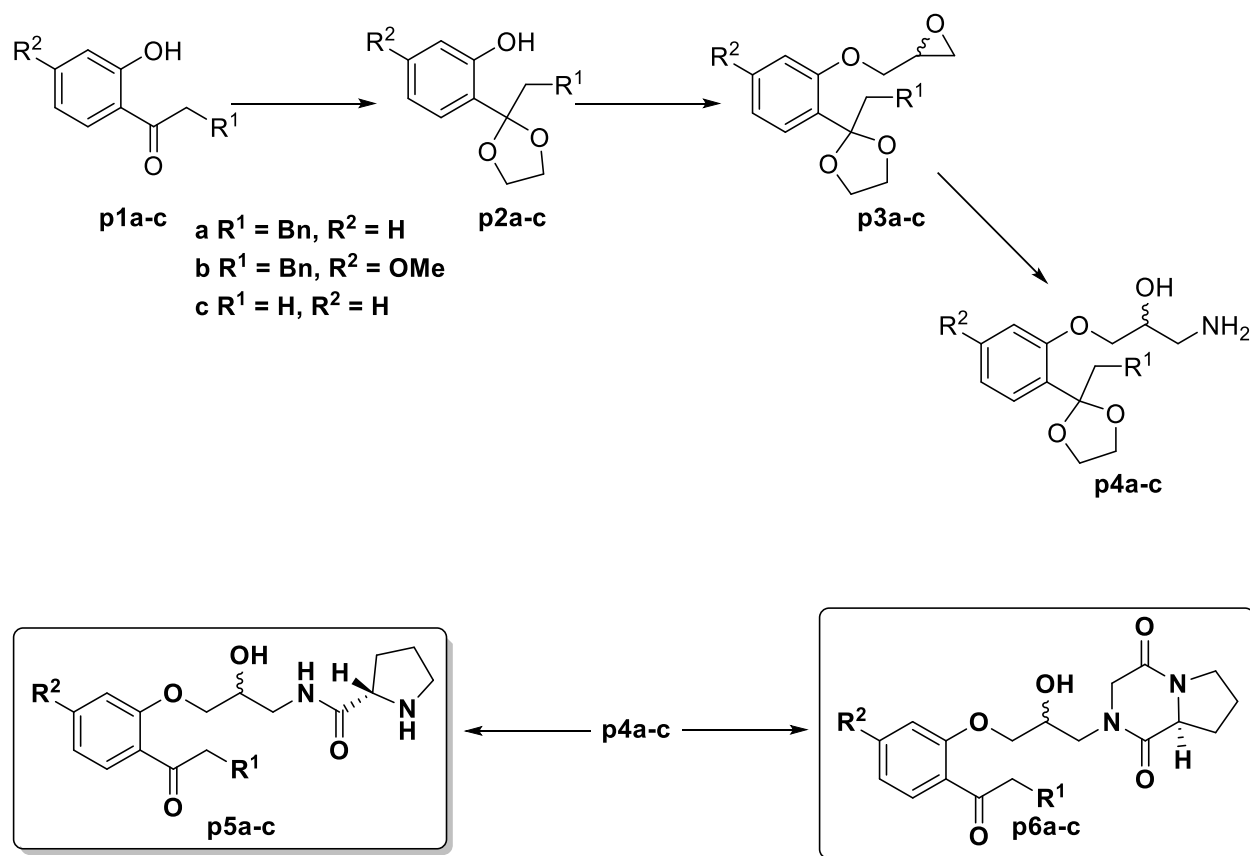
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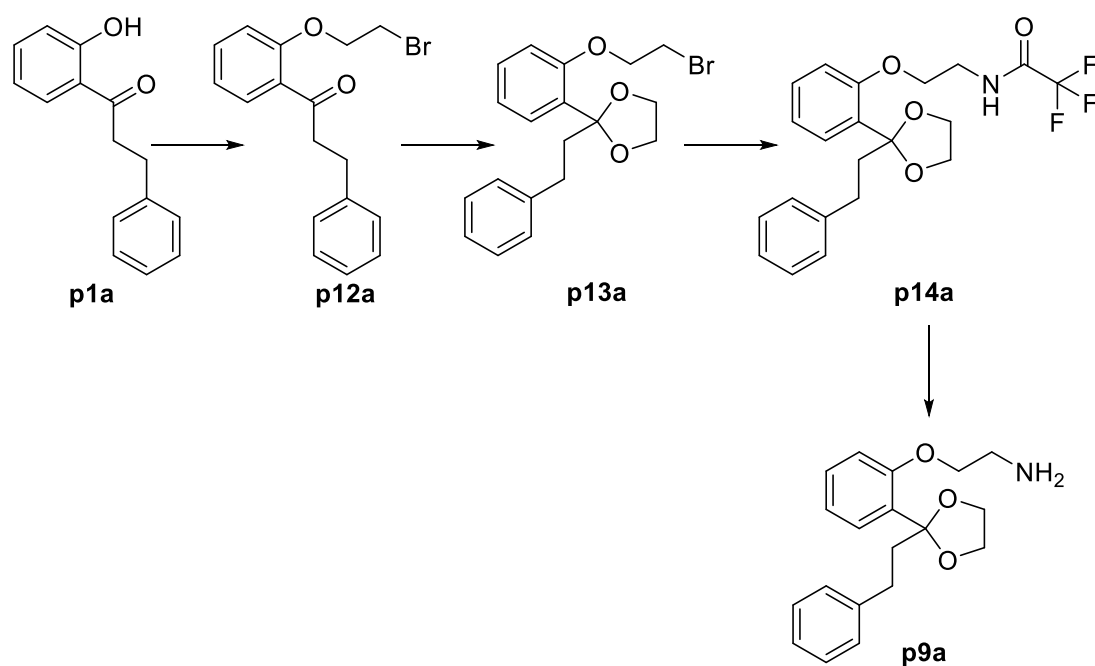
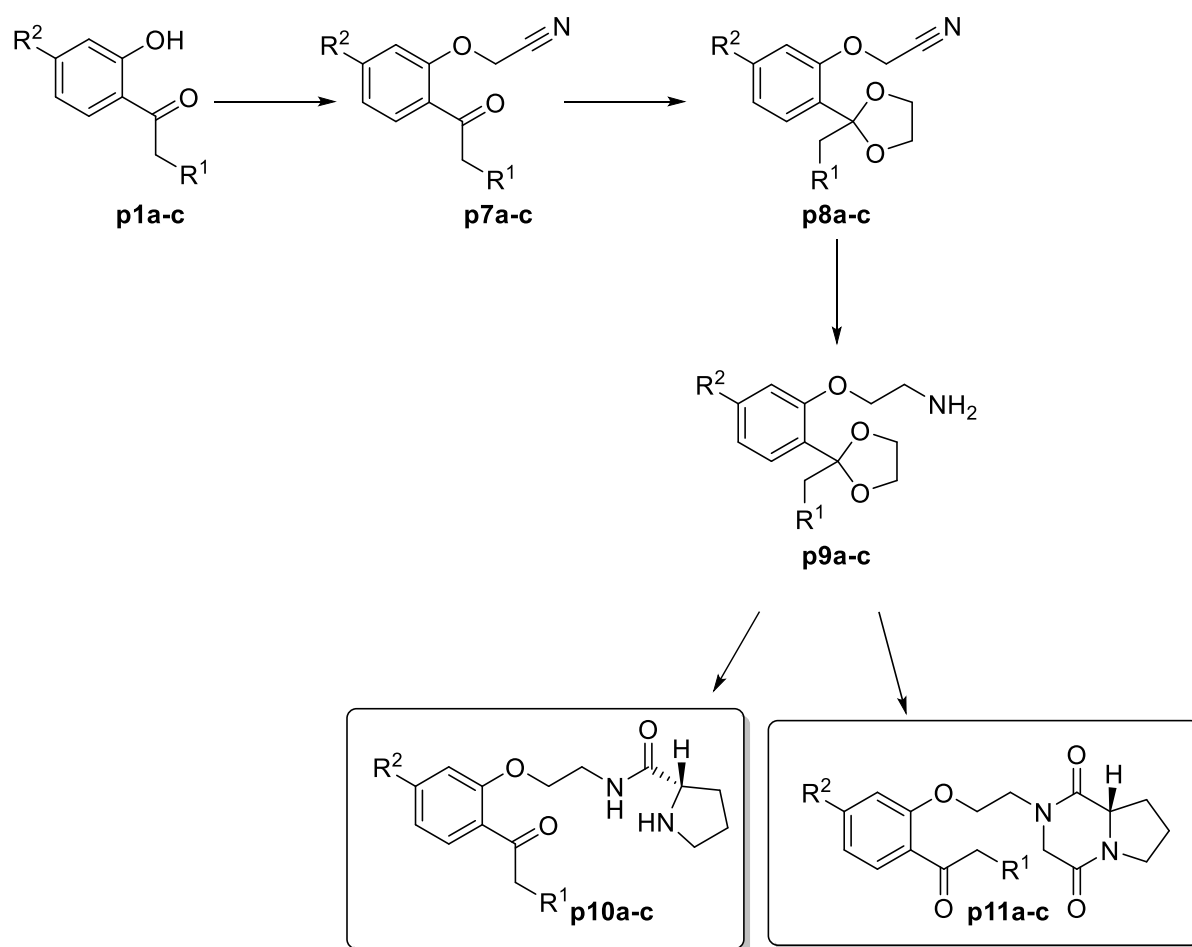
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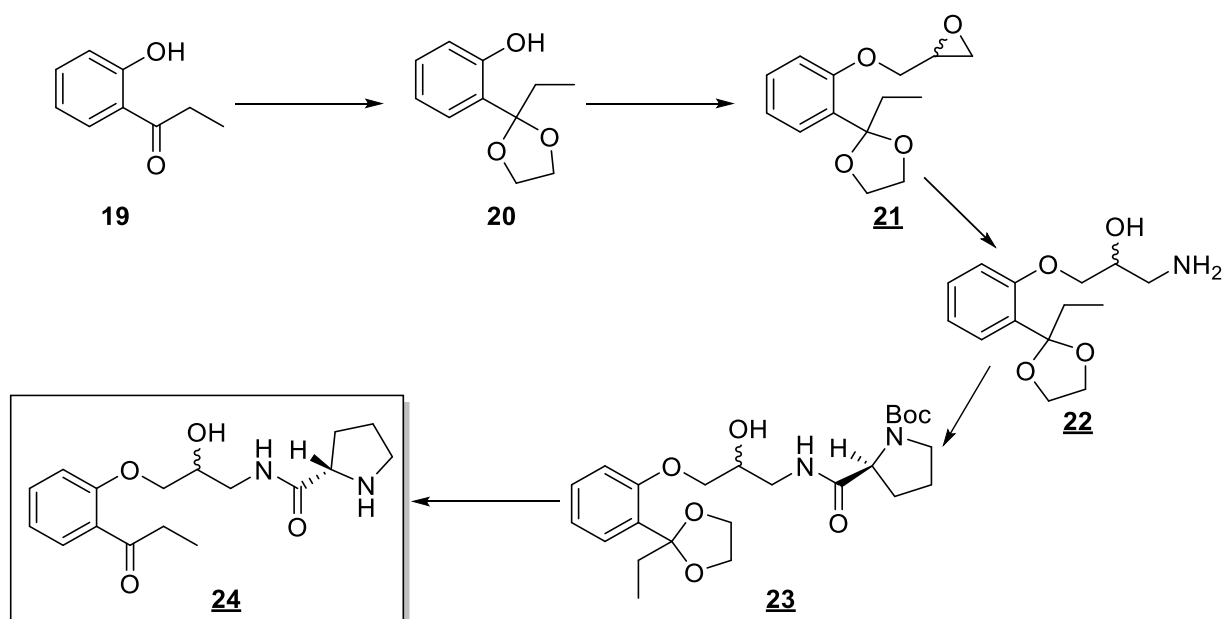
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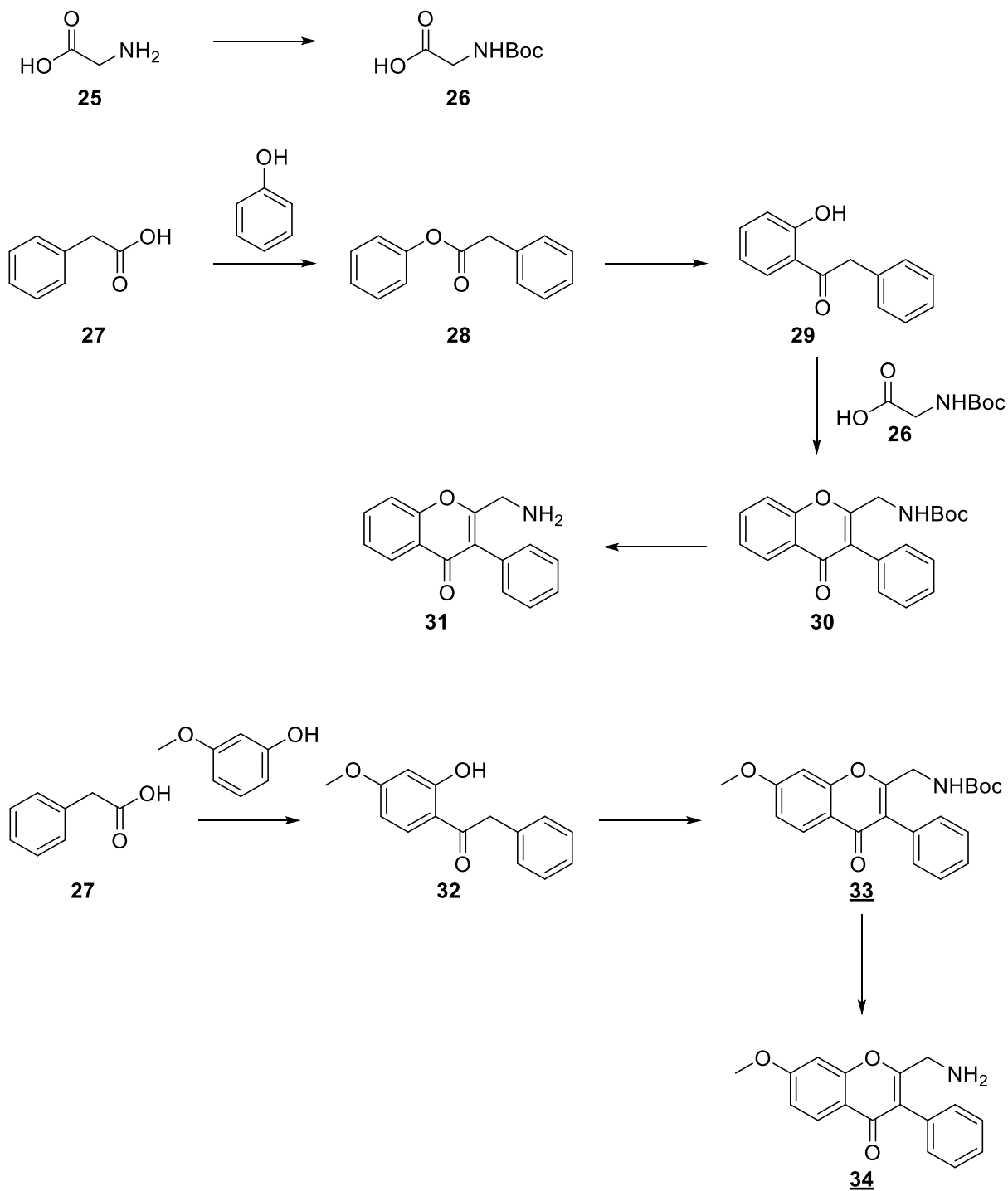


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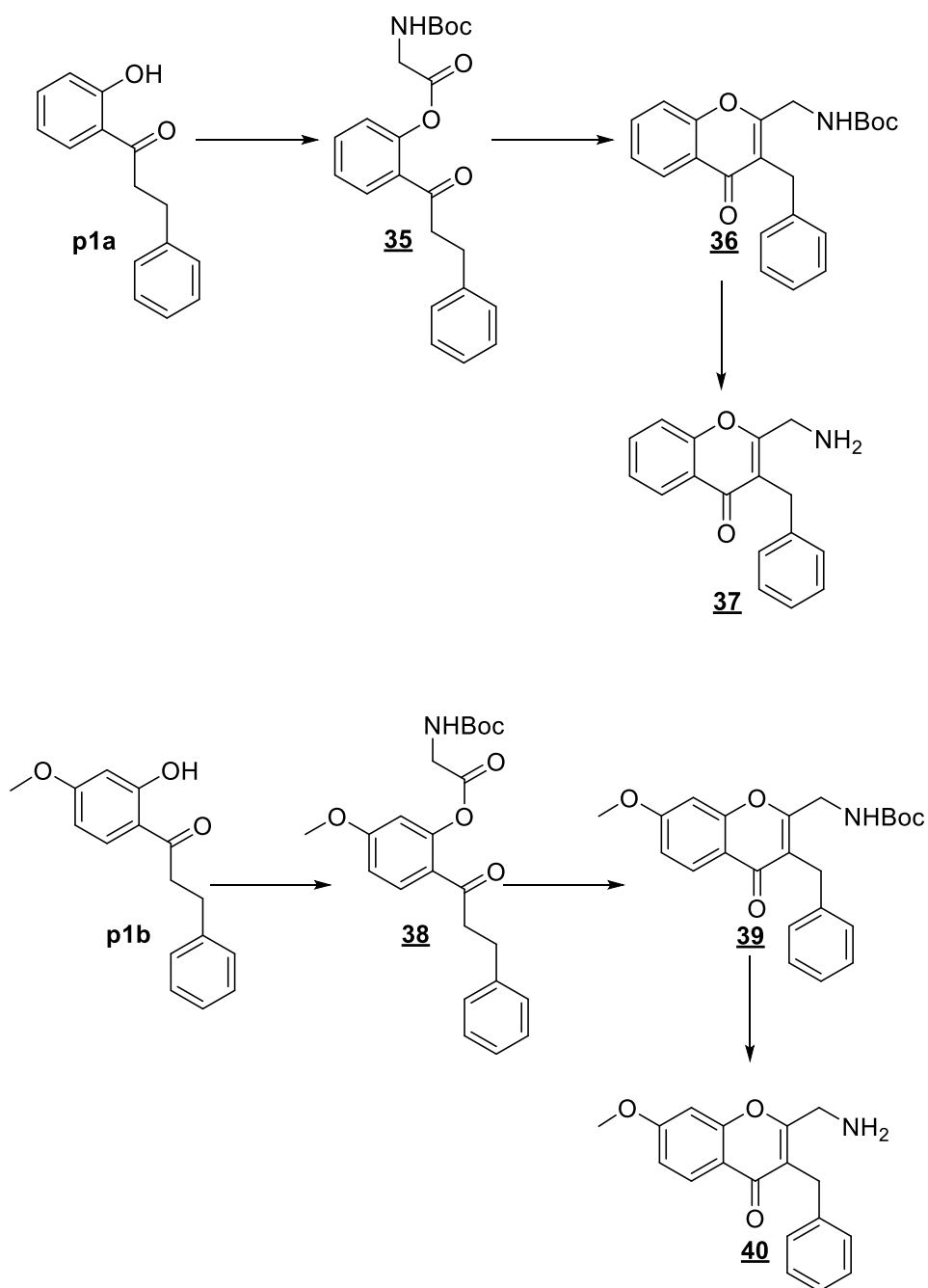


A.3. Synthesis of chromone derivatives

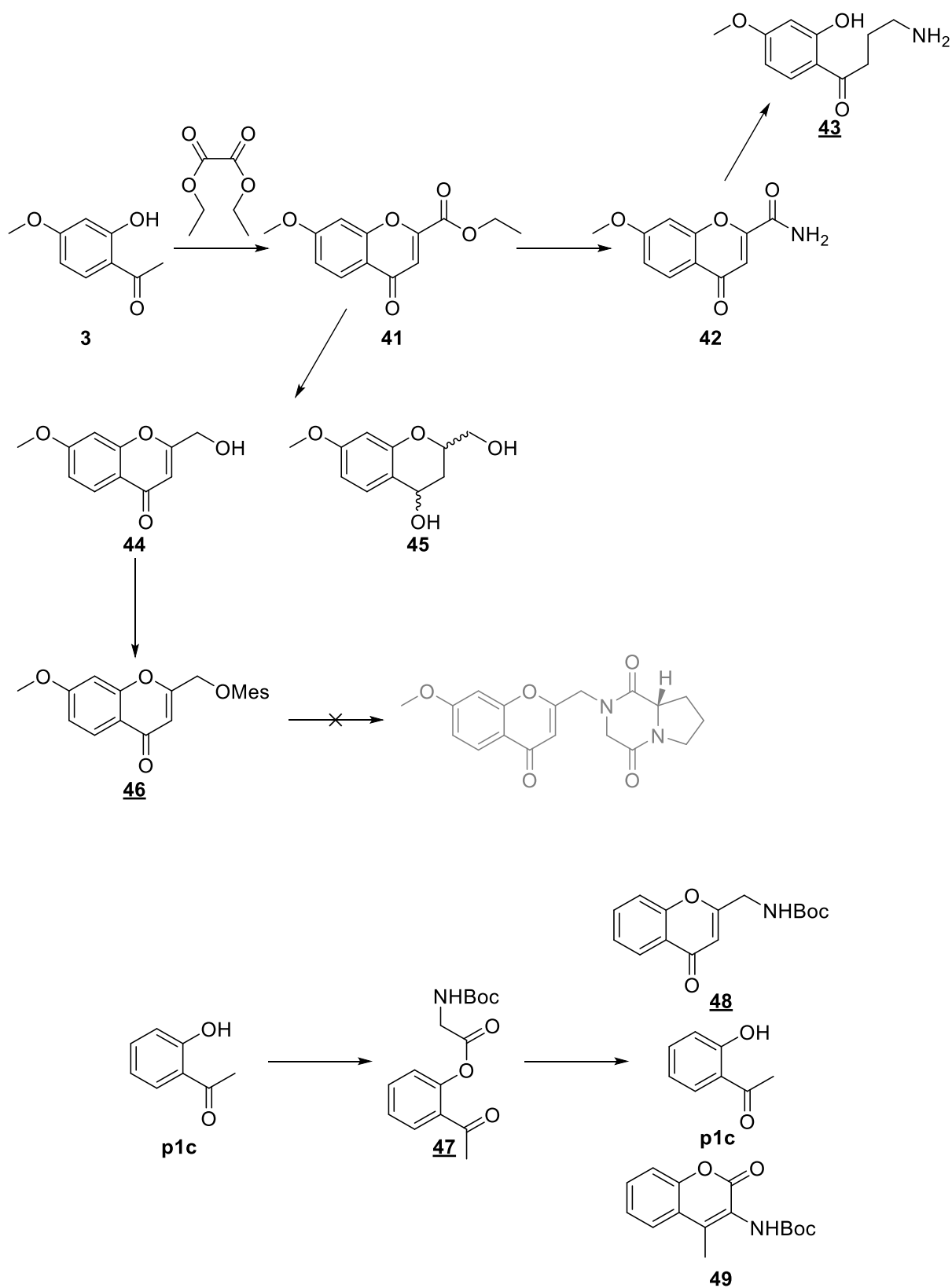
A.3.1. Synthesis of 2-(aminomethyl)-3-phenyl-4H-chromen-4-one and 2-(aminomethyl)-3-phenyl-7-methoxy-4H-chromen-4-one



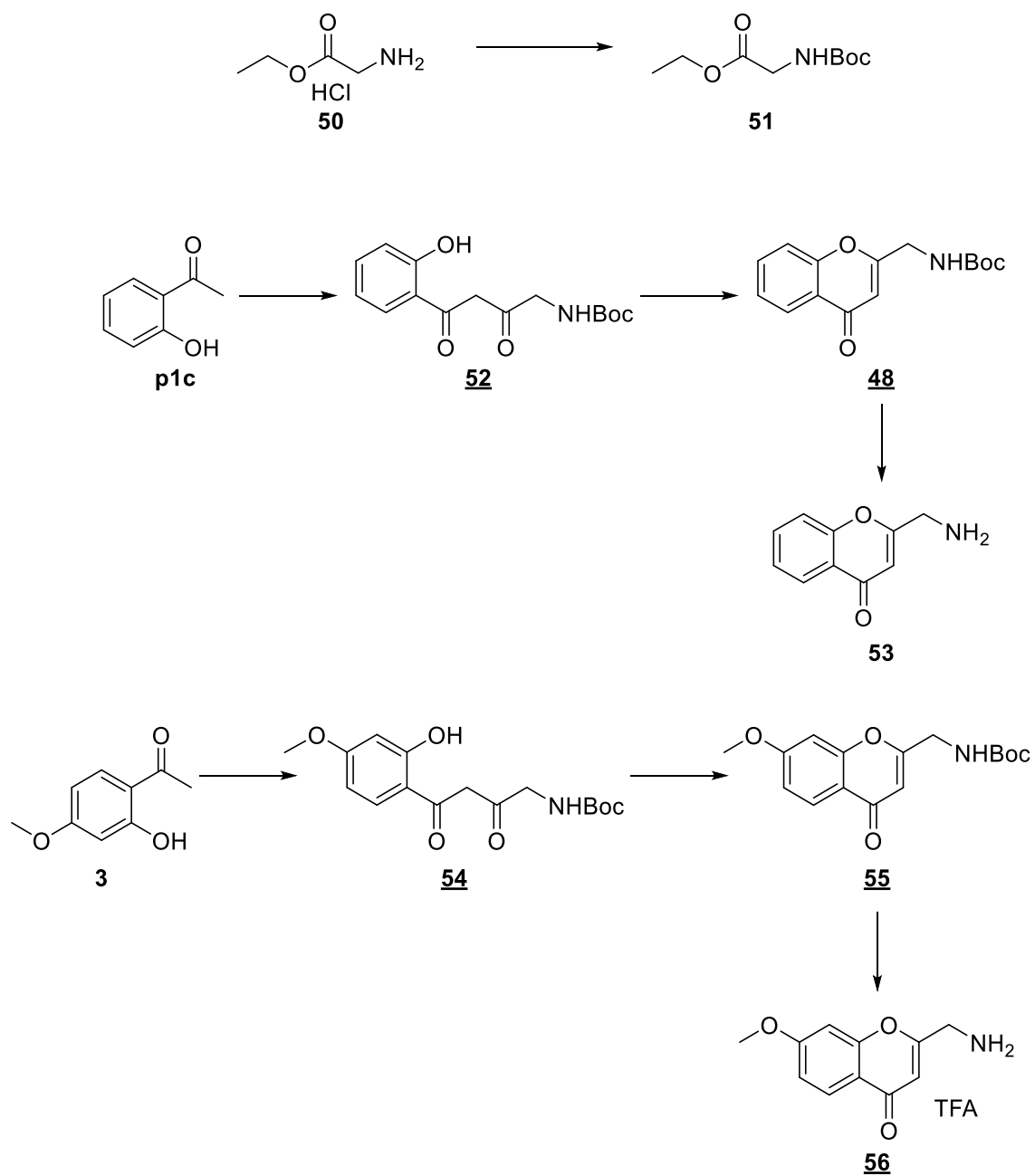
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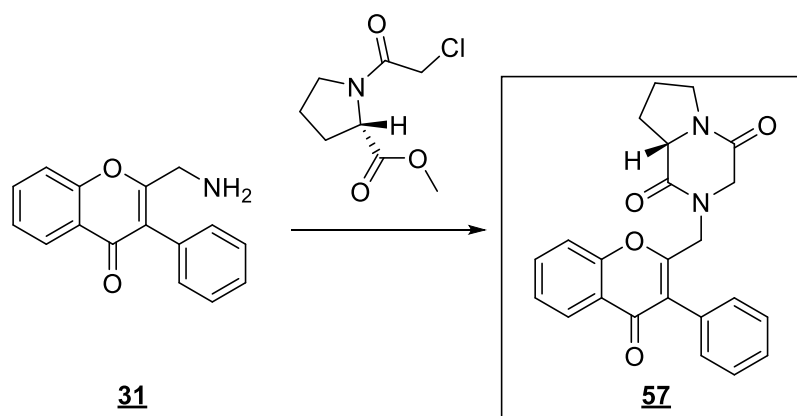
A.3.3. Synthesis of 2-(aminomethyl)-4H-chromen-4-one and 2-(aminomethyl)-7-methoxy-4H-chromen-4-one

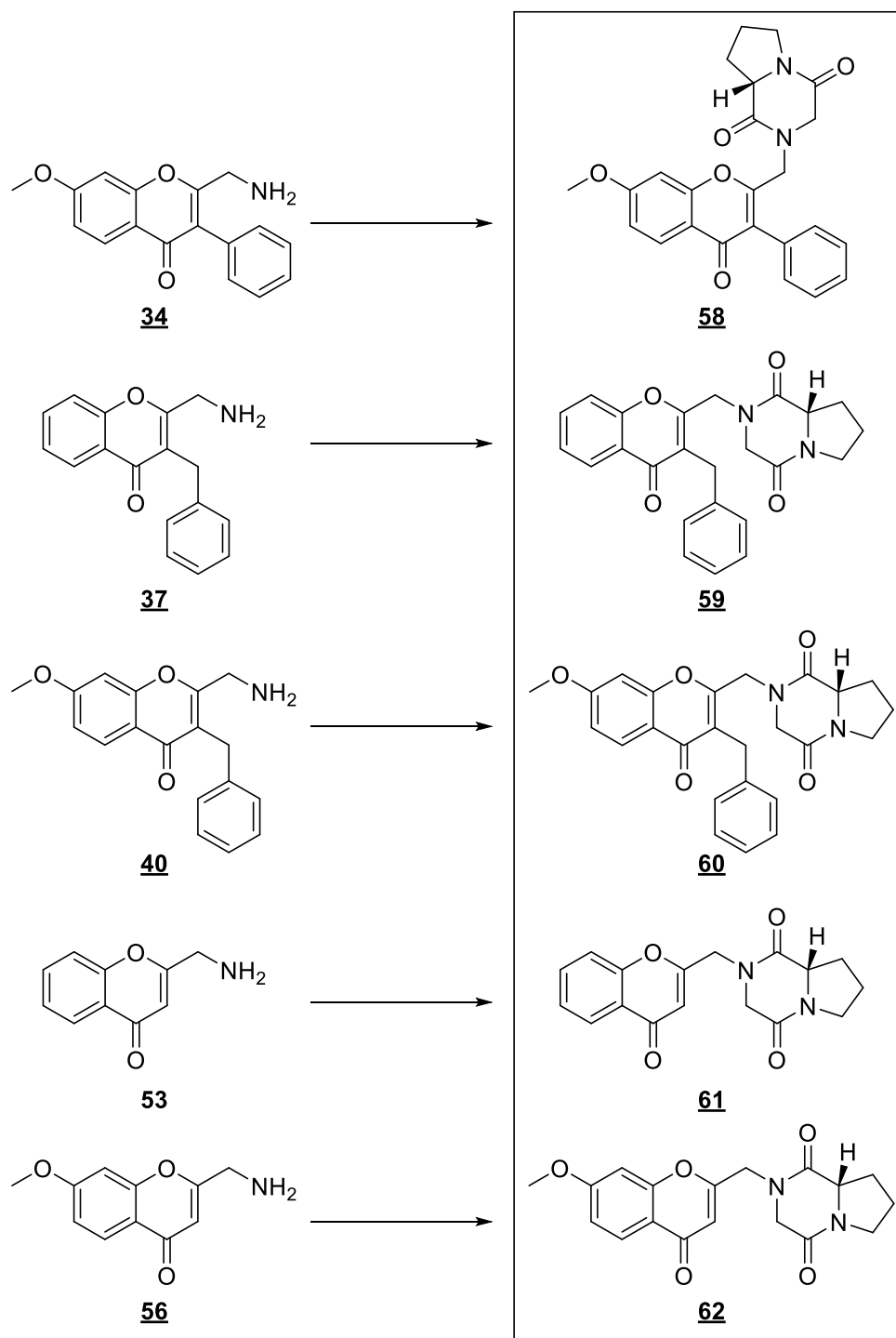


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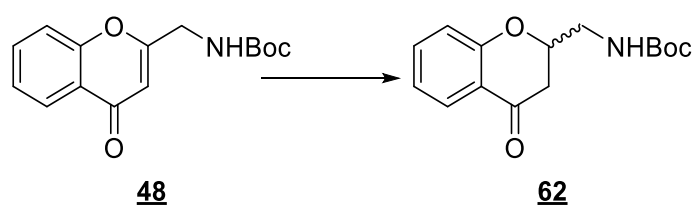


A.3.4. Synthesis of diketopiperazine derivatives of chromones

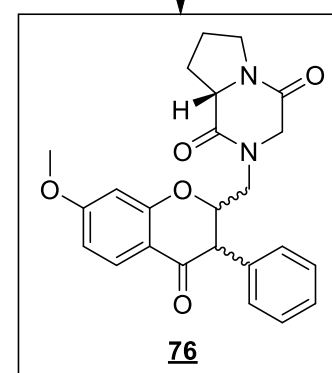
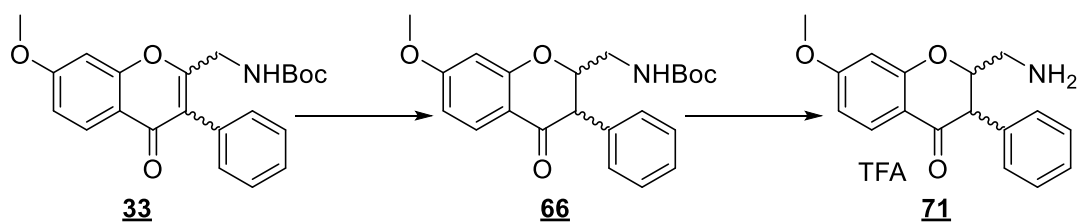
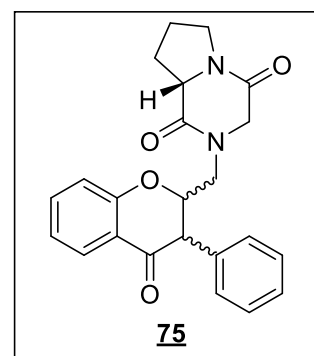
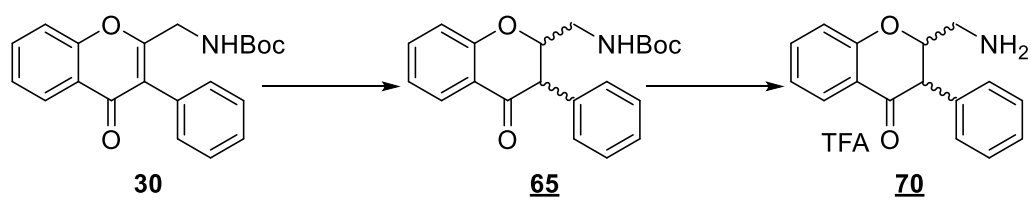
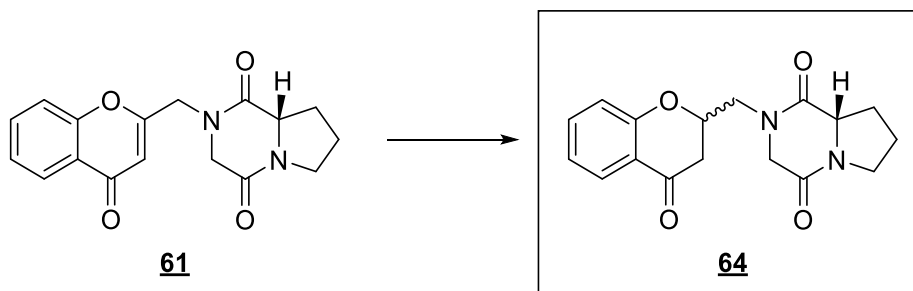




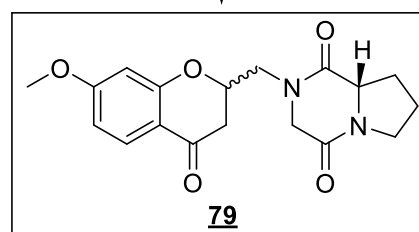
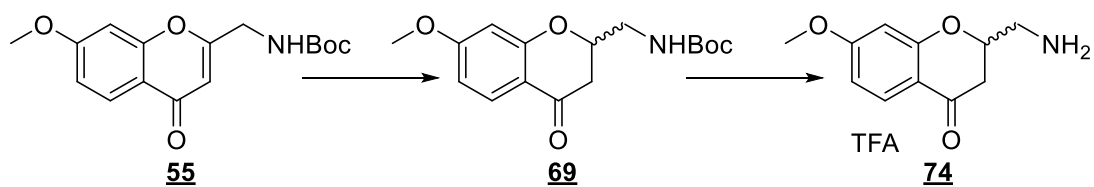
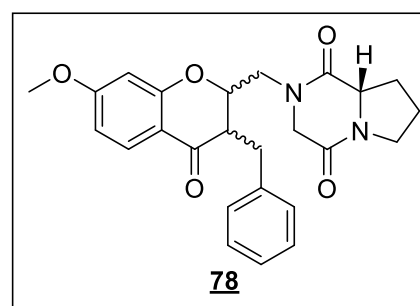
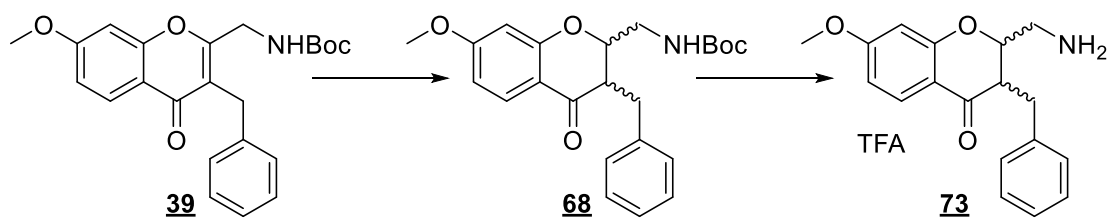
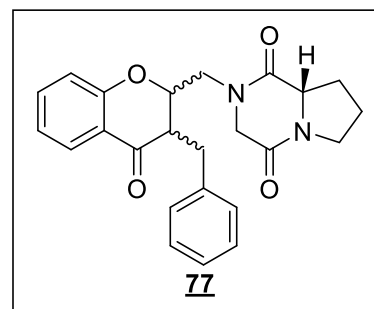
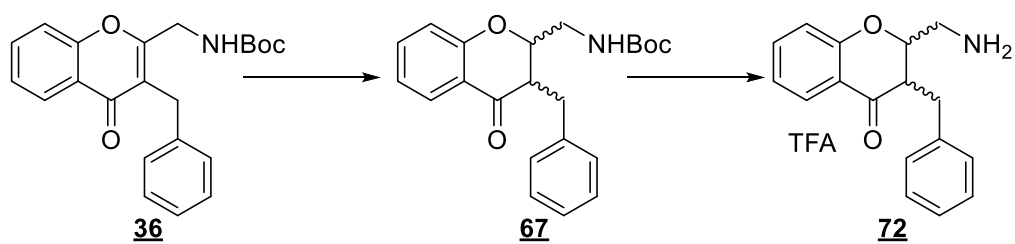
A.4. Synthesis of chromanone derivatives



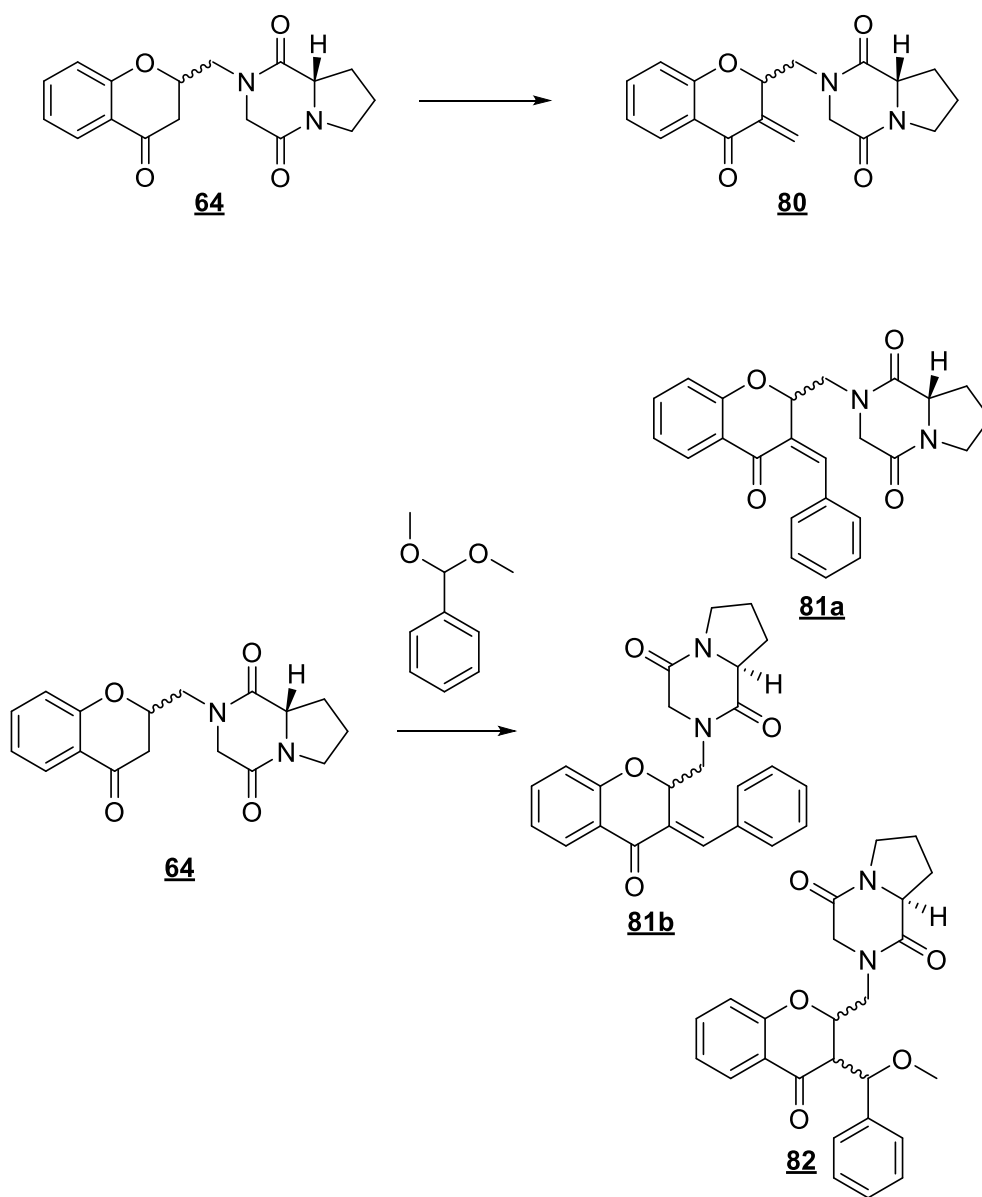
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Synthetic Schemes



A.5. Attempts at the synthesis of final target molecule G



Introduction

A.6. General motivation

ABC transporters (adenosine-triphosphate binding cassette transporters) use the energy of ATP hydrolysis for the transport of different substrates across biological membranes. Depending on their transported substrates, which range from metal ions to peptides, the functions and tissue distributions of the different proteins vary ¹. Among the most prominent representatives are P-glycoprotein (ABCB1, Pgp) and Breast Cancer Resistance Protein (ABCG2, BCRP). These two ABC transporters exhibit generally protective functions for various tissues, which is shown by their localisation at the blood-brain barrier, maternal-foetus barrier, blood-testis barrier and in the intestine and liver ². By excreting toxic substances, these transporters play a vital role in protecting different tissues. However, they transport a highly diverse range of substrates including various drugs like for example cytotoxic agents used in chemotherapy. This becomes a problem in multidrug resistant cancer cells, which can show overexpression of Pgp and/or BCRP and thus limit the amount of drug which reaches its intracellular target ³. In addition, ABC transporters like P-glycoprotein and BCRP can also be seen as antitargets, as their expression can lead to interference with the pharmacokinetics of compounds which are pump substrates ⁴.

Because of these physiological roles, it is important to identify compounds which are inhibitors and/or substrates of these transporters. So far, neither for human Pgp nor for human BCRP a high-resolution crystal structure has been published, therefore limiting the studies to homology models or ligand-based analyses. It has been shown that Pgp and BCRP show overlapping, but slightly different substrate and inhibitor patterns. Propafenone derivatives have long been known to be modulators of Pgp, and the effects of various substituents have been studied. However, it was discovered that some propafenones inhibit BCRP as well ⁵. This inhibitor overlap can be desired or undesired, in any case further investigation of this observation is needed.

A.7. ABC transporters

ABC transporters (adenosine-triphosphate binding cassette transporters) are transmembrane proteins involved in the ATP-dependent transport of different substrates across biological membranes. They exist across all domains of life, indicating their various physiological roles. Mutations in genes responsible for ABC transporters result in or play a role in many human genetic disorders like for example cystic fibrosis, cholesterol and bile transport defects or neurological issues ¹. By binding ATP (adenosine triphosphate) and using the energy gained from hydrolysis, a wide range of substrates (e.g. sugars, amino acids, glycans, sterols, phospholipids, peptides, proteins, toxins, antibiotics and xenobiotics) can be transported across cytoplasmatic membranes as well as intracellular membranes of the endoplasmic reticulum (ER), peroxisome, and mitochondria ^{1, 6}. While most members of the superfamily facilitate the ATP-driven transport of substrates, some also function as receptors or ion channels or mediate other physiological phenomena like the control of translation and DNA repair ⁷. It is characteristic of the transporters to always be unidirectional; however, in

bacteria, they can be either importers or exporters, while in eukaryotes only exporters are observed ⁸.

Regarding their structure, common features were identified for all ABC transporters. The core parts are transmembrane domains (TMDs) with between 4 and 10 α -helices, and ATP binding and hydrolysis domains located in the cytosol (nucleotide binding domains, NBDs). An active prokaryotic or eukaryotic transporter has to consist of two TMDs and NBDs each. To achieve this minimum requirement, one single polypeptide chain (full transporter) can suffice, or two equal (homodimeric) or unequal (heterodimeric) polypeptide chains can be assembled. One transport protein can be assembled from one, two, three or four separate polypeptides. The existence of an additional TMD or soluble regulatory domains associated with the NBDs has been observed for some ABC transporters ^{8b}. While the amino acid sequence of the NBDs is highly conserved throughout the ABC transporter family, the TMDs show variations allowing for different substrates to be transported ⁶.

In the human genome, 49 ABC genes could be found, which are grouped into seven subfamilies (named ABCA to ABCG) according to their amino acid sequence and structure homology. The TMDs from various subfamilies of ABC transporters are not homologous, thus explaining the different substrate specificity of different transporters. In contrast, the NBDs are homologous throughout the family ^{2,9}.

A.7.1. P-glycoprotein (Pgp, ABCB1)

ABCB1 is also known as permeability-glycoprotein (Pgp) and belongs to subfamily B of the ABC transporter family. It has a molecular mass of 170 kDa ¹⁰. Pgp was one of the first ABC transporters known in humans. The phenomenon leading to its discovery was that colchicine-resistant Chinese hamster ovary cells also showed cross-resistance to various other cytostatic drugs. Originally, it was believed that a membrane alteration led to a reduced rate of permeation ¹⁰⁻¹¹. However, a cell surface glycoprotein was found by Juliano and Ling in 1976 which only occurred in drug-resistant cells ¹⁰. 10 years later, Roninson *et al* were able to clone the gene which encodes Pgp named *mdr1* ¹². Structurally, Pgp is a full transporter consisting of two bundles of six transmembrane helices and two cytosolic nucleotide-binding domains ¹³.

A model of Pgp based on the elucidated murine crystal structure, which shows 87% sequence identity to human Pgp ¹³, is depicted in figure B-1 ¹⁴. Substrate transport starts with a substrate (magenta) entering the membrane and afterwards diffusing from the inner leaflet of the bilayer into the drug-binding pocket of the transporter through a portal. In the next step, ATP (yellow) binds to the nucleotide-binding domains, which leads to a conformational change in which the drug-binding pocket is opened to the extracellular space and/or outer leaflet of the membrane bilayer ¹³⁻¹⁴. ATP binding probably causes a dimerization in the NBDs, which is disrupted again by ATP hydrolysis, resetting the system back to its inward facing state (A) ¹³.

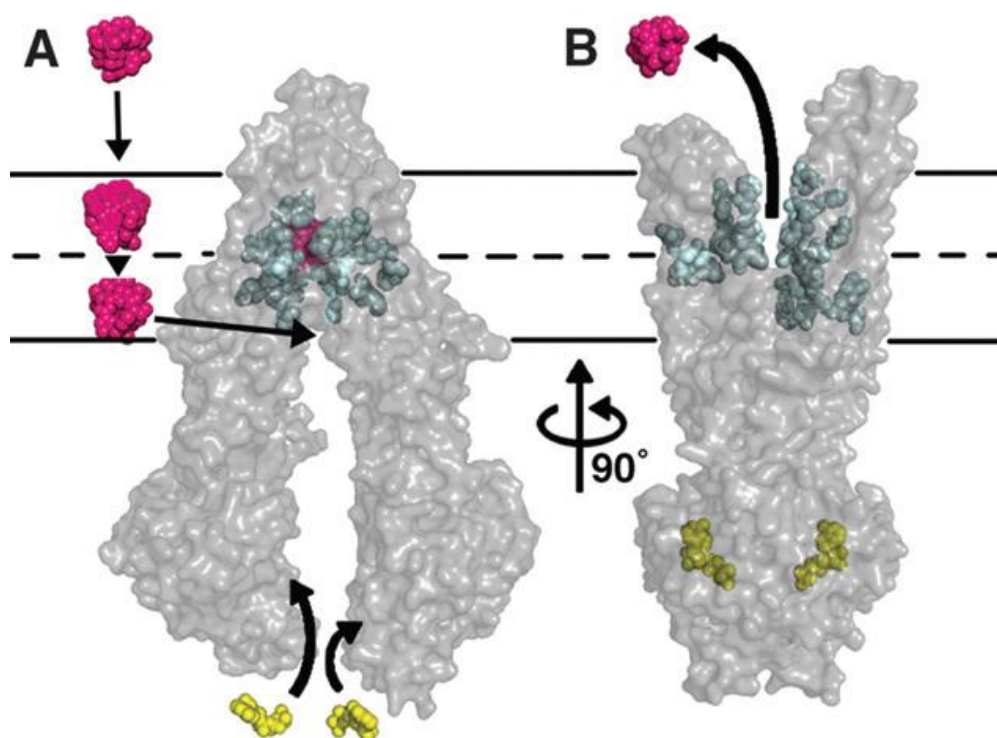


Figure B-1: Model of Pgp depicting substrate transport (magenta: substrate, yellow: ATP, blue: drug-binding pocket), source: ¹³⁻¹⁴, reprinted with permission from AAAS.

Regarding to its expression in normal human tissues, Pgp was found in specialized epithelial cells with secretory/excretory functions, trophoblasts in the placenta, and on endothelial cells of capillary blood vessels at blood-tissue barrier sites ¹⁵. In more detail, this includes liver cells (at the canalicular surface of hepatocytes), pancreas, kidney and the apical surface of mature enterocytes in the small intestine and colon. Additionally, ABCB1 was found in testes, in the brain on the luminal surface of capillaries and at the choroid plexus, on various leukocytes including T and B lymphocytes, and on dendritic cells ^{8b, 9}. This specific tissue expression underlines the importance of Pgp for the maintenance of protective barriers. In the intestine, it limits the absorption of xenobiotics, and in the biliary tract and kidney it takes part in the elimination of metabolic waste products and toxins. ABCB1 is also of key importance for the maintenance of the blood-testis, maternal-foetal, blood-brain, and blood-cerebrospinal fluid barriers ^{9, 16}. In addition, Pgp expression was found in many cancers, including tumours of the lung, genitourinary system, gastrointestinal tract, hematopoietic system, as well as childhood cancers and has been linked to negative clinical outcomes ³.

So far, no high-resolution crystal structure of human Pgp is published, however, there is the structure of murine Pgp (apo and drug-bound, inward-facing) ¹³ and *Staphylococcus aureus* Sav1866 (ATP-analogues bound, outward-facing) ¹⁷, based on which homology models are built (e.g. ¹⁸).

ABCB1 is known to pump many endogenous substances from the cytoplasm or cell membrane to the extracellular space. Pgp shows an overlap in substrate and inhibitor specificity with cytochrome P4503A4/5, although the overlap is not absolute ¹⁹. Pgp substrates include a number of drugs used in cancer chemotherapy (cytotoxic drugs like taxol, actinomycin D,

vinblastine and anthracyclines), immunosuppression (e. g. cyclosporine), protease inhibitors for the treatment of HIV infection (amprenavir), heart conditions (cardiac glycosides like digoxin), hypertension (calcium channel blockers like verapamil and diltiazem), allergy (second-generation antihistamines like cetirizine ²⁰), infection and inflammation (e.g. glucocorticoids like dexamethasone) ^{19a}, but also steroid hormones (e.g. aldosterone, estradiol-17 β -D-glucuronide), bilirubin and naturally occurring toxins and xenobiotics (e.g. colchicine) ^{19a, 21}.

A.7.2. Breast cancer resistance protein (BCRP, ABCG2)

ABCG2, which is also known as BCRP (Breast cancer resistance protein), MXR (mitoxantrone resistance protein) or placental ABC protein (ABCP1), is a half transporter protein with a mass of 72 kDa ^{16, 22}. BCRP was nearly simultaneously discovered by three research groups in 1998. Allikmets *et al* found it in placenta ²³, Doyle *et al* found it in MCF-7/AdrVp, a multidrug-resistant human breast cancer subline which lacked known multidrug resistance transporters such as P glycoprotein or the multidrug resistance protein ²⁴, and Miyake *et al* found it in a gene cloned from the mitoxantrone-selected colon carcinoma cell line S1-M1-80 ²⁵. In order to be functional, BCRP has to form homodimers/homooligomers. In contrast to Pgp and most other ABC proteins, it shows a reverse configuration with an N-terminal ATP-binding domain and a C-terminal transmembrane domain consisting of six helices ²⁶.

BCRP is found in different tissues of the human body, with its distribution being similar to the one of Pgp ¹⁶. High levels of ABCG2 are expressed in the placenta (especially in the syncytiotrophoblasts), central nervous system, liver, colon, biliary canaliculi, venous endothelium and capillaries, alveolar pneumocytes of the lung, breast, adrenal gland, sebaceous glands, prostate, testes and uterus, whereas lower levels are found in the brain (at the blood-brain barrier), prostate, small and large intestine, stomach, kidney and ovary ^{24, 26-27}. As for Pgp, the tissue localisation indicates an important role at the barrier tissues of the body, where its transport activity has a major influence on the ADME-Tox (absorption, distribution, metabolism, excretion and toxicity) properties of numerous pharmacological agents and natural compounds ²⁸.

Apart from its expression in normal tissues of the body, like Pgp, BCRP is also found in cancer cells, for example Ross *et al* found it in 33% of 21 patients with acute myelogenous leukaemia (AML) ²⁹. Nakanishi *et al* obtained similar results for blast cells from AML patients and could link BCRP mRNA expression to *in vitro* resistance to the cyclin-dependent kinase (CDK) inhibitor flavopiridol ³⁰. BCRP was also found in other types of leukaemia like acute lymphoblastic leukaemia (ALL) and chronic myelogenous leukaemia (CML), although the data is more limited and no clear connection between BCRP expression and poor prognosis could be drawn ³¹. In general, expression of Pgp and BCRP seems to be higher in leukaemia stem cells ³² and in primitive cell populations in CML ³³. Also in solid tumours, BCRP has been reported to be widely overexpressed ³⁴. While expression was found in all tumour types, it

appeared to be more frequent in adenocarcinomas of the digestive tract, endometrium, and lung, and melanoma.

Owing to the difference in structure from many other ABC transporters described above, structural predictions and homology models for BCRP are even harder to obtain and less frequently described than for Pgp (e.g. ³⁵). Only last year, the structure of a G-family heterodimer, namely the human sterol transporter ABCG5/ABCG8, was determined in a lipid bilayer by X-ray crystallography (nucleotide-free, inward-facing) ³⁶. A new transporter fold, which seems to be unique for ABCG proteins, could be discovered, and a new homology model for ABCG2 was built based on this structure ³⁷. Two out of the six equilibrated conformations together with docked substrates and identified sites are shown in figure B-2. Both substrates and non-substrates could dock at site 1 (blue), whereas solely substrates bound at site 2 (red). The central site 3 (yellow) is located between the two monomers. Evidence for a potential off-site at the extracellular part was found as well (site 4, magenta). Docking poses of sulfasalazine are depicted in the case of two ABCG2 conformations ³⁷.

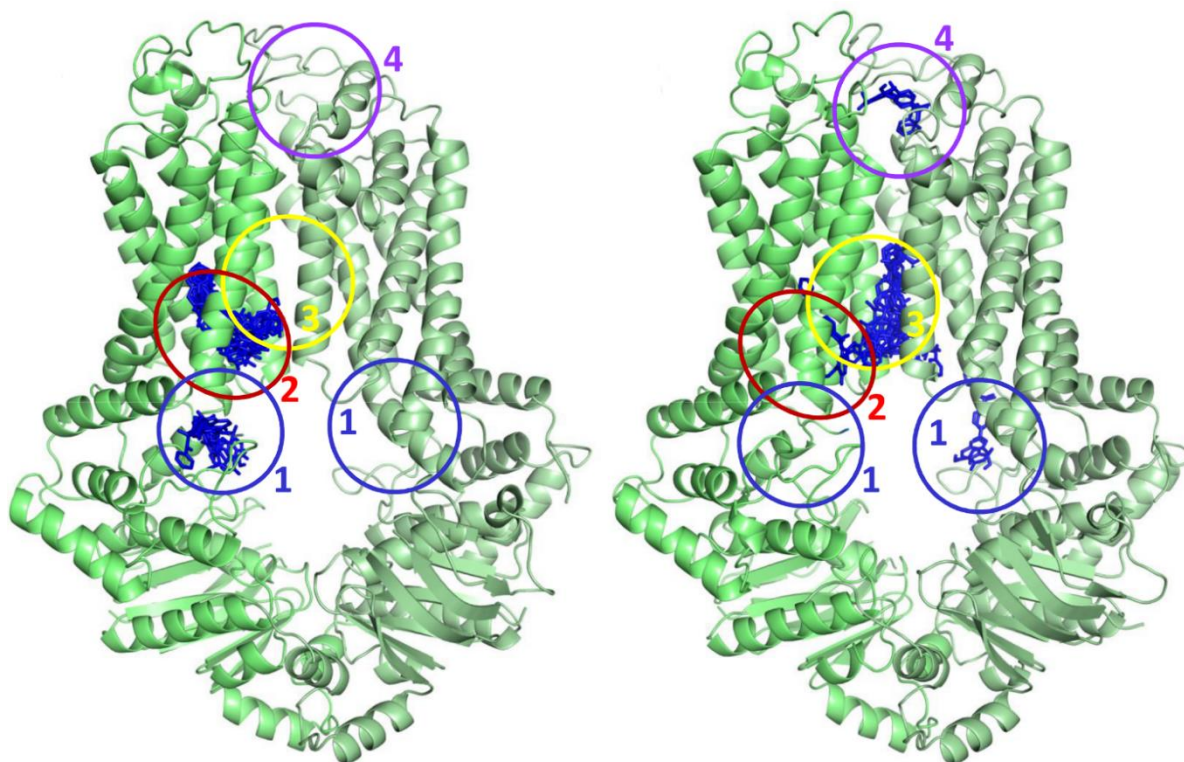


Figure B-2: Homology model of BCRP by László *et al* with docked substrates showing a potential substrate pathway (2 conformations) ³⁷: Site 1 (blue) – both substrates and non-substrates could dock, Site 2 (red) – only substrates could bind, Site 3 (yellow) – central site, Site 4 (magenta) – potential extracellular off-site

Substrates of BCRP are structurally diverse and interact with different targets. Among these are many naturally occurring toxic xenobiotics, for example dietary mutagens and carcinogens like heterocyclic amines and polycyclic aromatic hydrocarbons, but also mycotoxins like aflatoxin B1. In addition, BCRP also transports folic acid and conjugates of steroid hormones. Like Pgp, BCRP also transports many approved chemotherapeutics like antifolates (e.g. mitoxantrone), tyrosine kinase inhibitors (e.g. imatinib and gefitinib), anthracyclines (e.g. doxorubicin, daunomycin) and topoisomerase I inhibitors (e.g. topotecan and irinotecan) ^{31a}.

A.8. Multidrug resistance

Long before the first transporter genes were cloned, the observation was made that incubation of cancer cell lines with chemotherapy agents led to sublines which were not only resistant to the selecting drug, but also to other cytotoxic agents of different structures²⁶. This ability of cells or organisms to resist treatment by various structurally unrelated drugs is known as multidrug resistance (MDR). Naturally, it can lead to serious problems in the treatment of cancer or infectious diseases and is often linked to poor therapeutic prognoses. There are different mechanisms responsible for MDR including the efflux of the administered drug, the inhibition of drug uptake into the cell, or the alteration of pathways leading to apoptosis³⁸. In cancer, the overexpression of ABC transporters like ABCB1, ABCG2 or ABCC2 (multidrug-associated protein, MRP2) can cause MDR by efflux of administered chemotherapeutics, leading to a decreased intracellular drug concentration. As a result, the antineoplastic agents cannot reach their intracellular target molecules and cannot interact with them³⁹. Cancer cells can be intrinsically resistant owing to their original genetic make-up, or acquire resistance in response to exposure to anticancer agents during treatment. Studies demonstrate a connection between ABC transporter expression and poor treatment outcome in several cancer types¹⁴.

Knowing the mechanism of action of ABC transporters in MDR, co-administration of an ABC transporter inhibitor with anticancer drugs to improve drug response seems straight-forward. Although combined therapies showed some encouraging clinical results, to date no effective MDR reversing agent without toxic effects has been found and approved⁴⁰. First-generation MDR inhibitors like verapamil and cyclosporine A were recognized to be also substrates of Pgp and, as they were not specifically developed for the modulation of ABC transporters, had their own pharmacodynamic effects. Combining these first-generation inhibitors with anticancer drugs like mitoxantrone and daunorubicin caused toxic side effects and only limited function or no benefits⁴⁰⁻⁴¹. Second-generation MDR inhibitors were optimized to increase the inhibitory effects and decrease unwanted toxicities, leading to compounds like PSC-833 and VX-710. However, also for these compounds co-administration with chemotherapeutic drugs was not successful as they increased the systemic exposure to anticarcinogens due to an alteration of their ADME, which led to more toxicity in cancer patients^{40, 42}. Co-administration of VX-710 could not cause a distinct increase in the effect of anticancer drugs, which leads to the conclusion that also other mechanisms apart from the expression of ABC transporters must be relevant in MDR⁴³. Third-generation inhibitors led to an enhancement of chemosensitivity in *in vitro* studies compared to their predecessors, however their effect on overall survival rates of patients remained limited as well. In spite of the fact that neither zosuquidar (LY335979) nor tariquidar (XR9576) increased toxicity, neither of them could lead to prolonged overall survival of patients with AML or metastatic cancers^{40, 44}.

Although the search for ABC transporter inhibitors successful in the reversal of MDR in the clinic has not been very fruitful, the added interest in these proteins has definitely been helpful and has driven researchers to pay more and more attention to chemosensitizer properties of

novel drugs. For example, many tyrosine kinase inhibitors (TKIs) are also Pgp and BCRP inhibitors ⁴⁵. It has been shown that they can enhance the efficacy of conventional chemotherapeutic drugs, for example nilotinib could, combined with paclitaxel or doxorubicin, significantly decrease the size of tumours which overexpressed Pgp or BCRP in mice xenograft models ⁴⁶. Other novel approaches at reversing MDR linked to ABC transporters include nanoparticle formulations of chemotherapeutic drugs and ABC transporter inhibitors ⁴⁷, targeted downregulation of MDR genes by small molecule inhibitors, natural drugs like for example curcumin, RNA interference, epigenetic regulation as well as studies of signal transduction pathways ⁴⁰.

A.9. ABC transporters at the blood-brain barrier (BBB)

Multiple ABC transporters, including Pgp and BCRP, are found in the brain capillary endothelium that makes up the blood-brain barrier ⁴⁸. This barrier controls the transcellular flux of small molecules. Tight junctions force all solutes to take the transcellular route ⁴, which has major implications for the development of CNS-active drugs (central nervous system). These drugs need to cross the BBB and should therefore not be transported by Pgp and/or BCRP. This constitutes a major problem for the treatment of brain tumours because most anticancer drugs are substrates of Pgp and BCRP ⁴⁹. However, inability to interact with CNS targets can also be a desired feature of drugs, which then are designed to favour their Pgp substrate properties. An example are antihistamines, where the first generation of compounds displayed CNS-related side effects like dizziness. In contrast, the optimized drugs of the third generation, such as fexofenadine, lack CNS side effects because of their Pgp substrate properties ^{4, 50}. It has been shown by Bauer *et al* that there is a functional interplay between Pgp and BCRP at the BBB, as only moderate increases in the brain distribution of dual Pgp/BCRP substrates were observed when Pgp alone was inhibited ⁵¹. Drug-drug interactions caused by Pgp and BCRP can be an important issue at the blood-brain barrier, which will be explained in further detail in the next section.

A.10. ABC transporters as antitargets

As described above, several inhibitors of Pgp were subject to clinical studies, however none of them has been approved so far. The question, if Pgp is a druggable target, is therefore still unanswered. However, P-glycoprotein's potential role as an antitarget has reached more and more attention ⁴. This means that if Pgp is affected by a drug, it can cause undesirable side-effects. These properties of ABC transporters have been widely recognized. For drug candidates, the FDA recommends standardized experiments assessing the probability of a compound to interact with P-glycoprotein or BCRP ⁵². As potential drugs can be both substrates and inhibitors of Pgp/BCRP, both properties need to be determined. Inhibitor activity can lead to drug-drug interactions, as the co-administration of a Pgp inhibitor with a Pgp substrate can lead to much higher blood levels of the latter and cause severe side effects ⁴. One example is loperamide, an opioid used for the treatment of diarrhoea which is normally

not active on the central nervous system because it is a substrate of Pgp. Co-administration of a Pgp inhibitor like for example quinidine or ritonavir can lead to serious side effects ⁴.

A.11. Molecular basis for polyspecificity in mechanisms of transport and inhibition

The ability of Pgp to interact with a wide variety of substances was discovered early on ⁵³. Among other hypotheses, the most plausible one seems to be that multiple binding sites exist in the drug-binding pocket of Pgp. Evidence for this theory was generated for example by Pascaud and colleagues ⁵⁴, who measured ATPase activity in the presence of one or more Pgp modulators and demonstrated that some of them could interact simultaneously with the transporter, indicating that there are several distinct binding sites. However, these binding sites could be sterically close as interactions in ATPase activity were observed. Also the X-ray structure of murine Pgp shows that different cyclic peptide inhibitors of Pgp bind in different positions ¹³. The presence of additional allosteric regulatory sites distinct from sites needed for transport seems likely, supported for example by the findings of Martin *et al* ⁵⁵, showing that adding SR33557 increased the ability of verapamil to modulate vinblastine transport. In another study, Martin *et al* ⁵⁶ concluded the existence of at least four distinct drug binding sites categorized as transport and regulatory sites. However, it was observed that some modulators, which were not transported, interacted at a transport rather than at a regulatory site. Pajeva and co-workers ⁵⁷ propose binding sites for rhodamine 123 and prazosine (regulatory site) at the TMD-NBD interface and a binding site for rhodamine 123 in the TMD region. When the transport cycle is considered, it is necessary that substrate binding sites change between high- and low-affinity conformations in order to move a drug, which may be the result of stimuli like drug binding and/or ATP hydrolysis ⁵⁶.

With respect to inhibitors, these can block the transport function in summary by blocking the drug binding site competitively, non-competitively or allosterically. In addition, they can affect ATP hydrolysis ⁵⁸ (flavonoids like quercetin) or alter the assembly and structure of cell membrane lipids ⁵⁹ (non-ionic detergents). It is believed that most drugs (apart from flavonoids) inhibit Pgp function by blocking drug binding sites. However, the existence of more than one binding site renders the development of a clear and general SAR (structure-activity relationship) for substrates or inhibitors difficult. If they bind to the same binding sites, substrates and inhibitors can be treated in the same way. However, then the question is how the systems differentiates between substrates and inhibitors at the molecular level ⁶⁰. Eytan *et al* ⁶¹ propose that inhibitors, which are often more lipophilic than substrates, can easily re-enter the inner leaflet of the membrane bilayer and be transported again, thus being cycled repeatedly and limiting the transport of more hydrophilic substrates which need more time to re-enter the inner leaflet of the membrane. ATPase assays can discriminate between inhibitors, which are transported repeatedly, and non-transported inhibitors. For example, propafenone modulators lead to a concentration-dependent increase of ATPase activity, while cyclosporine A does not ⁶².

Although the majority of data was generated for Pgp, much of it can probably be extrapolated to BCRP. László *et al*³⁷ provide evidence for several binding sites along a potential transport pathway which is also supported by knowledge about mutations important for the substrate specificity of BCRP⁶³.

A.12. Inhibitors/modulators of Pgp and BCRP

As already outlined above, numerous substances have been identified to be substrates of either P-glycoprotein, BCRP, or both. In addition to being transported, some substrates also inhibit these transporters. Additionally, compounds which are solely inhibitors but which are not transported, and compounds which induce transporter activity, are known as well.

A.12.1. General requirements for modulators of P-glycoprotein

In general, a lot of substances interact with Pgp, which also led to the transporter being given the title “hydrophobic vacuum cleaner”⁶⁰. Therefore, describing the structural requirements for modulation of the transporter has been difficult. Ligand-based approaches have shown that both Pgp substrates and inhibitors are of a lipophilic nature, which can be explained by their need to be able to enter the membrane bilayer before interaction with the transporter. In addition, they contain hydrogen bond acceptors or basic functionalities⁶⁴. Planar aromatic rings and a nitrogen atom which confers the basicity are often observed as well^{8b}. A comprehensive study conducted by Seelig⁶⁵ found that the strongest interacting compounds contain two or three electron donor groups, and in addition, that the distance between these groups remains constant. The transmembrane helices of Pgp contain a high number of hydrogen bonding donor and acceptor moieties, which supports these requirements. The differences between substrates and inhibitors seem to lie in their propensity to form hydrogen bonds. Inhibitors seem to form stronger and more numerous hydrogen bonds with Pgp, thus leading to a stronger affinity for interaction. This leads to slower dissociation rates for inhibitors compared to substrates which was shown by radioligand binding studies with [³H]-vinblastine (substrate) and [³H]-XR9576 (inhibitor)^{8b, 66}

A.12.2. General requirements for modulators of BCRP

With respect to BCRP, a similar preference towards lipophilic compounds is observed. For inhibitors, the average molecular weight lies between 350 and 550 g/mol. Most structures contain less than five hydrogen-bond donors and five hydrogen-bond acceptors. One common structural trait, which is shared by many specific inhibitors of BCRP, is a substituted polyaromatic entity, frequently containing an indole ring or an equivalent. Like for Pgp, the presence of at least one nitrogen atom is a frequent structural element, with the nitrogen atom being a tertiary amine or a tertiary amide. Piperazine rings appear to be a privileged entity ensuring the presence of nitrogen atoms. On the other hand, flavonoids have also been found to be potent inhibitors of BCRP without bearing any nitrogen atoms⁶⁷. A comprehensive review by Peña-Solórzano *et al*² identified selective and general modulators of BCRP among

many substance classes, for example acrylonitriles, anti-HIV-based modulators, flavonoids and chromone derivatives, fumitremorgin C analogues, natural product derivatives (antibiotics, curcuminoids, stilbenes and fungal metabolites) and tyrosine-kinase inhibitor based compounds.

A.12.3. Selectivity profiling/polyspecificity

As shown above, the structural requirements for inhibition of the human ABC transporters Pgp and BCRP, and also MRP2 are at least partly similar. Several studies have been already conducted pointing in this direction.

Matsson *et al*⁶⁴ studied 122 registered drugs for their inhibition of Pgp, BCRP and MRP2 and identified 23 specific (11 specific for Pgp, 8 specific for BCRP, 4 specific for MRP2), 19 completely overlapping and 24 partially overlapping inhibitors of these three ABC transporters (one overlapping inhibitor for BCRP/MRP2, 21 overlapping inhibitors for Pgp/BCRP and two overlapping inhibitors for Pgp/MRP2). Interestingly, they also found that the well-known BCRP inhibitor Ko143 is only specific for BCRP at low concentration ($IC_{50}=0.2\ \mu M$), whereas it also inhibits MRP2 at higher concentrations ($IC_{50}=1.5\ \mu M$) and, to a lesser extent, even Pgp as well. They defined important structural characteristics of inhibitors of all 3 transporters, which includes lipophilicity and aromaticity, with general inhibitors being more lipophilic than specific inhibitors. The number of aromatic bonds was similar for general and specific inhibitors (12-18 aromatic bonds) with the exception of specific Pgp inhibitors (usually contained 6-12 aromatic bonds). Docking experiments of multi-specific inhibitors to the ATP-binding domain suspecting inhibitor competition with ATP as a general mechanism of action only gave good results for the most hydrophilic general inhibitors (MK571, quercetin and silymarin) whereas the prototypical lipophilic inhibitors showed low docking scores indicating no interaction with the ATP-binding domain. Comparison of Pgp- and BCRP-specific inhibitors showed that BCRP-selective compounds contained a larger number of nitrogen atoms, in particular aromatic nitrogens, and that specific Pgp inhibitors were less aromatic. To the best of my knowledge, this is the only study where the inhibitory properties of structurally diverse compounds are compared and quantified.

Lanzotti *et al*⁶⁸ and Peña-Solórzano *et al*² summarize work on selective and general modulators of Pgp and BCRP, which are often inspired by plant compounds like for example flavonoids. In the following section, I will focus on studies of compound series for which inhibitory properties on both Pgp and BCRP were tested.

Among the first studied compounds were taxoids⁶⁹, derivatives of paclitaxel optimized for losing its cytotoxicity by removing the C-13 sidechain of paclitaxel that binds β -tubulin and mediates cytotoxicity. 20 compounds which inhibited Pgp were also tested for their inhibition of BCRP and MRP2, giving the result that most derivatives (17) also modulated BCRP to some extent, and some (4) also had an effect on MRP2. 4 compounds which exhibit broad-spectrum inhibitory activity were identified, with the most promising being TRA98006 (see figure B-3),

the only molecule of this series bearing a tertiary amine highlighting the importance of nitrogen atoms for activity.

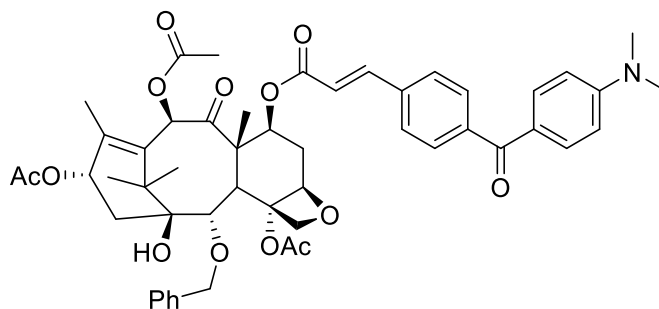


Figure B-3: Structure of tRA98006, a non-cytotoxic taxane derivative which inhibits Pgp, BCRP and MRP2

A study of Cramer *et al*⁷⁰ showed for the first time that propafenone analogues, which had previously only been known as Pgp inhibitors, were in some cases also modulators of BCRP (see figure B-4 for examples). Conclusions which could be drawn from the SAR of the 23 compounds were that while hydrogen-bond acceptor strength in the vicinity of the nitrogen atom is highly important for the activity towards ABCB1, it clearly is not for ABCG2. Charge of the nitrogen is irrelevant for Pgp and BCRP. It could be shown that by changing the substitution within the same chemical scaffold, selectivity indices (SI) can span three orders of magnitude.

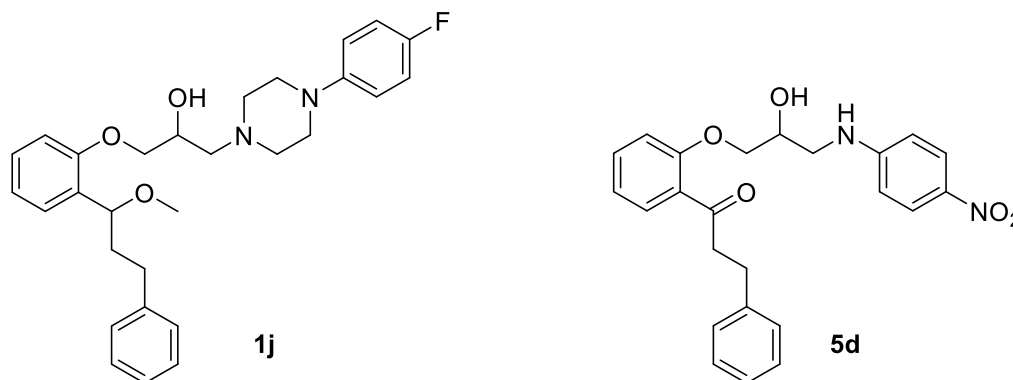


Figure B-4: Structures of propafenone derivative 1j (most active on Pgp, IC₅₀=0.09 μ M, SI=0.006) and 5d (most active on BCRP, IC₅₀=1.24 μ M, SI=5.8)

Colabufo and co-workers put their focus on the search of selective Pgp inhibitors, the synthesized structures are depicted in Figure B-5. First, they synthesized arylmethoxy- and arylmethylamine-phenyl derivatives (section A in figure B-5)⁷¹ and showed that compounds with a nitrogen linker were generally more active on both transporters. However, compounds bearing an oxygen linker were more selective for Pgp inhibition. These findings were used⁷² to synthesize a series of compounds with an oxygen linker, varying spacer lengths and different nitrogen-bearing substituents giving compounds which selectively inhibited Pgp (see section B, figure B-5). This group also synthesized⁷³ 2-aryloxazole and 2-arylthiazole derivatives (section C), giving a series of compounds which showed activity on BCRP, to a bit lesser extent on Pgp, and in some cases also on ABCC2. In addition, naphthalenyl derivatives bearing oxazole, thiazole or furyl heteronuclei⁷⁴ were presented by this group as bioisosters of the previously published aryl-oxazoles and -thiazoles (section D). It could be shown that this

Introduction

introduction of steric hindrance led to retained Pgp activity, however, BCRP and MRP2 activity was diminished.

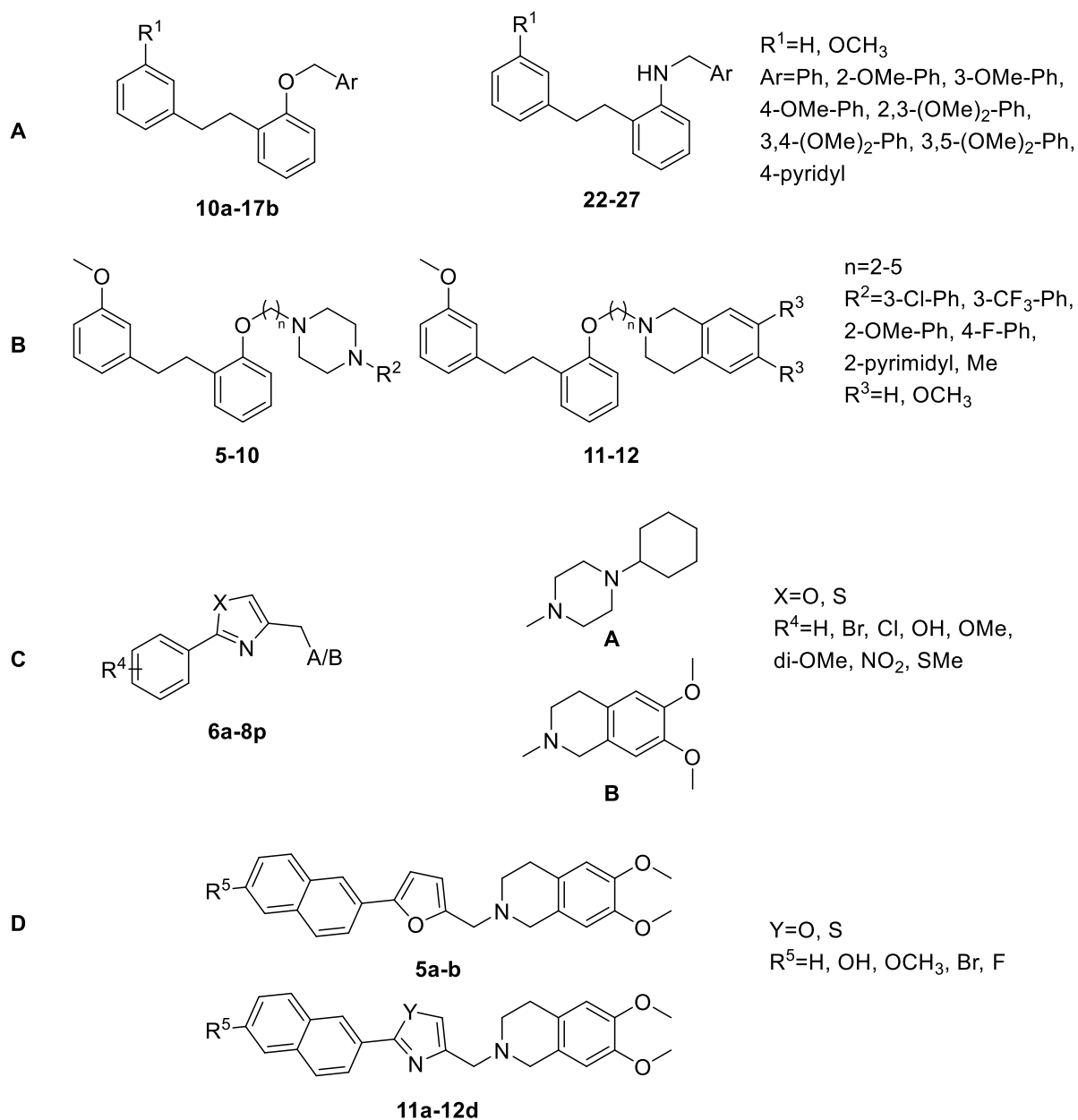


Figure B-5: Compounds synthesized by Colabufo and co-workers as inhibitors of Pgp, BCRP and MDR2, A: arylmethoxy- and arylmethylamine-phenyl derivatives, B: compounds selectively inhibiting Pgp with an O-linker, C: 2-aryloxazole and 2-arylthiazole derivatives, D: naphthalenyl derivatives bearing oxazole, thiazole or furyl heteronuclei

Kühnle *et al*⁷⁵ showed that modification of tariquidar analogues known as dual ABCB1/G2 modulators led to selective and highly potent inhibitors of BCRP with compound 6 being the most active one (see figure B-6).

Jekerle and colleagues⁷⁶ report the synthesis of similar compounds (see figure B-6). While all compounds inhibit BCRP, only WK-X-34 and XR9577 also have an inhibitory effect on Pgp. WK-X-34 also was evaluated as a broad-spectrum modulator *in vitro* and *in vivo*⁷⁷.

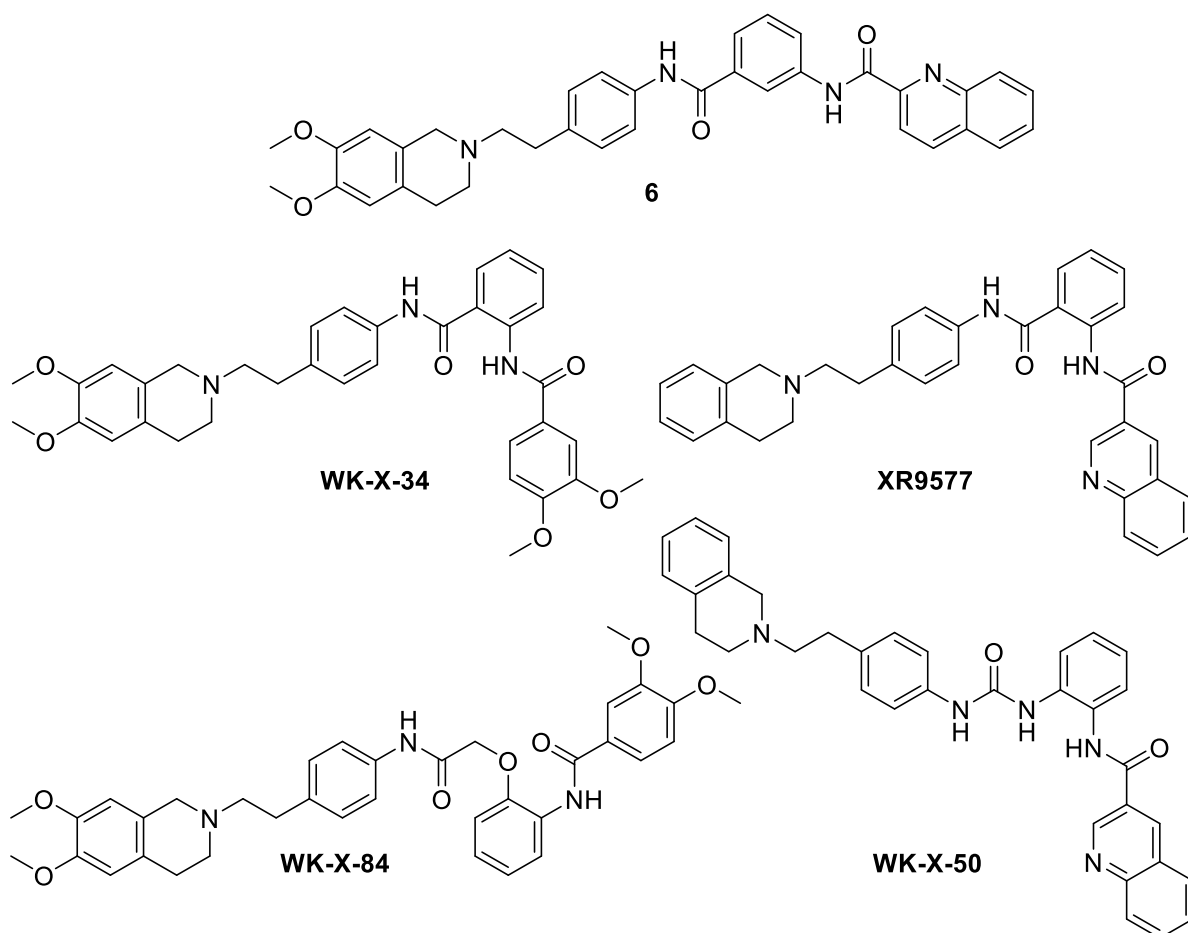


Figure B-6: Compound 6, a tariquidar analogue selective for BCRP, and WK and XR compounds presented by Jekerle and co-workers which are selective for BCRP (WK-X-84 and WK-X-50) or dual inhibitors (WK-X-34 and XR9577)

A substance class for which much literature on Pgp and BCRP inhibition can be found is flavonoids. Many of the naturally occurring flavones, isoflavones, chromones, chalcones or rotenoids have been identified to inhibit Pgp, BCRP, or both ⁷⁸. For example, it has been shown by Fleisher *et al* ⁷⁹ that the ingredients of fruit juices inhibit Pgp moderately (tangeretin and nobiletin) and BCRP more pronouncedly (bergamottin, 6',7'-dihydroxybergamottin, tangeretin, and nobiletin). Inhibition of ABC transporters by flavonoids has been linked to binding at the NBDs ⁸⁰.

Quite a lot of research has been conducted in the field of chalcones, biosynthetic precursors of flavonoids. Already in 1998, Bois *et al* ⁸¹ reported substituted chalcones as inhibitors of Pgp and studied the effect of variation of substituents in position 4' on ring B (see scheme B-6, section A for the general structure). They found that the iodo-substituted compound had the highest activity, their ranking being I > Br > Cl > OMe > F > H₂O. Liu *et al* ⁸² synthesized a library of functionalized chalcones and showed that compounds bearing one basic substituent were potent inhibitors of Pgp (cpds 6-130, 6-131, see figure C-6), but showed no activity on BCRP. Differently substituted derivatives with no basic functionality, on the contrary, were shown to inhibit BCRP (cpd 5-14). In addition to these selective inhibitors, also a general inhibitor (3-100) could be found.

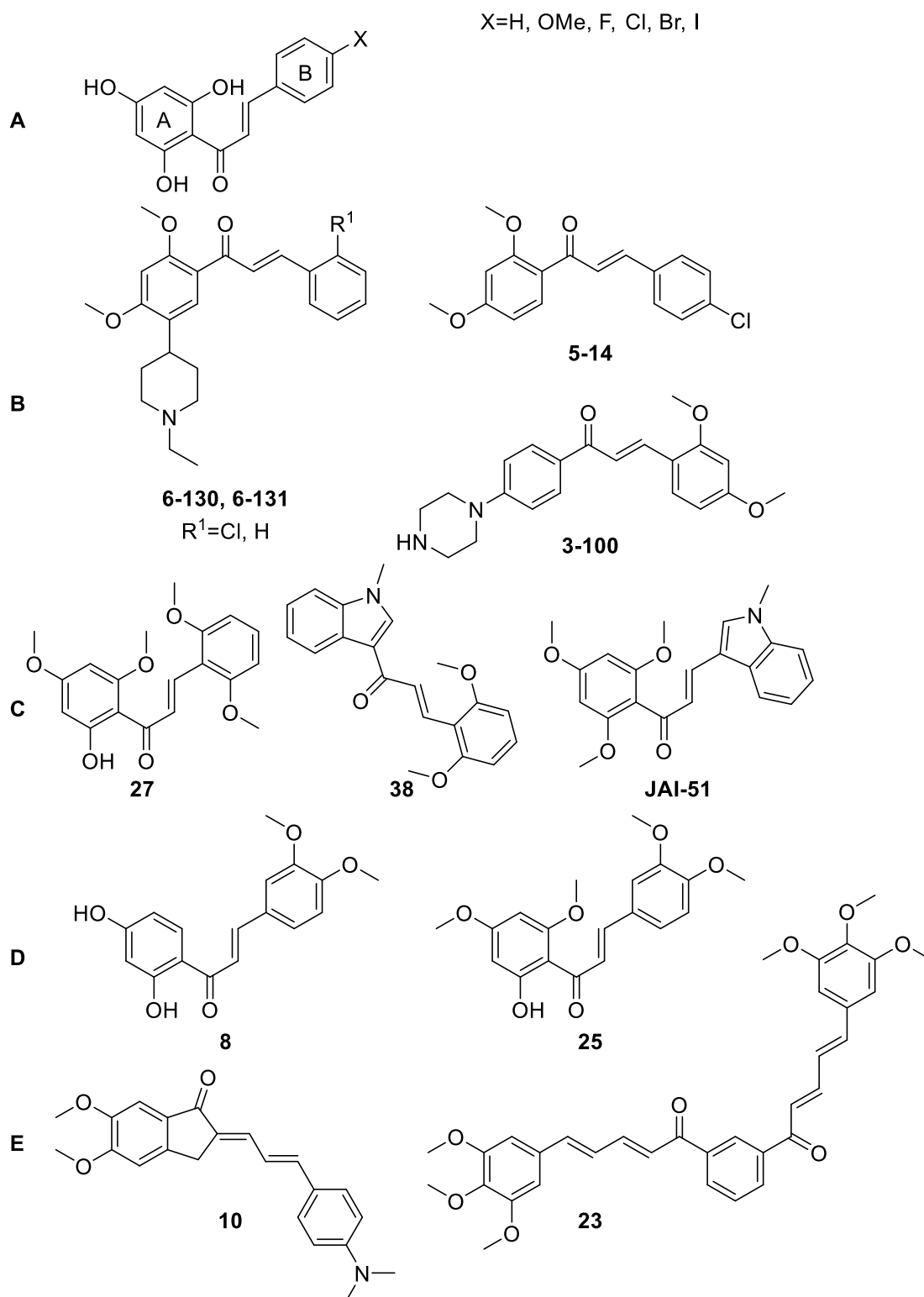


Figure B-7: Chalcone compounds, A: Chalcones as Pgp inhibitors synthesized by Bois, B: Compounds synthesized by Liu *et al* (Pgp-selective – 6-130, 6-131, BCRP-selective – 5-14 or dual inhibitors – 3-100), C: BCRP-selective chromones synthesized by Valdameri *et al* (27, 38) and Boumendjel *et al* (JAI-51), D: BCRP-selective compounds by Juvalle *et al*, E: Pgp-inhibiting chalcones by Parveen *et al*

Based on these findings, Han *et al*⁸³ investigated a series of chalcones bearing no basic substituent based on 5-14 (varying degree and position of substitution by methoxy and hydroxy on ring A, chloro-groups on ring B) and got selective inhibitors of BCRP. 2,4-dimethoxy

or 2,4-dihydroxy substitution on ring A, and mono-chloro-substitution on ring B in position 2' or 3' gave the most active compounds.

Valdameri *et al*⁸⁴ synthesized a large library of chalcones and their indolyl analogues evaluating the effect of methoxy substituents (see figure B-6, section C) and observed that best inhibitory activity on BCRP was observed for a N-methyl-1-indolyl (cpd 38) or a 6'-hydroxyl-2',4'-dimethoxy-1-phenyl (compound 27) moiety (ring A) and a B-ring substituted with two methoxy groups at positions 2 and 6. Highly active compounds were additionally tested on Pgp and MRP2 and it was shown that they were selective for BCRP. Boumendjel *et al*⁸⁵ also present structurally similar JAI-51, which, in addition to being an inhibitor of Pgp and BCRP, also acts as a microtubule depolymerizing agent.

Juvalé *et al*⁸⁶ also show for a series of chalcone and benzochalcone derivatives that compounds highly active on BCRP are also selective, and that methoxy substitution has a big impact, with compounds 8 and 25 (scheme B-6, section D) being the most active ones.

Apart from these extensive studies focusing mainly on BCRP inhibition, studies showing chalcones as Pgp inhibitors are published as well. For example Parveen *et al*⁸⁷ report chalcones and analogues as highly potent Pgp inhibitors (structures of the most active derivatives 10 and 23 given in figure B-6) and also did 3D-QSAR for these compounds.

Chromone derivatives were first identified by Hadjeri *et al*⁸⁸ as Pgp inhibitors, with the most active one being the methylated derivative 4a (see scheme B-8, section A). In addition, they showed that dimethoxyaurone 6 also is a potent inhibitor of Pgp. Further research on the inhibitory activity of similar compounds on BCRP⁸⁹ showed that the substituent R¹ is crucial for selectivity – while the unsubstituted or ethyl- and propyl-substituted derivatives showed no activity on BCRP, the n-butyl- and n-pentyl-substituted ones were active. Higher activity on BCRP was observed for the phenalkylamino-substituted chromones 5a-c. In contrast, a similar compound (4-chloro-benzylchromone 4i) showed no effect on Pgp⁸⁸.

Valdameri *et al*⁹⁰ also report the synthesis of a series of chromones, which they identified as highly active and selective inhibitors of BCRP. Among the most active compounds were 6f-6h as shown in figure B-8, section B, with the bromo-substituted compounds being the most active ones. They stress the importance of two substituents in order to obtain a high-activity compound. The most active compounds were found to be at least 2-3 fold more efficient than previously reported flavones⁹¹, acridones⁹², boeravinones⁹³ (a subclass of rotenoids, see figure B-8 for the most active compounds, boeravinone G) or natural products (e.g. Pick *et al* did a SAR for flavonoids⁹⁴).

With respect to aurones and similar derivatives, a large study was conducted by Sim and colleagues⁹⁵ including more than 100 compounds from subclasses of differently substituted aurones, indanones, aza-aurones, dehydro-aurones, isoaurones, indolinones, chalcones and flavones. First, the inhibitory effect on BCRP was tested, with the result that some series, namely the ring A hydroxylated and unsubstituted aurones, dehydroaurones, and isoaurones and indolinones, were inactive with respect to BCRP inhibition. The most active compounds across the series were found among the dimethoxyaurones, indanones and flavones, and were observed to be disubstituted with methoxy or methoxy and hydroxy groups on ring B

(see figure B-8, section C). In addition, Pgp inhibition was determined, giving the result that only 14 compounds inhibited Pgp above a certain threshold. These belonged to the classes of methoxyaurones, indanones, azaaurones, and flavones, showing that compound series which were inactive on BCRP also showed no activity on Pgp. As most compounds showed similar (less than 4-fold difference) inhibition for BCRP and Pgp, the conclusion was drawn that features which were beneficial for ABCG2 also promoted interaction with ABCB1.

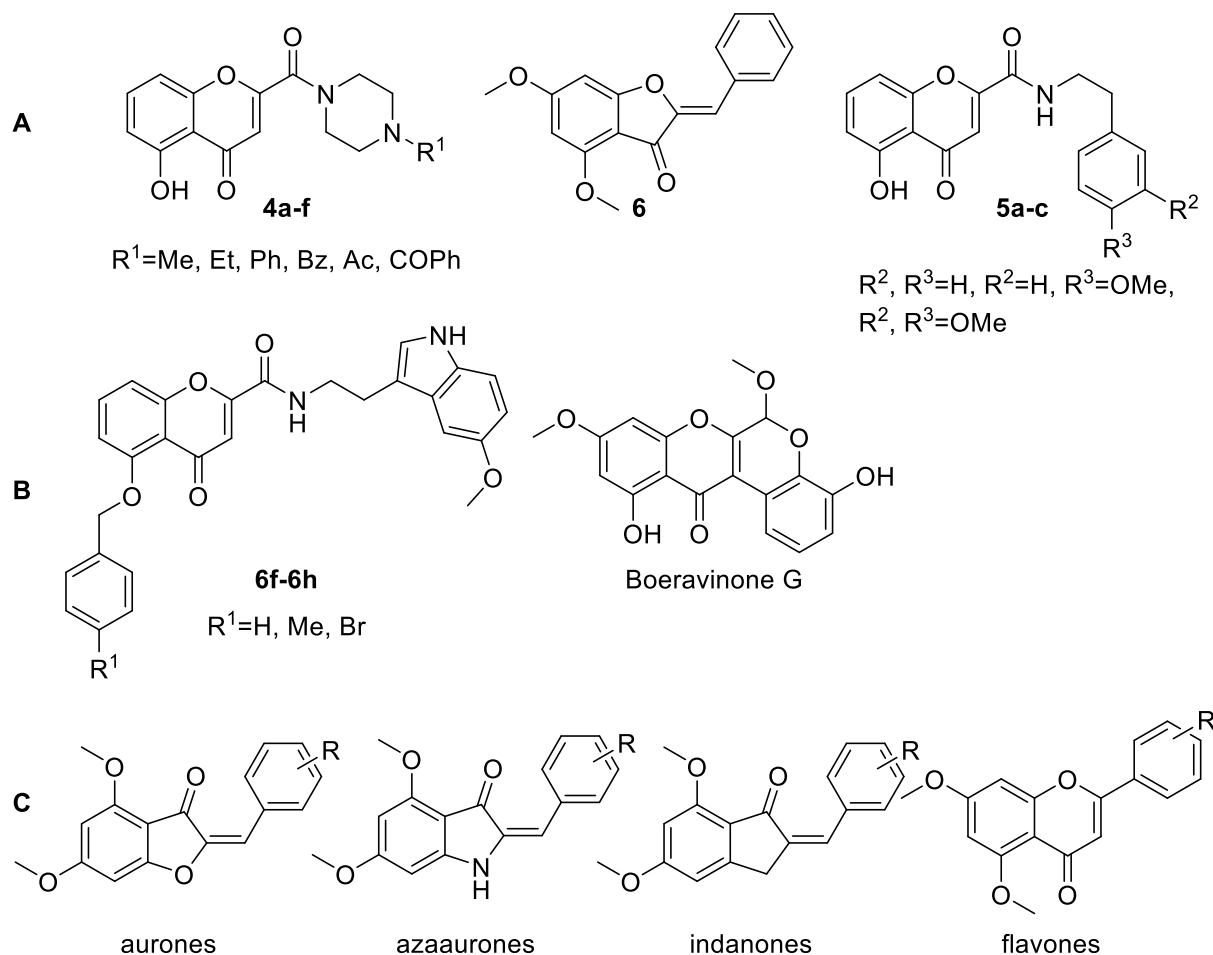


Figure B-8: Chromones, aurones and rotenoids as Pgp/BCRP inhibitors, A: Chromones (4a-f) and aurones (6) identified by Hadjeri as Pgp inhibitors, chromones (5a-c) are BCRP inhibitors (Boumendjel), B: Chromones 6f-6h as selective BCRP inhibitors presented by Valdameri et al, and boeravinone G, a rotenoid which is a BCRP inhibitor, C: compound classes inhibiting both Pgp and BCRP

In summary, it can be concluded that many compounds and compound series have been identified as inhibitors of P-glycoprotein, BCRP, or both. However, SAR predictions, if possible at all, often remain limited to compound series or restricted scaffolds. With respect to the importance of Pgp and BCRP as both targets and antitargets, further studies, which could maybe even fill the gaps in the chemical space between compound series, are desirable.

Aim of the thesis

As outlined above, the ways how substances interact with ABC transporters, and the criteria which make a compound an inhibitor or substrate remain not fully understood and difficult to predict, thus necessitating further research in this area. Most compound series focus on synthetically easily accessible molecules, therefore leaving big gaps in the chemical space where the inhibitory properties of possible compounds remain unknown. In addition, the ABC transporters most recognized for their involvement in MDR and drug-drug interactions, P-glycoprotein and BCRP, show overlapping, but slightly different substrate and inhibitor patterns which have not been explained in a satisfactory way.

Therefore, the aim of this thesis was to synthesize molecules which cover a chemical space between two known inhibitors of BCRP or Pgp. On the one side, the inspirational molecule is GPV005 (top left in figure C-1), a propafenone derivative, which was proven to be a selective inhibitor of Pgp. However, the study by Cramer and co-workers already presented above ⁷⁰ shows that even some propafenone derivatives are already BCRP inhibitors as well, indicating that both selective and general inhibitors are possible among propafenones. The other endpoint of our chosen compound spectrum is fumitremorgin C (FTC, top right in figure C-1), which was one of the first discovered inhibitors of BCRP, which it inhibits selectively. The aim of this thesis is now to synthesize compounds which lie structurally between these extremes and which are stepwise made more similar to FTC. Starting with compounds with the propafenone scaffold, stepwise more and more features of FTC are introduced, while, however, some of GPV005's core features are retained. The envisioned target structures are shown in scheme C-1, the arrows indicate that the scaffolds become more and more similar to FTC. In more detail, this includes first the replacement of the cyclic amine in GPV005 by proline (**A**), then the shortening of the 2-hydroxypropylene linker to an ethylene linker (**B**). For both scaffolds with the 2-hydroxypropylene and the ethylene linker, the proline moiety is then replaced by a diketopiperazine, a small cyclic dipeptide consisting of proline and glycine (**C** and **D**). This structural element is also found in FTC. In the next step, another ring fused to the benzene ring is introduced, generating chromone or isoflavone compounds (**E**). The role of a double bond in this ring should be evaluated by generating also the reduced chromanones/isoflavanones (**F**). The last compound, most similar to FTC, would have the final ring closure, thus also showing a pentacyclic fused ring system like FTC (**G**).

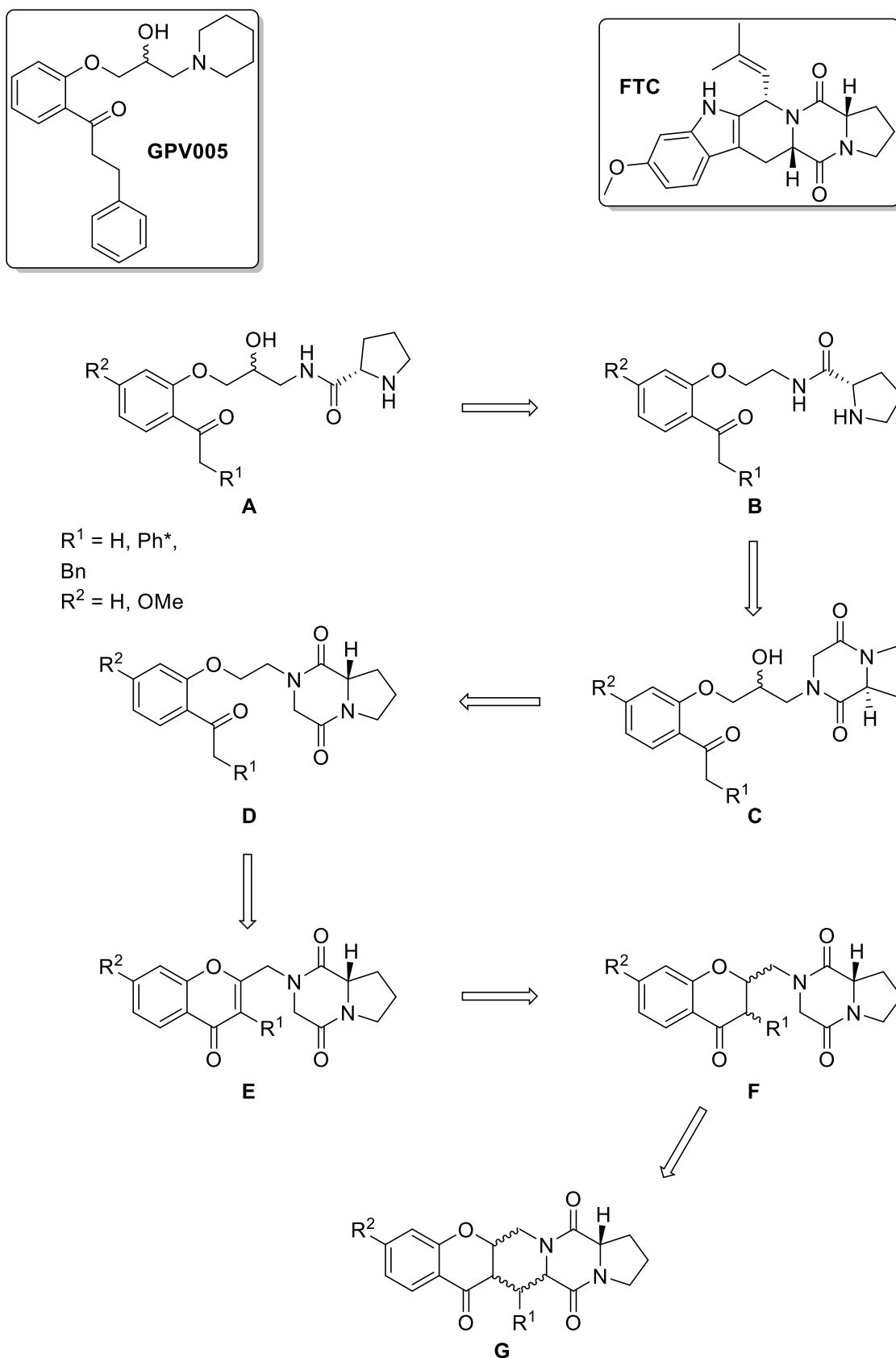


Figure C-1: Structures of GPV005, fumitremorgin C and planned target molecules, *: Ph only for E & F, for G only R¹=H or Ph, R²=H

The expected output of this thesis is the identification of functional groups or molecular patterns necessary for the interaction with one or the other ABC transporters. A possible

overlap of inhibitors for these two transporters or a switch of selectivity might be observed. New selective or dual inhibitors should be found. The aim is not to find especially active compounds, but to gain more understanding for the requirements to make a compound an inhibitor of Pgp or BCRP. In the future, this knowledge could be used to design selective or general inhibitors, or to omit inhibitor-triggering features if no inhibition is desired.

To this effect, synthetic routes for the desired compounds should be developed, and the characterized and purified compounds should be tested for their inhibitory activity on both Pgp and BCRP.

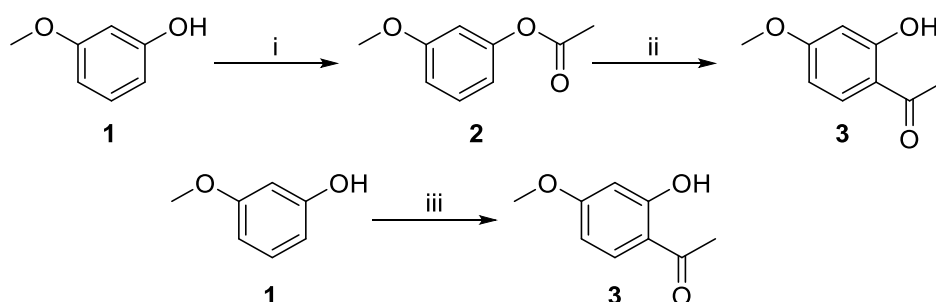
Although there are extensive records of inhibitors of Pgp and BCRP in the literature, no study has been conducted so far in which a substrate or inhibitor of one has been transformed stepwise into a substrate or inhibitor of the other transporter. Therefore, we expect to get a better insight into the specificity and the functionality of these transporters. In addition, these results can help to “fill the structural gaps” between different substance classes and could therefore help to improve the *in silico* prediction of inhibitors and/or substrates in the future. Generally, research and new findings in the field of inhibitors of Pgp or BCRP helps the development of new, safer drugs in all fields of medicine, but especially in oncology.

Results and Discussion

A.13.Synthesis of starting materials and reagents

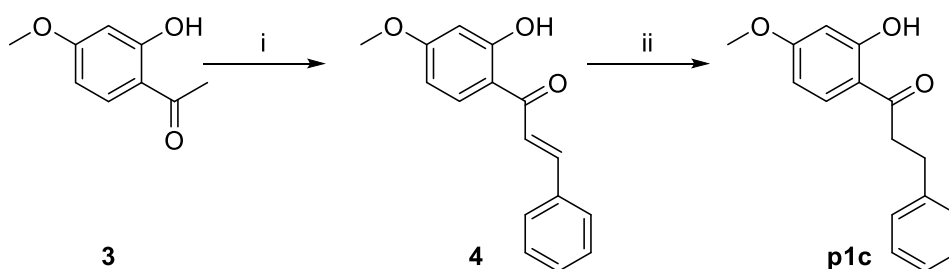
A.13.1. Synthesis of 2-hydroxyacetophenone derivatives

The first step of the synthesis, the preparation of 2-hydroxy-4-methoxyacetophenone, was accomplished via different methods. In a first attempt, according to ⁹⁶, 3-methoxyphenol (**1**) was first acylated with acetic anhydride in 88% yield. The next step in this procedure was a Fries rearrangement using aluminium chloride, which gave the desired product **3** in 49% yield. A second, alternative method was the direct Friedel-Crafts-acylation of **1** using titanium(IV)chloride and acetyl chloride, which gave **3** in 64% yield following ref ⁹⁷. Boron trifluoride etherate was also used as a Lewis acid following the procedure by Kim ⁹⁸, giving similar results with also 64% yield. In general, the procedure by Kim was found to be the most feasible, giving the product in high purity, acceptable yield and under the mildest conditions.



Scheme D-1: Synthesis of 2-hydroxy-4-methoxyacetophenone. Reaction conditions and yields: method1: i) Ac_2O , pyridine, 3 h, 100 °C, 88%; ii) AlCl_3 , 2.25 h, 130 °C, 49%; iii) method 2: AcCl , TiCl_4 , 1 h, 120 °C, 64%, method 3: AcCl , $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 3 h, 85 °C, 64%

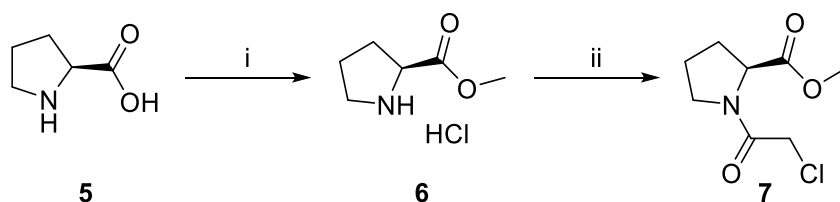
1-(2-hydroxy-4-methoxyphenyl)-3-phenylpropan-1-one (**p1c**) was prepared following a two-step protocol according to ref ⁹⁹. First, the chalcone **4** was obtained by condensation of **3** with benzaldehyde under basic conditions in 91% yield. The conjugated double bond was subsequently reduced using palladium on charcoal and hydrogen gas at atmospheric pressure (68% yield).



Scheme D-2: Synthesis of 1-(2-hydroxy-4-methoxyphenyl)-3-phenylpropan-1-one, reaction conditions and yields: i) benzaldehyde, NaOH , EtOH 96%, 2 d, RT (twice), 91%; ii) Pd/C , THF , H_2 (1 atm), 3 h, 68%

A.13.2. Synthesis of methyl (2-chloroacetyl)-L-prolinate

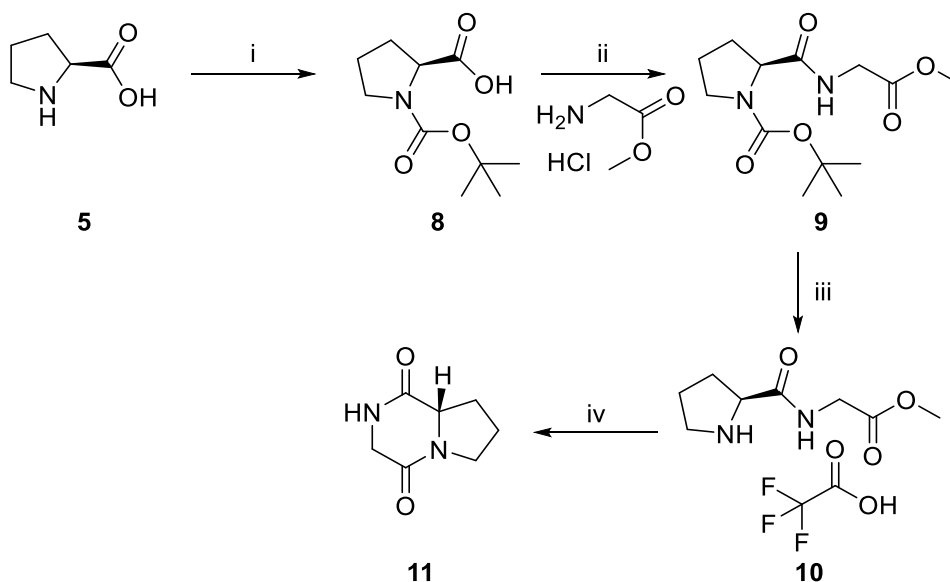
As described in ref ¹⁰⁰, reagents similar to methyl (2-chloroacetyl)-L-prolinate (**7**) are useful for the preparation of diketopiperazines in one step from primary amines. To obtain **7**, first L-proline methyl ester hydrochloride (**6**) was prepared from L-proline in 96% yield ¹⁰¹, which was then transformed to **7** using chloroacetyl chloride in DCM in 53% yield following ref ¹⁰².



Scheme D-3: Synthesis of methyl (2-chloroacetyl)-L-prolinate, reaction conditions and yields: i) SOCl_2 , MeOH, 0 °C, 1.25 h, then RT overnight, 96%; ii) chloroacetyl chloride, Et_3N , DCM, 0 °C, then RT, 2 d, 53%

A.13.3. Synthesis of the diketopiperazine (S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione

The diketopiperazine **11** was prepared as a reference substance, and also with a possible substitution at the amide NH in mind. First, the amine functionality of L-proline was protected using di-tert-butyl dicarbonate (97%, see Scheme D-4, ¹⁰³). N-Boc-proline (**8**) was subsequently coupled to glycine methyl ester using DCC (N,N'-dicyclohexylcarbodiimide, quant., ¹⁰⁴). After quantitative deprotection using trifluoroacetic acid, the intermediate product **10** was cyclised to the final product **11** by refluxing in MeOH in the presence of triethyl amine (both according to ref ¹⁰⁵, 78%, over two steps). The NMR spectra of the amide intermediates showed the presence of two rotamers, however the final product **11** seems to exist only in one form due to the fixed conformation of the amides in the ring.

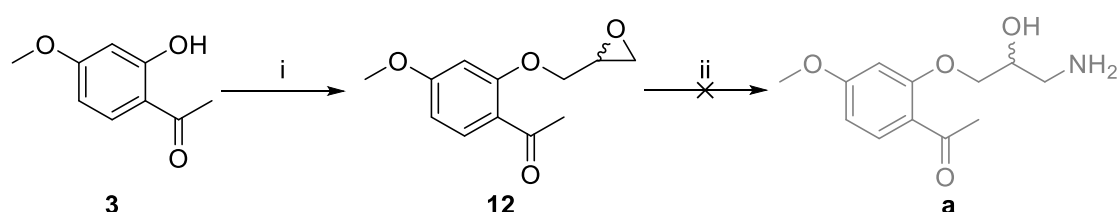


Scheme D-4: Synthesis of (S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione, reaction conditions and yields: i) Boc_2O , sat. NaHCO_3 , THF, 0 °C, 1 h, then RT, 21 h, 97%; ii) 4-methylmorpholine, DCC, DCM, DMF, 0 °C, 1 h, then RT, overnight, quant.; iii) TFA, DCM, RT, 45 min; iv) Et_3N , MeOH, reflux, 16 h, 78% (2 steps)

A.14.Synthesis of proline and diketopiperazine derivatives of propafenones and 2-ethoxyacetophenones

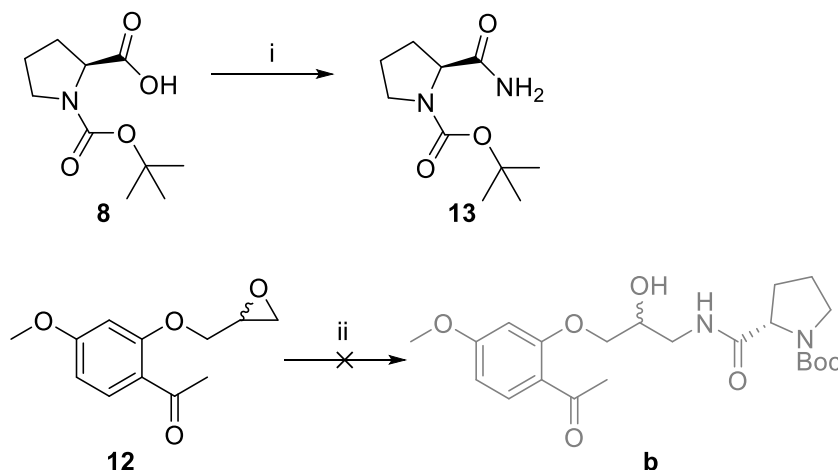
A.14.1. First attempts at the synthesis

The first attempts at the synthesis of proline derivatives of propafenones followed the established routes for the synthesis of propafenones. Therefore, first the phenol **3** was reacted with epichlorohydrin to produce the epoxide **12** in 98% yield according to ref ¹⁰⁶. A first attempt to open the epoxide with ammonia in methanol as proposed by ref ¹⁰⁷ led to a complex mixture of products, probably due to intra- or intermolecular reaction of amine and ketone. This problem was avoided by protection of the ketone functionality as an ethylene ketal as shown in chapter D.2.2.



Scheme D-5: Attempted synthesis of 1-(2-(3-amino-2-hydroxypropoxy)-4-methoxyphenyl)ethan-1-one, reaction conditions and yields: i) epichlorohydrin, NaOH, reflux, 24 h, 98%; ii) NH₃ in MeOH (2M), RT, 3 d

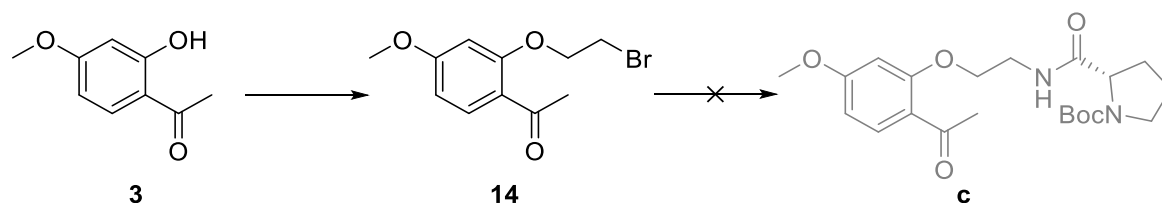
The next approach, which was chosen because of its simplicity, was to try a direct substitution of the primary amide **13**. **13** could be easily obtained from Boc-proline (**8**) in 60% yield following ref ¹⁰⁸. Different reaction conditions were tried for the reaction of the epoxide **12** with the amide **13** (see table D-1), however none led to product formation. Thus, a new synthetic strategy was developed (see chapter D.2.2).



Scheme D-6: Synthesis of N-Boc-D-prolineamide and attempted synthesis of **b, reaction conditions and yields: i) ethyl chloroformate, -12 °C, 30 min, aq. NH₄OH, -20 °C, then RT, 19 h, 60%; ii) reaction conditions summarized in table 1**

Also for the synthesis of the ethylene analogue, the first strategy was to try the substitution of the amide. The ethylene bromide **14** was synthesized for this reason according to ref ¹⁰⁹ in 65% yield. Also in this case, the N-alkylation of the amide was not successful, probably due to the low reactivity of acyclic primary amides ¹¹⁰. The used reaction conditions for both the bromide and the epoxide are summarized in table 1. It is visible that prolonged reaction times

did not lead to product formation either. Although there are several records in the literature for successful substitutions of primary amides with bromides or other nucleophiles, it seems that the reaction is only applicable under many restrictions (e.g. activation of amide through electron-withdrawing substituents, see chapter D.2.2).



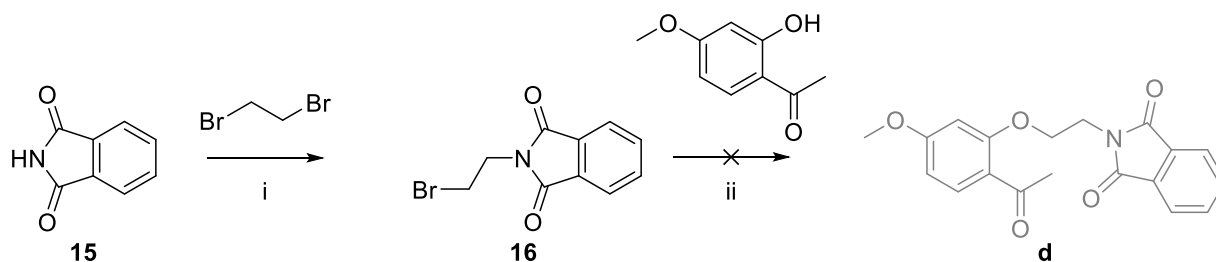
Scheme D-7: Synthesis of the bromoethylene derivative 15 and attempted synthesis of c, reaction conditions and yields: i) 1,2-dibromoethane, K_2CO_3 , Bu_4NBr , toluene, water, reflux, 2 d, 65%; ii) reaction conditions summarized in table 1

Table D-1: Reaction conditions employed for the substitution reaction of 13 with 12 or 14

STARTING MATERIAL	LITERATURE	REAGENTS	TEMPERATURE, TIME	OUTCOME
12	¹¹¹	NaH 60%, 1.1 eq (2x), DMF	85 °C, 24 h, then addition of 1.1 eq NaH, 50 °C, 20 h	Degradation of starting materials
12	¹¹²	K_2CO_3 0.5 eq, Bu_4NBr 0.1 eq, DMF (tried twice)	100 °C, 69 h	Degradation of starting materials
12	¹¹³	K_2CO_3 0.4 eq, $BnEt_3NCl$ 0.1 eq, dioxane	50 °C, 4 d, then 90 °C, 5d	Degradation of starting materials
12	¹¹⁴	Pyridine, EtOH	RT, oN, then 50 °C, 3d	Degradation of starting materials
12	¹¹⁵	NaOH, DMF	RT, oN, then 50 °C, 3d, then 90 °C, 2 d	Degradation of starting materials
14	¹¹⁶	K_2CO_3 1.5 eq, Bu_4NBr 0.4 eq, DMF	80 °C, 47 h	Degradation of starting materials
14	¹¹⁷	KOH 1.5 eq, DMF	RT, 24 h	Degradation of starting materials
14	¹¹⁸	NaOH 3.5 eq, K_2CO_3 2.0 eq, Bu_4NHSO_4 0.1 eq, toluene	RT, 3d, then 50 °C, 3 d	Degradation and dimerization of 15
14	¹¹⁹	K_2CO_3 2 eq, MeOH	RT, oN, then 50 °C, 1d	Degradation and dimerization of 15
14	¹²⁰	NaH 60% 1.1 eq, DMF	50 °C, 3 d, then 90 °C, 1 d	Degradation of starting materials
p1a	-	BuLi, THF, DMF	-78 °C, 4.5 h, then RT, On	Starting materials recovered, no reaction

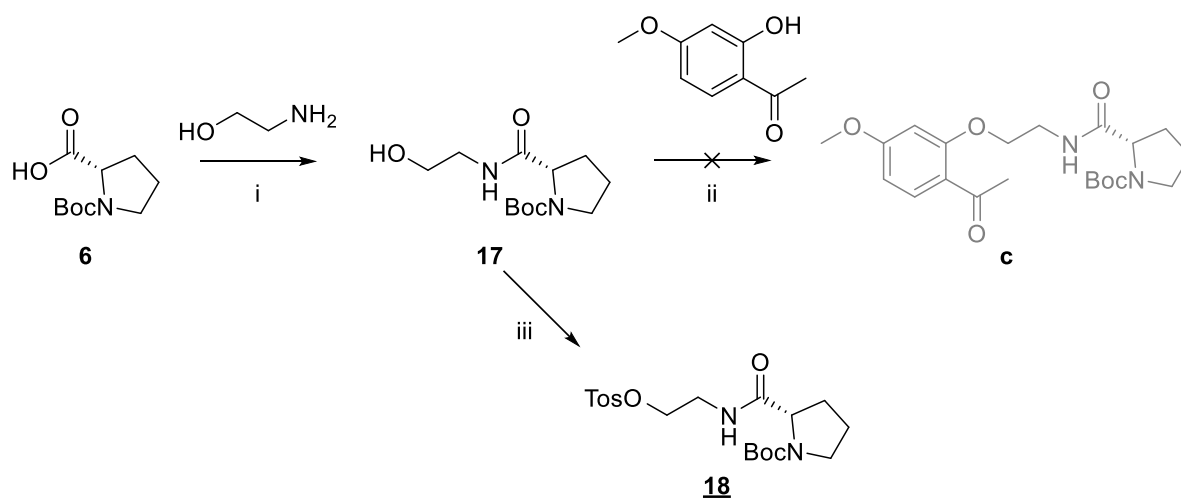
For the synthesis of the ethylene derivatives, other strategies were envisioned. The first approach started with the formation of 2-(2-bromoethyl)isoindoline-1,3-dione (**16**) by reaction of 1,2-dibromoethane and phthalimide (87%, according to ref ¹²¹). The planned

strategy was to react this reagent with the phenol **3** and afterwards to release the masked amine by reaction with e.g. aqueous hydrazine, maybe after protection of the ketone functionality. Unfortunately, already the reaction of **16** and **3** following ref ¹²² did not yield the desired product, as no conversion of **3** could be seen. Switching the base used from potassium to caesium carbonate did not lead to formation of the desired product either. It could be shown that the neighbouring ketone was not the reason for the absent reactivity, as also the ketal **p2a** did not yield the desired product in the reaction with **16**.



Scheme D-8: Attempted synthesis via the 2-(2-bromoethyl)isoindoline-1,3-dione (**16**) intermediate, reaction conditions and yields: i) 1,2-dibromoethane, K_2CO_3 , phthalimide, DMF, 3 h, RT, 86%; ii) a) K_2CO_3 , 120 °C, 1.5 d, no visible conversion, b) Cs_2CO_3 , DMF, 100 °C, 5d, no visible conversion, c) reaction of **p2a** with **16**: K_2CO_3 , Bu_4NI , DMF, 6 d, 80-100 °C, starting materials recovered and partial degradation

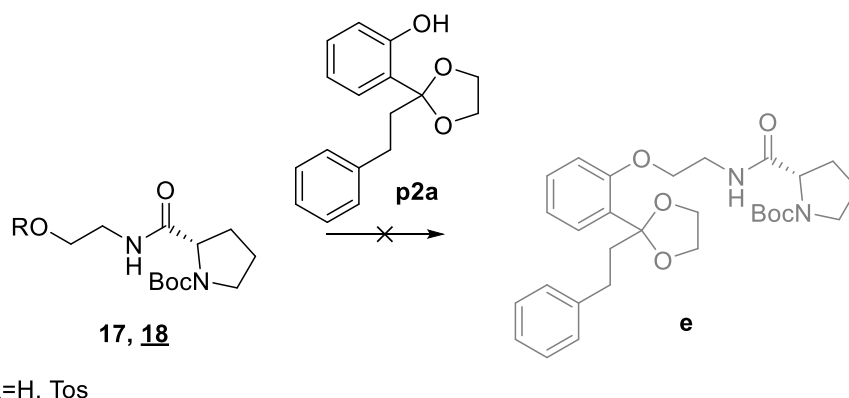
In a similar approach, **17** was prepared from Boc-proline (**6**) and aminoethanol (99%, according to ref ¹²³). It was envisioned to react **17** with **3** under Mitsunobu conditions. Unfortunately, this strategy was not successful for compound **3** (several attempts, see Scheme D-9).



Scheme D-9: Approach at the synthesis of **c** via tert-butyl (S)-2-((2-hydroxyethyl)carbamoyl)pyrrolidine-1-carboxylate (**17**), reaction conditions and yields: i) N-hydroxysuccinimide, DCC, DCM, 0 °C, 2 min, then RT, oN, 99%; ii) a) DIAD (diisopropyl azodicarboxylate), PPh_3 , toluene, RT, 4 d, no conversion, according to ref ¹²⁴, b) DIAD, PPh_3 , THF, 1.25 h, RT, ultrasonic bath, no conversion, according to ref ¹²⁵

Like for the reaction of **16** and **3**, it was suspected that the hydrogen-bonding interaction between the ketone and the phenol and the vinylog carbonic acid character of the phenol decreased its reactivity. However, also the Mitsunobu reaction of the ketal **p2a** was not successful. Transforming the alcohol to the tosylate **18** with tosyl chloride was only possible in poor yield (14%, according to ref ¹²⁶), as tosylation of the amide occurred as well and led

mainly to the di-tosylated product. Attempts at the reaction of **p2a** and the tosylated product **18** did not yield the desired product either.

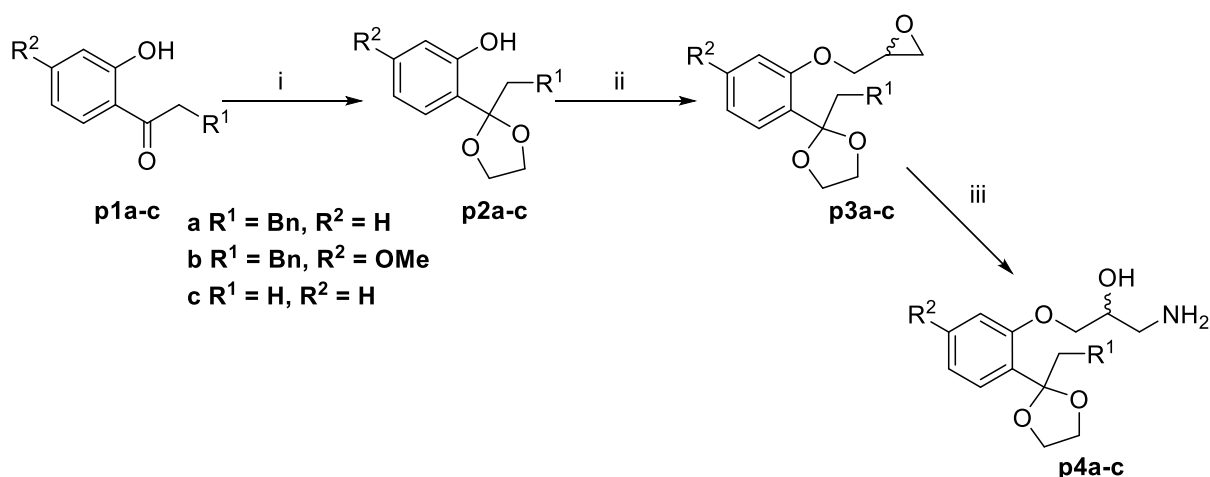


Scheme D-10: Attempted reaction of 2a with 17 and 18, reaction conditions and yields: a) reaction with 17: DIAD, PPh₃, THF, 0 °C, 30 min, then RT, 3 d, no conversion, according to ref ¹²⁷; b) reaction with 18: Cs₂CO₃, DMF, RT, oN, then 65 °C, 4 d, no conversion, according to ref ¹²⁸

A.14.2. Publication “Subtle Structural Differences Trigger Inhibitory Activity of Propafenone Analogues at the Two Polyspecific ABC Transporters: P-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)”, Synthesis of proline and diketopiperazine derivatives of propafenones and 2-ethoxyacetophenones (A, B, C & D)

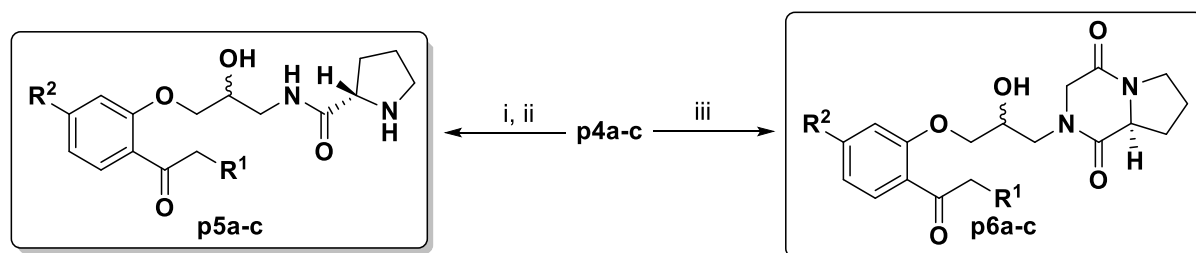
In the following section, the successful syntheses of proline and diketopiperazine derivatives of propafenone and ethoxyacetophenone compounds are described. These results were already published (see publication in the appendix). The substance numbers with the prefixed “p” are the ones used in the publication.

To avoid undesired side reactions of the amine as described above, the ketone in **p1a-c** was first protected as the ethylene ketal. The subsequent reaction with epichlorohydrin and ring opening with ammonia in methanol followed the originally planned route to give the amine **p4a-c**.



Scheme D-11: Synthesis of intermediate products p4a–c, reagents and conditions: i) ethylene glycol, TfOH, trimethyl orthoformate, hexane, 40 °C, 3 days (63-99%); ii) epichlorohydrin, NaH, DMF, 70 °C overnight (74-99%); iii) ammonia in MeOH (14-54%)

p4a-c could now be reacted with proline using the peptide coupling reagent DCC, or with methyl (2-chloroacetyl)-L-prolinate (**7**) to obtain the diketopiperazine derivative. Both reactions were successfully accomplished and led after deprotection to the desired products. In the case of all proline derivatives, the hydrochloride was formed before testing to increase the solubility in aqueous media.

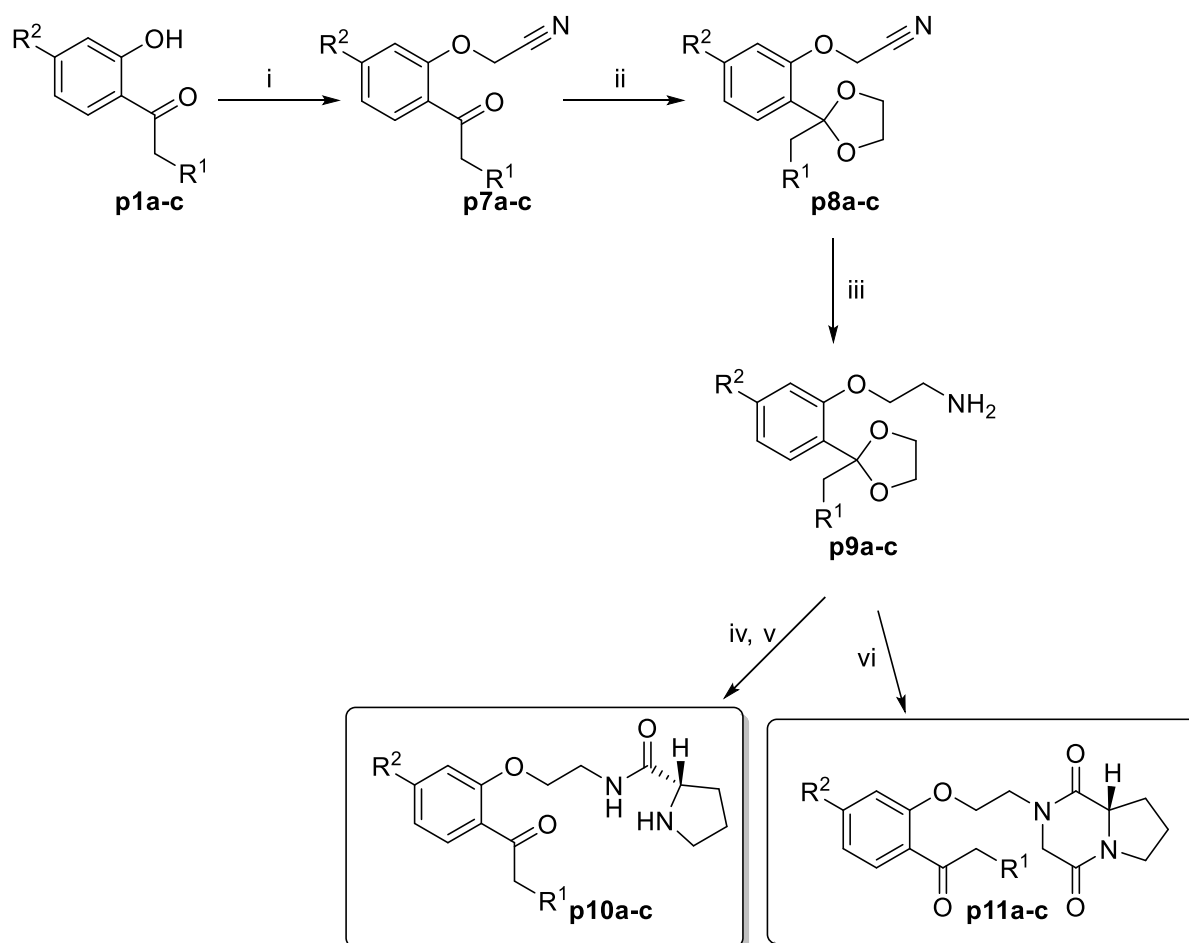


Scheme D-12: Synthetic route for final compounds p5a-c and p6a-c, reagents and conditions: i) Boc-proline, DCC, DMAP (4-dimethylaminopyridine), dichloromethane, 2h to overnight (41-71%); ii) 2 N HCl, ACN (98% - quantitative); iii) methyl (2-chloroacetyl)prolinate, triethyl amine, 2 days, then 2N HCl, ACN (6-61%, 2 steps)

The first successful route to the ethylene amine derivative **p9a-c** was based on the work of Bremner *et al*¹²⁹ and started with a reaction of the phenols with bromoacetonitrile, followed by a protection of the ketone and reduction of the nitrile using LiAlH₄. The obtained amine could, in the same manner as for **p4a-c**, be transformed to the proline or diketopiperazine derivative.

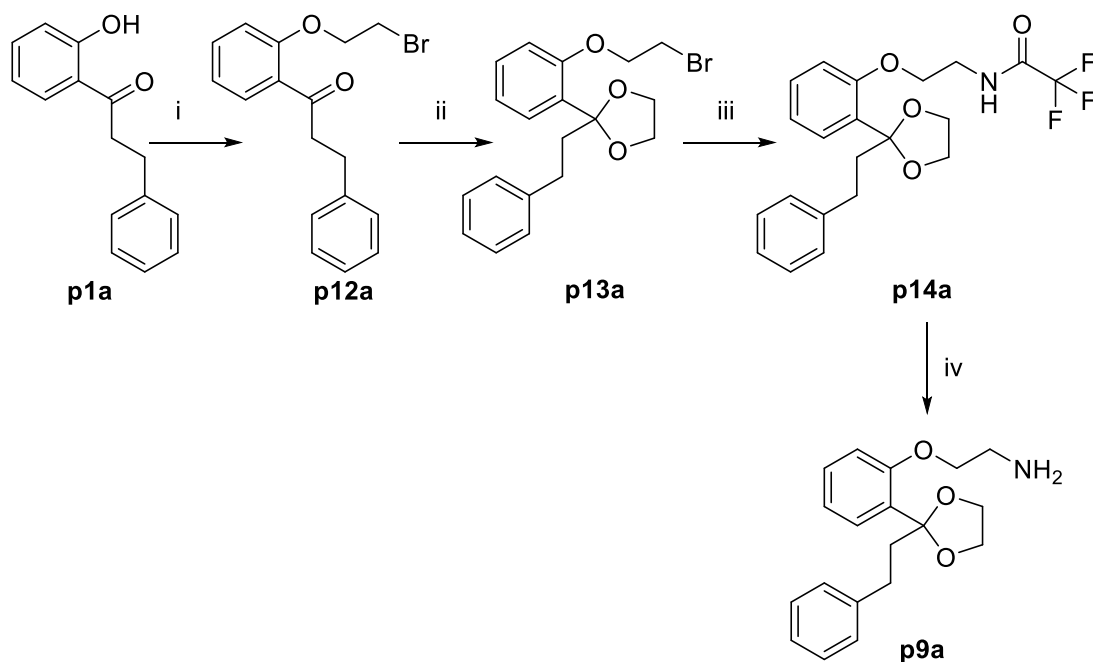
Due to the fact that the nitrile reduction was not very high-yielding, and the reaction conditions (LiAlH₄ in Et₂O) were potentially dangerous, a second route for the synthesis of ethylene amine derivatives was developed. This route started with the reaction of **p1a** with dibromoethane followed by protection of the ketone. It became apparent that reaction of the protected ketone **p2a-c** with dibromoethane was not a feasible way, as at least partial deprotection occurred in the course of the reaction. The protected bromide was consequently reacted with trifluoroacetamide, which could be hydrolysed to give the amine under basic

conditions ¹¹⁶. It should be noted that the reaction with trifluoroacetamide is also a substitution reaction of a primary amine, which works very well under mild conditions due to the electron-withdrawing effect of three fluorine atoms.

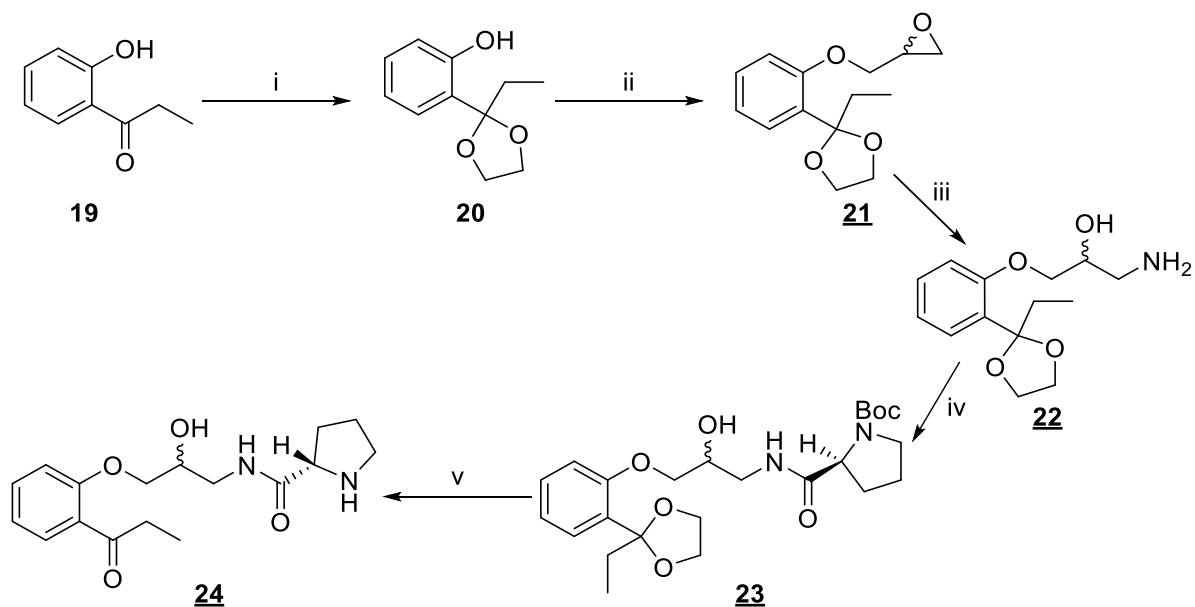


Scheme D-13: Synthesis of compounds 10a-c and 11a-c, reagents and conditions: i) bromoacetonitrile, potassium carbonate, acetone, 50 °C, 4 days (88-100%); ii) ethylene glycol, p-toluenesulfonic acid (pTosOH), toluene, reflux, overnight (72-97%); iii) LiAlH₄ or Red-Al (sodium bis(2-methoxyethoxy)aluminumhydride), Et₂O, reflux or RT, 3-4 h (15-42%); iv) Boc-proline, DCC, DMAP, dichloromethane, 2h to overnight (57-78%); v) 2 N HCl, MeCN (97% - quantitative); vi) methyl (2-chloroacetyl)prolinate, triethylamine, 2 days, then 2N HCl, MeCN (19-39%, 2 steps)

The same reaction conditions which were used for the synthesis of the compounds **p5a-c** were also used on 2'-hydroxypropiophenone (**19**) to obtain the ethyl-substituted target molecule **24** (see Scheme D-15). Unfortunately, this molecule, like all the compounds with a methyl next to the ketone, lacked activity on both Pgp and BCRP (see also chapter E). The reason for this behaviour was probably the low lipophilicity of these compounds. Therefore, it was decided to stop further syntheses of ethyl substituted compounds, as the possibility that they were inhibitors of one or the other transporter was very low. This is the reason why the diketopiperazine derivatives, and also the derivatives with the ethyl linker, were not formed for the ethyl series.



Scheme D-14: Alternative route to compound 9a, reagents and conditions: i) 1,2-dibromoethane, K_2CO_3 , Bu_4NBr , toluene, water, reflux, 6 d, 79%; ii) ethylene glycol, $TfOH$, trimethyl orthoformate, hexane, 40 °C, 3 d, 99%; iii) trifluoroacetamide, K_2CO_3 , Bu_4NBr , DMF, 80 °C, 4.5 h, 65%; iv) KOH , MeOH, water, RT, 2 h, 99%



Scheme D-15: Synthesis of the ethyl derivative 24. Reagents and conditions: i) ethylene glycol, $TfOH$, trimethyl orthoformate, hexane, 40 °C, 4 days (quant.); ii) epichlorohydrin, NaH , DMF, 70 °C overnight (88%); iii) ammonia in MeOH, RT, 4 d (40%); iv) Boc-proline, DCC, DMAP, dichloromethane, RT, overnight (35%); v) 2 N HCl, dioxane (99%)

A.15.Synthesis of chromone derivatives (E)

The next target molecules were diketopiperazine derivatives of chromones of the structure **E** depicted in Figure D-1. Relying on the information already gained in the synthesis of the first target molecules, key intermediate **I** was the synthetic target. Despite its simplicity and close relation to isoflavonoids, there are only few records of syntheses for compounds like **I**. As shown in this chapter, different synthetic routes had to be found for the differently substituted chromones

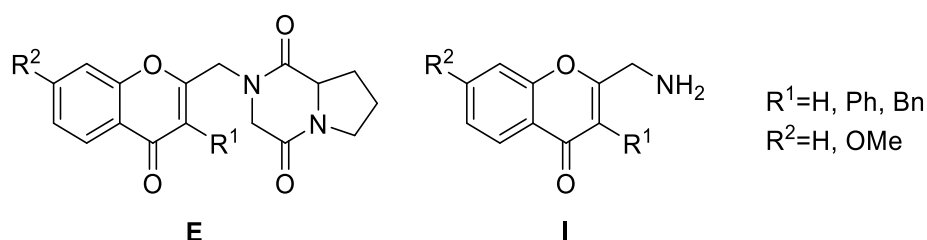
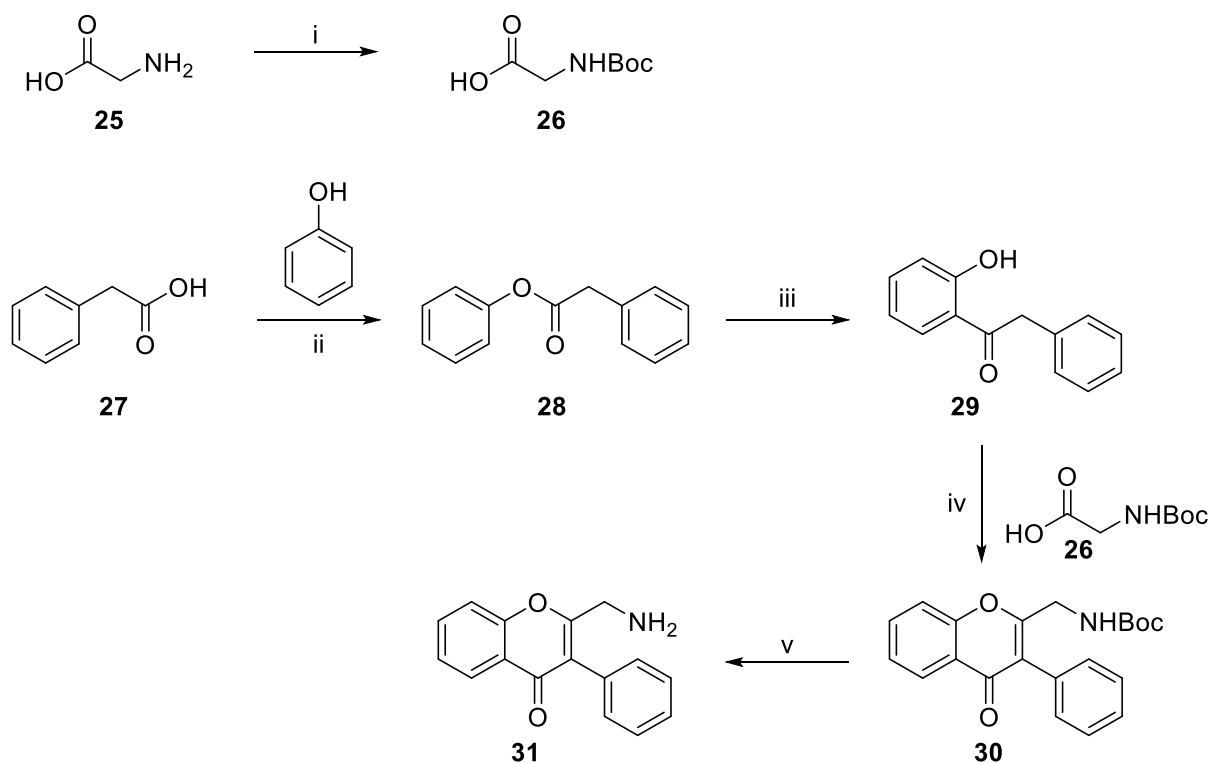


Figure D- 1: Chromone target structures

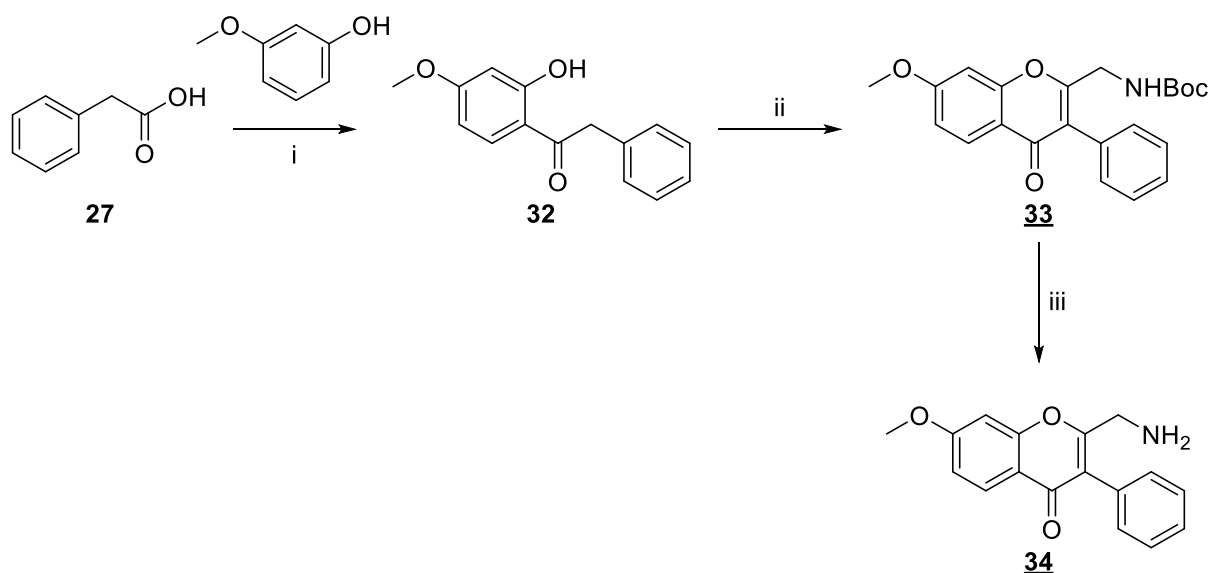
A.15.1. Synthesis of 2-(aminomethyl)-3-phenyl-4H-chromen-4-one and 2-(aminomethyl)-3-phenyl-7-methoxy-4H-chromen-4-one (R¹=Ph, R²=H, OMe)

For R¹=Ph, R²=H, a synthesis for **I** is described ¹³⁰. First the starting materials had to be synthesized. Boc-Glycine (**26**) was obtained in quantitative yield from glycine and di-tert-butyl dicarbonate. The phenol **29** was synthesized via first formation of the ester of phenol and phenylacetic acid (99%, according to ref ¹³¹), and then Fries rearrangement by reaction with AlCl₃ (36%, according to ref ¹³¹). Reaction of the phenol **29** with Boc-glycine and a peptide coupling reagent (HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) under basic conditions (Et₃N) gave directly the cyclised product **30** (66% yield, following ref ¹³⁰). The cyclisation in situ probably occurred due to the conjugation of the system. Deprotection was achieved with TFA in DCM.



Scheme D-16: Synthesis of the phenyl-substituted key intermediate **31**, reaction conditions and yields: i) Boc_2O , Et_3N , dioxane, water, oN, RT, quant.; ii) SOCl_2 , CHCl_3 , 4 h, reflux, then phenol, CHCl_3 , RT, oN, 99%; iii) AlCl_3 , 10 min, RT, then 100°C , 1 h, 36%; iv) Et_3N , HATU, DCM, 42 h, RT, 66%; v) TFA, DCM, RT, 2 h, 35%

This route could also be used for the methoxy-substituted compounds ($\text{R}^1=\text{Ph}$, $\text{R}^2=\text{OMe}$). The methoxy-substituted derivative **32** was obtained by direct Friedel-Crafts acylation using $\text{BF}_3\cdot\text{Et}_2\text{O}$ as a Lewis acid (22% yield, following ref ¹³²). The cyclisation reaction of **32** was achieved with 63% yield following the same procedure as for **29**.



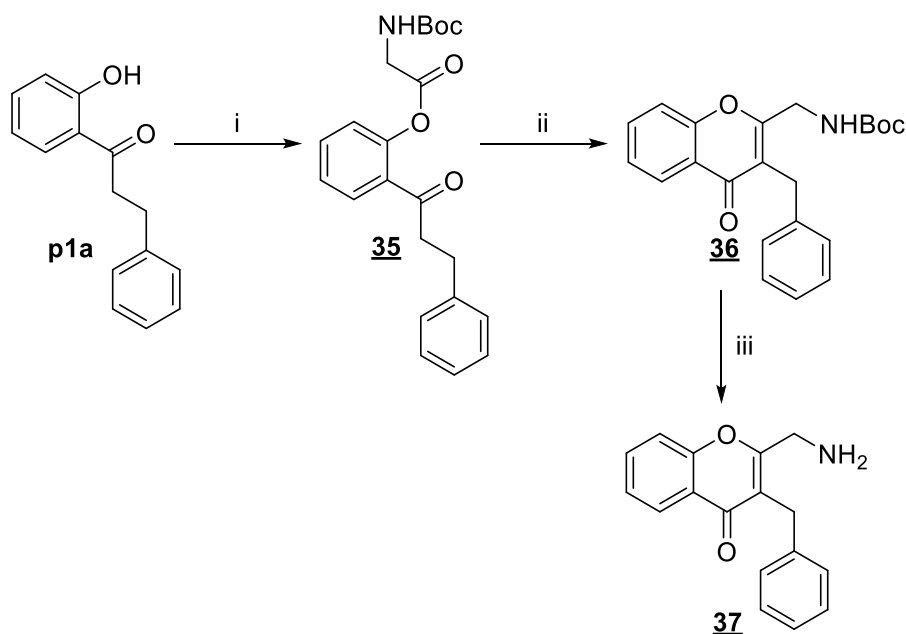
Scheme D-17: Synthesis of the methoxy-substituted intermediate **34**, reaction conditions and yields: i) $\text{BF}_3\cdot\text{Et}_2\text{O}$, 5 h, 85°C , 22%; ii) Et_3N , HATU, DCM, 42 h, RT, 63%; iii) TFA, DCM, RT, 2 h, 45%

A.15.2. Synthesis of 2-(aminomethyl)-3-benzyl-4H-chromen-4-one and 2-(aminomethyl)-3-benzyl-7-methoxy-4H-chromen-4-one ($R^1=Bn$, $R^2=H$, OMe)

Compounds like **1** with $R^1=Bn$ were not described in the literature, but a synthesis described for compounds substituted on the CH_2N -group (i.e. using valine instead of glycine) could be adapted¹³³. It could be shown that, no matter which reaction conditions were used, the reaction of **p1a** and Boc-glycine with esterification reagents and base stopped at the ester stage and no direct cyclisation like for the phenyl-substituted derivatives could be achieved. A screening of different esterification reagents and amounts of triethyl amine showed that the best results were obtained with HATU and 3 eq of Et_3N (see table C-2). The rearrangement and cyclisation were achieved by treatment with potassium carbonate in DMF at 130 °C. Although ester cleavage and coumarin formation occurred as side reactions, sufficient amounts of the desired product could be obtained. The obtained Boc-protected products were deprotected by reaction with TFA.

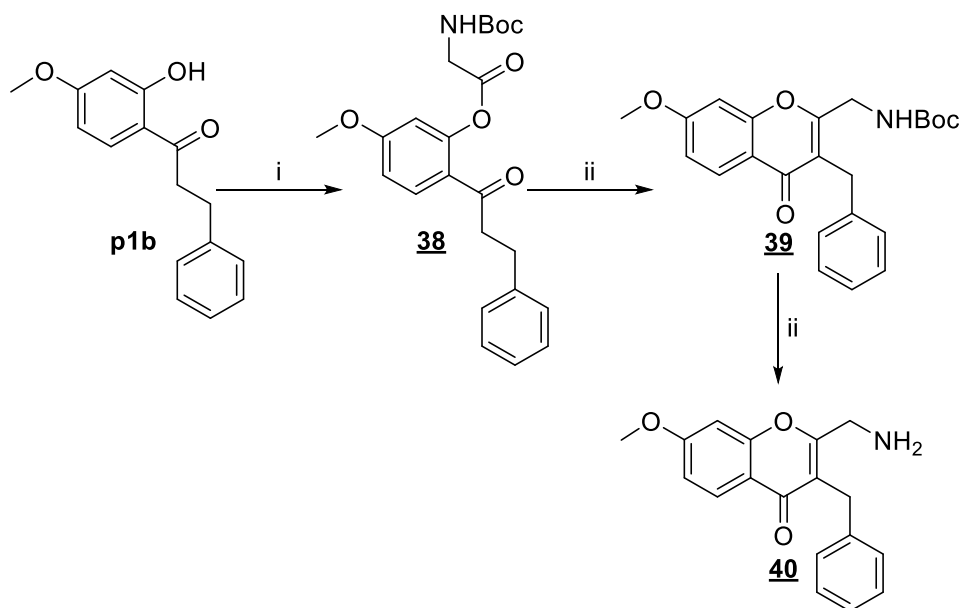
Table C-2: Reaction conditions evaluated for the esterification of **p1a** and Boc-glycine

LITERATURE	REAGENTS	REACTION CONDITIONS	ISOLATED YIELD (35)
134	Et_3N (7.5 eq), HATU (2 eq), DCM	RT, 6 d	5%
134	Et_3N (7.5 eq), HATU (2 eq), DCM	RT, 22 h	17%
-	DCC (1.2 eq), DMAP (0.1 eq), DCM	RT, 4 d	33%
133	Et_3N (3 eq), HATU (1.2 eq), DCM	RT, 4 d	62%
-	Et_3N (1.5 eq), TBTU (1.2 eq), DCM	RT, 4 d	24%



Scheme D-18: Synthesis of the benzyl-substituted key intermediate **37**, reaction conditions and yields: i) Et_3N , HATU, DCM, RT, 4 d, 62%; ii) K_2CO_3 , DMF, 130 °C, 20 min, 27%; TFA, DCM, RT, 2.5 h, 68%.

Again, the same reaction conditions could be applied for the methoxy-substituted derivatives (see Scheme D-19) resulting in similar yields.



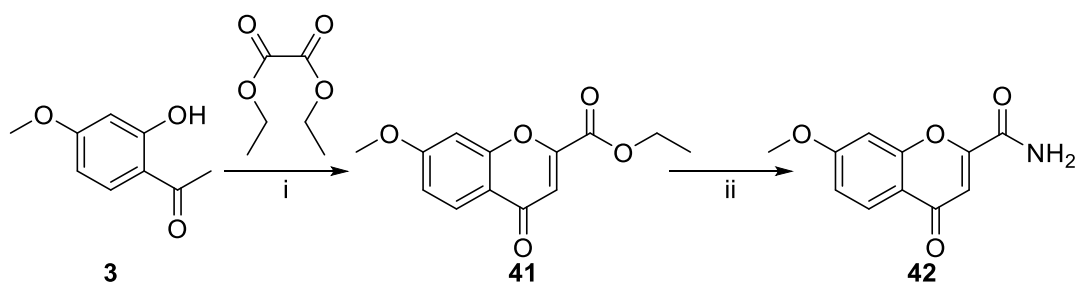
Scheme D-19: Synthesis of the methoxy-substituted intermediates, reaction conditions and yields: i) Et₃N, HATU, DCM, RT, 4 d, 85%; ii) K₂CO₃, DMF, 130 °C, 20 min, 20%; TFA, DCM, RT, 2.5 h, 58%.

A.15.3. Synthesis of 2-(aminomethyl)-4H-chromen-4-one and 2-(aminomethyl)-7-methoxy-4H-chromen-4-one

For compound **I** with R¹=H, R²=H there was one single-step synthesis described which could not be reproduced ¹³⁵. This article describes the direct reaction of glycine ethyl ester, 2'-hydroxyacetophenone and sodium in cyclohexane. The spectral shifts given in the publication differ strongly from the ones obtained for compound **53**, and two signals are missing altogether, therefore it is questionable if the same product was synthesized.

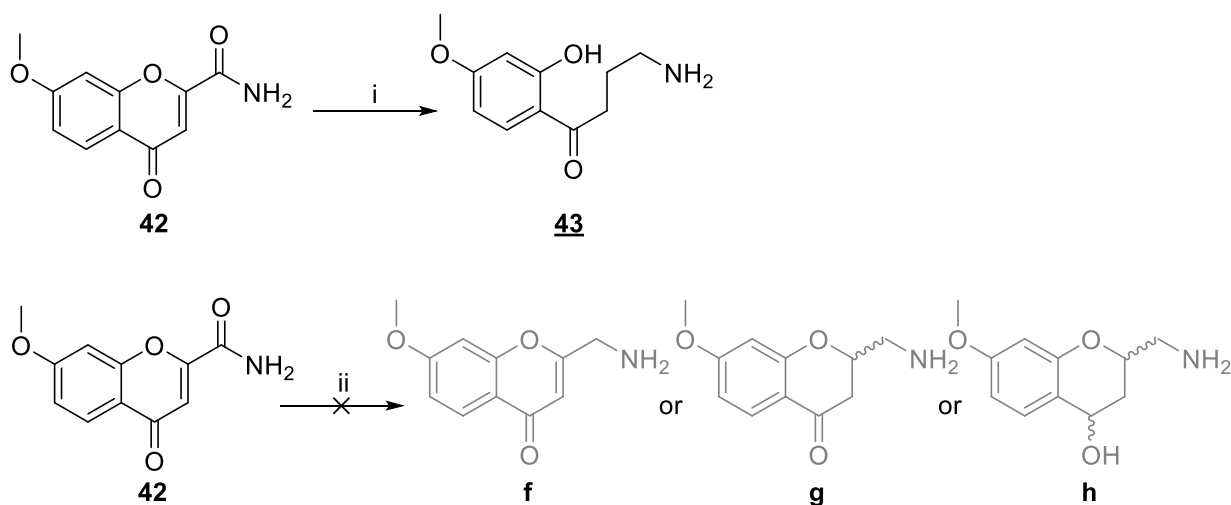
In the patent by Rowbottom ¹³⁶, different strategies for obtaining aminomethylchromones were described. The first one consists of forming the diketone from 2'-hydroxyacetophenone and diethyl oxalate, which is cyclized to the chromone ethyl ester, reducing the ester to obtain the alcohol, transforming it to the chloride, which is subsequently transformed to the azide and then the amine (six steps altogether). Another strategy described involves forming 2-methylchromone in 2 steps via the acetyl ester, bromination on the methyl using NBS, and transforming the bromide to the azide and subsequently the amide (5 steps).

In attempts to find a faster and more straight-forward synthesis, the first approach was the synthesis of the chromone amide **42**, and to try to reduce it to the amine. For this, I followed the strategy by Rebitzer ¹³⁷, in which, starting from the 2-hydroxyacetophenone, the chromone ethyl ester was obtained by reaction with diethyl oxalate and sodium hydride. The intermediate diketone was not isolated and directly cyclized under acidic conditions. This reaction proceeded as described in the literature with 69% yield (Scheme D-20). The next step was the transformation of the ester to the amide, which was accomplished by reaction with ammonia in methanol in 92% yield.



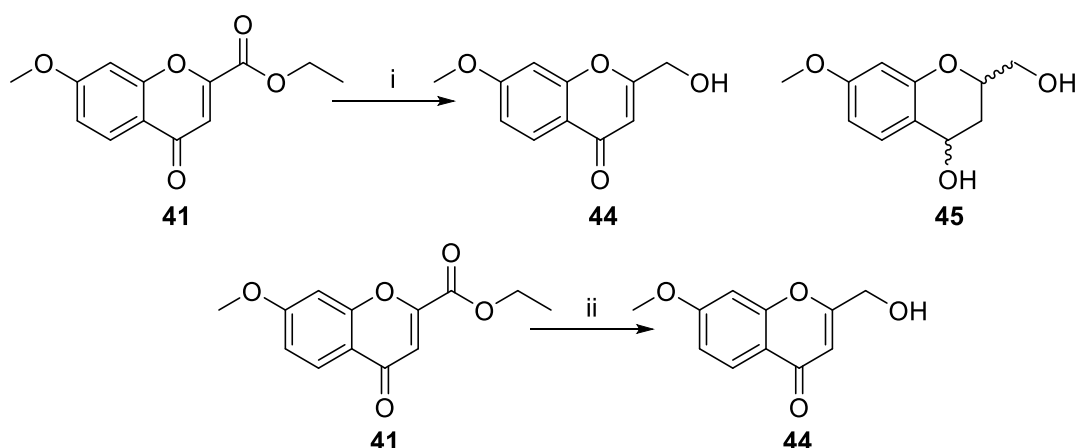
Scheme D-20: Synthesis of the chromone ethyl ester and amide. Reaction conditions and yields: i) Na, EtOH, reflux, 1 h, then conc. H₂SO₄, EtOH, 80 °C, 1 h, 69%; ii) NH₃ in MeOH, MeOH, 0 °C, then RT, oN, 92%

Subsequently, the reduction of the amide to an amine, accompanied by optional reduction of the double bond and/or the ketone, was desired. It was known from literature that the ketone, if it should be reduced to the benzylic alcohol, could be easily re-oxidized using for example PCC¹³⁷. However, the reduction of the amide proved to be difficult. In a first attempt to reduce the amide **42** with LiAlH₄ in THF, an amine was obtained, but structure elucidation showed that it was the ring-opened product **43**. NaBH₄ as a milder reducing agent in MeOH at room temperature did not lead to full conversion, probably mainly due to the poor solubility of **42** in methanol. A small amount (10 mg) of what was thought to be the fully reduced derivative **h** was obtained. Attempts to fully convert the starting material by using different solvents (mixture of methanol, DMF and DMSO) led to the formation of different products, none of which had the desired product mass.



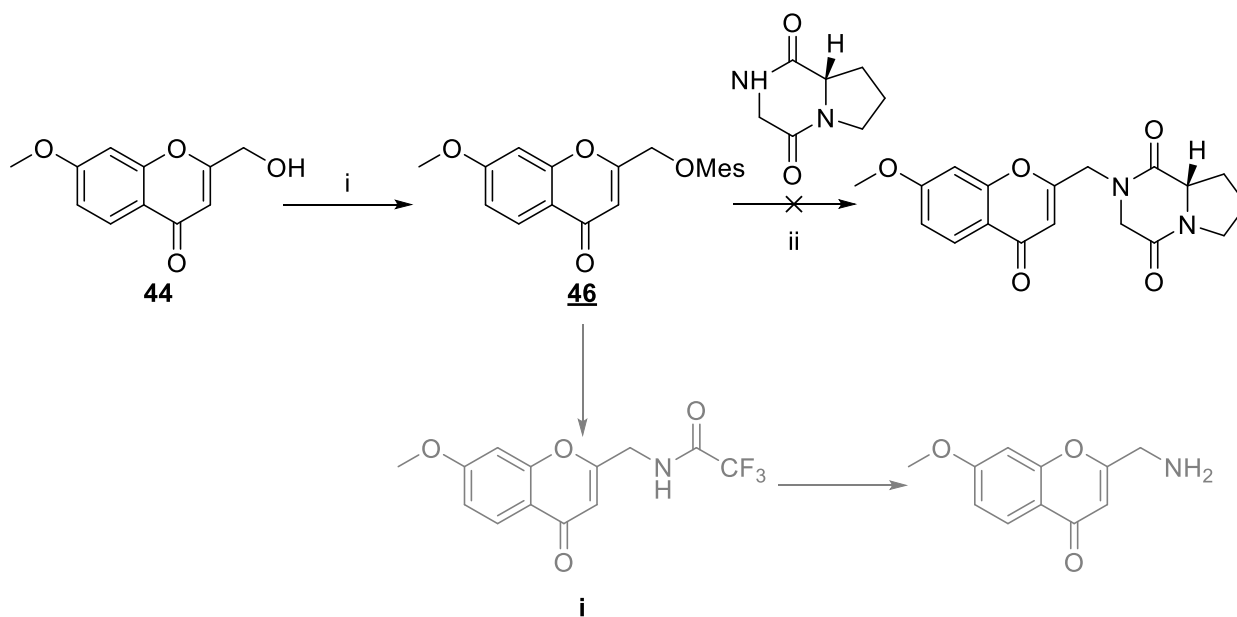
Scheme D-21: Attempts at the synthesis of 2-(aminomethyl)-7-methoxychroman-4-one, reaction conditions and yields: i) LiAlH₄, reflux, 4 h, 8%; ii) NaBH₄, first attempt: RT, 2 d – mostly starting material recovered, 5% of probably **h** obtained (no structure elucidation conducted), second attempt: NaBH₄, DMF:MeOH:DMSO=10:2:6.5, 50 °C, 1 d, mixture of unidentified products (none with desired product mass)

Because the desired product could not be obtained in this fashion or not in sufficient yield, the strategy originally shown by Rebitzer was followed for another step. The ester **41** was reduced with sodium borohydride in methanol which led to different outcomes depending on the reaction temperature. Cooling the reaction with an ice-salt bath led to reduction of solely the ester functionality (**44**), while reaction at room temperature led to a 50:50 mixture of this product and the completely reduced diol **45**.



Scheme D-22: Reduction of the ester **41**, reaction conditions and yields: i) NaBH₄, MeOH, RT, 3.5 h, quant. (1:1 mixture of **44** & **45**), ii) NaBH₄, MeOH, ice-salt bath, 2.25 h, 96%

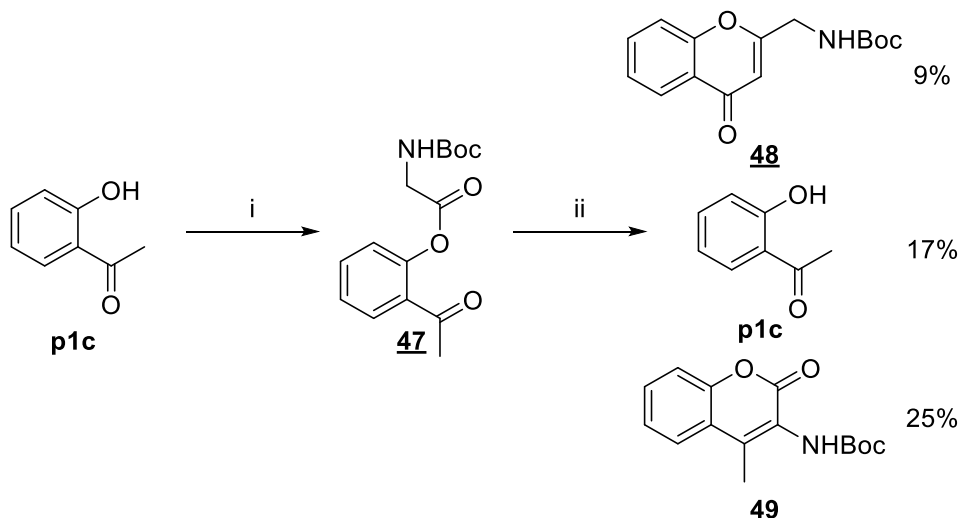
The alcohol **44** was transformed to the mesylate in 80% yield. An attempt to react the mesylate with the diketopiperazine did not lead to the desired product. Although it would have been possible to transform the mesylate to the amine like shown above for the bromide (or via the azide as shown in the patent ¹³⁶), this strategy was abandoned because a faster, more straightforward synthesis was desired.



Scheme D-23: Synthesis of the mesylate **46** and attempted reactions, reaction conditions and yields: i) Et₃N, MesCl, DCM, 0°C, 30 min, then RT, 2 h, 80%, ii) Bu₄NBr, K₂CO₃, ACN, RT, 2 d – degradation of reactants, no desired product formed

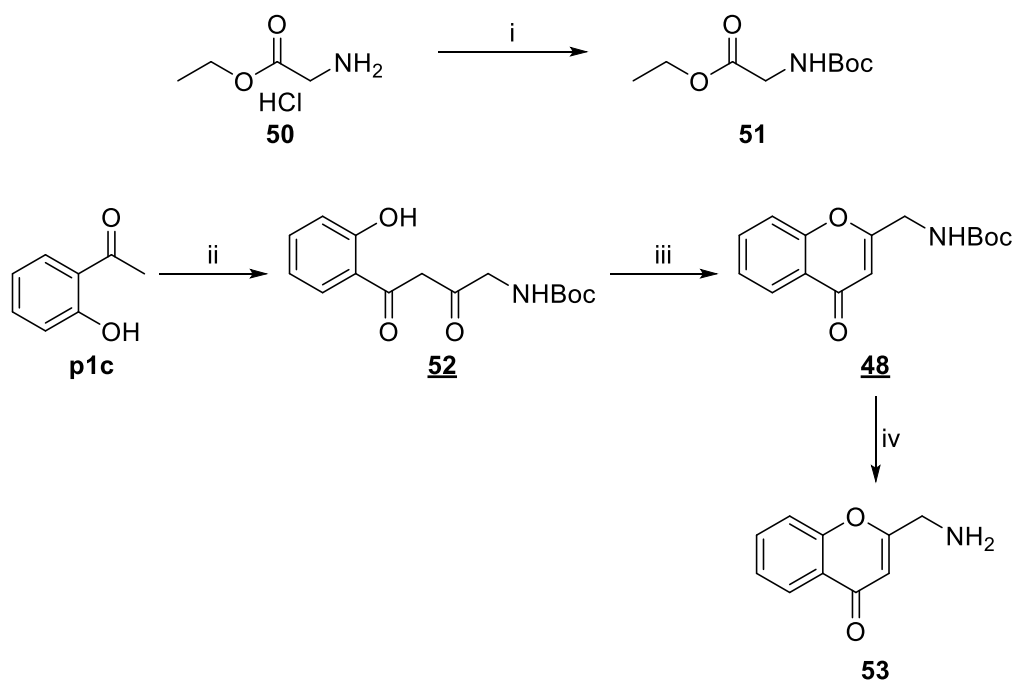
The next envisioned route was to try the same strategy as for the benzyl-substituted derivatives **37** and **40**. The first step, the esterification, worked well also for the unsubstituted 2-hydroxyacetophenone. However, the second step, which should consist of a Baker-Venkatarman rearrangement and cyclisation, could not be achieved successfully for this compound. Treatment with potassium carbonate in DMF led mostly to a cleavage of the ester bond and only formation of **p1c**, while DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as a base led to a complex mixture with only little yield of the desired product **48** (9%) next to 2-

hydroxyacetophenone (**p1c**) and the coumarin **49**. While coumarin formation occurred also in the case of the benzyl derivatives, there the desired chromone was the predominant product.



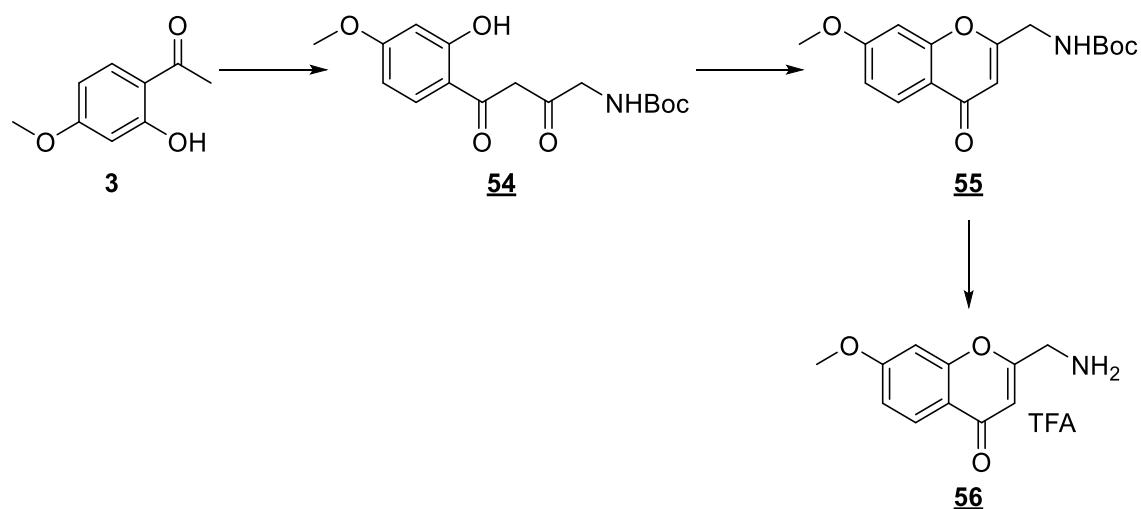
Scheme D-24: Synthesis of **49** via the ester **47**, reaction conditions and yields: i) Boc-glycine, HATU, Et_3N , DCM, 18 h, 92%; ii) DBU, pyridine, 85 °C, 1 h, 9%

In attempts to get access to a higher-yielding synthetic route, diketone formation followed by cyclisation was the next idea. This route is not described for amino-substituted derivatives, and the main obstacle seemed to be that the cyclisation is usually conducted under strongly acidic conditions incompatible with the Boc protecting group (see e.g. preparation of **41** following ref ¹³⁷). However, records of mild, basic conditions for the cyclisation could be found (e.g. ref ¹³⁸), therefore this route seemed promising. The diketone was formed following ref ¹³⁹ via the reaction of **p1c** with Boc-proline ethyl ester and NaH. The obtained mixture, which consisted of the diketone existing in keto- and enol forms, starting material and the cyclised product, was transformed completely to the cyclised product **48** by treatment with a catalytic amount of pyrrolidine (41%, over 2 steps). Workup and not too high catalyst loading was crucial in this step to avoid the formation of an addition and ring opening product with pyrrolidine. Deprotection was achieved like described before.



Scheme D-25: Successful synthesis of **53**, reaction conditions and yields: i) Et_3N , Boc_2O , THF, $0\text{ }^\circ\text{C}$, 40 min, then RT, oN, 96%; ii) NaH (washed), Boc-glycine ethyl ester, THF, RT, 2.25 h; iii) pyrrolidine, MeOH, H_2O , $55\text{ }^\circ\text{C}$, 19 h, 41%, over 2 steps; iv), TFA, DCM, RT, 2.5 h, 75%

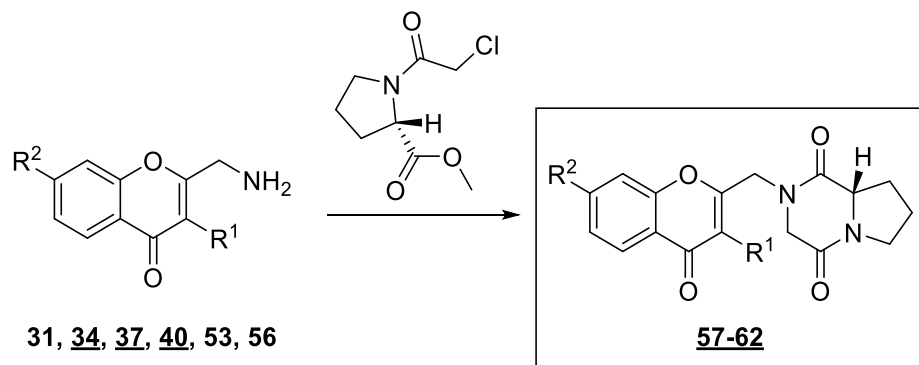
The same reaction conditions were applied for the synthesis of the methoxy-substituted derivative **56**. In a first attempt to increase the conversion of **3** to **54**, the reaction mixture was heated to reflux for 1.5 h. However, this led to a partial transformation of the tertbutyl-carbamate to the ethyl carbamate which was subsequently impossible to cleave. Therefore, in a second attempt, the reaction mixture was only stirred at room temperature, which did not lead to the undesired ethyl carbamates. An attempt at the diketone formation with **p1a** did not lead to any conversion, indicating that this reaction might only work for unsubstituted derivatives. As the final amines **53** and its methoxy analogue are highly polar and require repeated extraction with organic solvents to remove quantitatively from the aqueous phase, **56** was not transformed to the free amine, but was isolated as the trifluoroacetate, which was directly used in the next step.



Scheme D-26: Synthesis of the methoxy-substituted key intermediate **56**, reaction conditions and yields: i) NaH (washed), Boc-glycine ethyl ester, THF, RT, oN; ii) pyrrolidine, MeOH, H_2O , $55\text{ }^\circ\text{C}$, 19 h, 40%, over 2 steps; iii), TFA, DCM, RT, 2.5 h, 97%

A.15.4. Synthesis of diketopiperazine derivatives of chromones

The formation of the diketopiperazine derivatives was accomplished like described above by reaction of the amines with the reagent **7**. In the case of trifluoroacetate **56**, the used amount of triethyl amine was doubled.



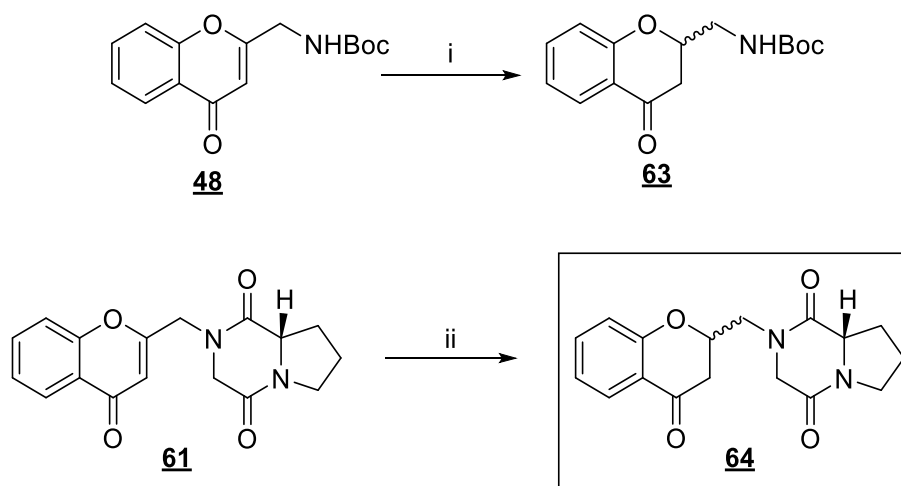
Scheme D-27: Synthesis of the diketopiperazine derivatives of chromones, reaction conditions: Et_3N , 2-ethoxyethanol, reflux, 43 h, yields summarized in table C-3

Table D-3: Yields for the synthesis of diketopiperazine derivatives

STARTING MATERIAL	PRODUCT	R^1	R^2	YIELD
31	57	Ph	H	28%
34	58	Ph	OMe	36%
37	59	Bn	H	31%
40	60	Bn	OMe	31%
53	61	H	H	64%
56	62	H	OMe	27%

A.16.Synthesis of chromanone derivatives (F)

To obtain the chromanone derivatives, the conjugated double bond of the chromone compounds had to be reduced. To study the reaction, intermediate **48** was used. A first attempt at the reduction with palladium on charcoal and an atmospheric hydrogen pressure following ref ¹⁴⁰ showed that not only the double bond, but also the ketone was reduced partially, thus not giving a selective reaction. The next tried reagent, L-selectride (lithium tri-sec-butylborohydride), inspired by the publication of Thomas *et al* ¹⁴¹, showed primarily a selective reduction of only the double bond (if no more than 1.1 eq were used). Therefore, this was the reducing agent of our choice. The first idea to reduce the final diketopiperazine derivatives obtained in the step before, only worked for the unsubstituted derivative **61**. Two diastereomers were obtained which could be separated by chiral preparative HPLC. The methoxy derivative **62** did not react at all even when the reaction mixture was warmed to room temperature overnight, probably due to solubility problems in THF. For the other derivatives, a full conversion could not be achieved, and in some cases also the ring-opened product of a retro-oxa-Michael reaction could be observed.

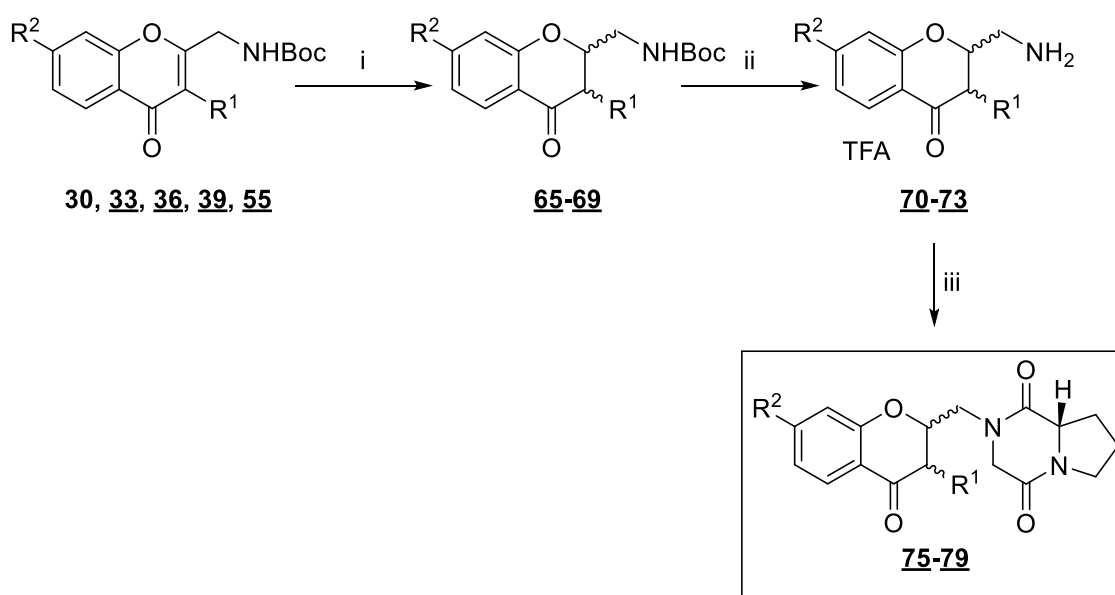


Scheme D-28: Reduction of chromone derivatives, reaction conditions and yields: i) L-selectride, THF, -78 °C, 1 h 10 min, 55%; ii) L-selectride, THF, -78 °C, 1 h 40 min, 59%;

Reductions at the stage of the Boc-protected intermediates, however, proceeded smoothly for all compounds. A clear preference for one isomer in the reduction was seen in all cases, the ratio always being approximately 1:0.2. For determining which isomer was the *cis* and which was the *trans* product, the gamma-gauche effect was used. Gamma effects are defined as the effects of a replacement of a hydrogen by a substituent X on the second atom relative to one carbon atom (*C-C-C-H $\rightarrow$ *C-C-C-X). These effects show a strong proximity component (syn γ -effect, γ -gauche effect) resulting in a dependence on stereochemistry. Syn γ -effects usually lead to a lower frequency ¹³C shift ($\Delta\delta$ is negative, upfield). This means that if in one isomer a γ -atom is close to a carbon, and remote in another, then the chemical shift of that carbon will be upfield (lower frequency) in the first isomer ¹⁴².

In our case, the ¹³C chemical shifts of the CH₂N- and the 1'-groups (C1 of phenyl ring) for the phenyl-substituted compounds, and the chemical shifts of the CH₂N and the CH₂Ph-groups for the benzyl-substituted compounds were compared for the two isomers (see table D-4). Isomer

1 was the major isomer obtained in the reduction. The isomers with the higher chemical shift are marked bold in the table. Interestingly, it can be seen that judging from the carbon chemical shifts, the major isomer would be assigned as *cis* for the phenyl-substituted compounds, and as *trans* for the benzyl-substituted compounds. For the phenyl-substituted compounds **65** and **66**, coupling constants of H-2 and H-3 could be determined, which were small (singlet when measured at 500 MHz, 2.3-3.5 Hz when measured at 200 MHz) for the major isomer and big ($J=11.3$ -11.8 Hz) for the minor isomers. A big coupling constant could be the result of a big dihedral torsion angle between the two vicinal hydrogens in a *trans* isomer.



Scheme D-29: Reduction and diketopiperazine formation, reaction conditions: i) L-selectride, THF, -78 °C, 1 h 10 min; ii) TFA, DCM, 2.5 h; iii) 7, Et₃N, 2-ethoxyethanol, reflux, 3 d; yields summarized in table C-4

Table D-4: Yields, ¹³C shifts of selected carbons and ratio of *cis*/*trans* isomers for the reduction with L-selectride (*trans* isomer ¹³C chemical shift in bold)

START. MAT.	R ¹	R ²	YIELD STEP I	PRODUCT STEP I, ISOMER MAINLY FORMED	¹³ C SHIFT CH ₂ N (ISOMER 1, MAJOR, PPM)	¹³ C SHIFT CH ₂ N (ISOMER 2, MINOR, PPM)	¹³ C SHIFT 1'/CH ₂ PH (ISOMER 1, MAJOR, PPM)	¹³ C SHIFT 1'/CH ₂ PH (ISOMER 2, MINOR, PPM)	RATIO CIS/TRANS
30	Ph	H	64%	65 , <i>cis</i>	42.36	42.97	133.41	134.13	1:0.18
33	Ph	OMe	83%	66 , <i>cis</i>	42.42	42.99	133.76	134.59	1:0.21
36	Bn	H	85%	67 , <i>trans</i>	41.48	40.30	32.46	30.62	0.20:1
39	Bn	OMe	82%	68 , <i>trans</i>	41.89	40.39	32.76	30.78	0.22:1
55	H	OMe	51%	69					

For the phenyl substituted derivatives, a racemisation of the stereo centre next to the ketone could be observed during the deprotection step, whereas the ratio stayed similar for the benzyl-substituted compounds. This seems plausible following the assumption that *trans* isomers are more stable, and racemisation is possible via the enol form. Opposed to the strategy for the chromone derivatives, the free amines were not generated, but the

trifluoroacetates were used directly in the reaction with the diketopiperazine forming reagent. This was done to avoid the presence of amine and unprotected ketone in the molecule and possible imine formation as a side reaction.

Table D-5: Yields, ^{13}C shifts of selected carbons and ratio of cis/trans isomers for the deprotection with TFA

STARTING MATERIAL STEP II	YIELD STEP II	PRODUCT STEP II, ISOMER MAINLY FORMED	^{13}C SHIFT CH_2N (ISOMER 1, PPM)	^{13}C SHIFT CH_2N (ISOMER 2, PPM)	^{13}C SHIFT $1'/\text{CH}_2\text{PH}$ (ISOMER 1, PPM)	^{13}C SHIFT $1'/\text{CH}_2\text{PH}$ (ISOMER 2, PPM)	RATIO CIS/TRANS
65	Quant.	70 , trans	41.77	42.58	131.90	132.55	1:1.20
66	Quant.	71 , trans	41.72	42.45	132.28	133.04	1:1.10
67	Quant.	72 , trans	41.47	40.26	32.55	30.67	0.25:1
68	Quant.	73 , trans	41.62	40.36	33.00	30.87	0.36:1
69	Quant.	74					

The formed diketopiperazines should be two or four diastereomers, respectively. Attempts to separate them by chiral or achiral preparative HPLC were difficult as they behave very similarly chromatographically. For **75**, a partial separation of the stereoisomers by chiral preparative HPLC was possible. As analytical runs over a chiral column did not seem promising for the other compounds, they were purified by reversed phase preparative HPLC. Unfortunately, for the methoxy-substituted derivatives **76** and **78**, an impurity could not be removed and they could not be obtained in the purity needed for biological testing.

Table D-6: Yields of the diketopiperazine formation

STARTING MATERIAL	YIELD STEP III (COMBINED YIELD OF ALL PRODUCT FRACTIONS AFTER PREPARATIVE HPLC)	PRODUCT STEP III	COMMENTS
70	6%	75	P1:1:0.11 mixture, mainly isomer 1 (trans) P2: 1:0.82 mixture of isomers 1 & 2 (cis) P3:1:0.08 mixture, mainly isomer 3 (cis)
71	5%	76	1:0.73:0.10:0.10 mixture of 4 diastereomers (main isomers trans), 18 mol% impurity
72	7%	77	1:0.79:0.46:0.23 mixture of 4 diastereomers (main isomers trans)
73	13%	78	1:1:0.45:0.38 mixture of 4 diastereomers (main isomers trans), 31 mol% impurity
74	16%	79	1:1 mixture of 2 diastereomers

A.17. Attempts at the synthesis of final target molecule G

The last step of the transformation from propafenones to fumitremorgin C would have been derivatives with five annulated rings as shown in Figure D-2. The general idea was to start with the reduced derivative **64**, and to try a condensation with benzaldehyde, formaldehyde or equivalent reagents.

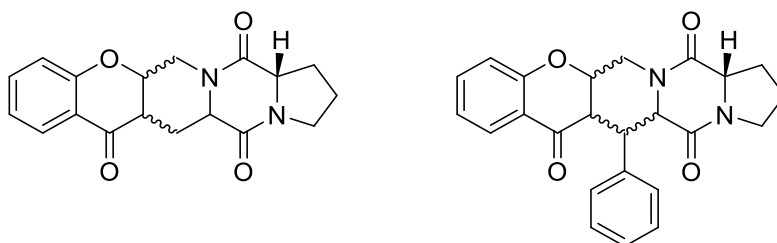
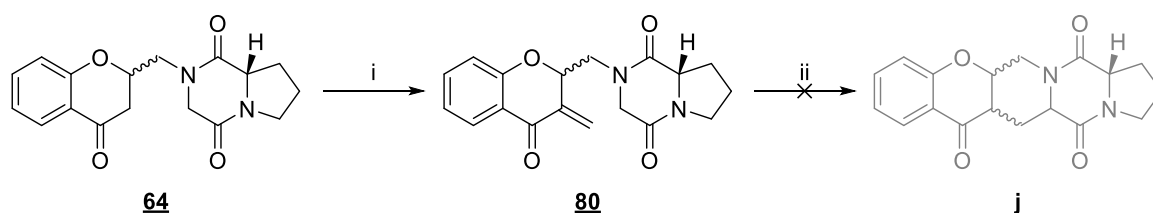


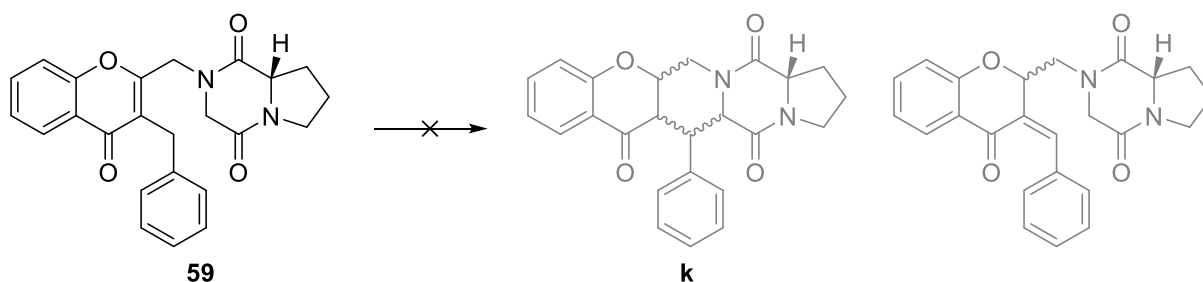
Figure D-2: Final target molecules with five annulated rings

For the formal condensation with formaldehyde, a procedure using FeCl_3 , potassium peroxodisulfate and dimethylacetamide or DMF was found, which gave the intermediate product **80** in 35% yield¹⁴³. Unfortunately, attempts to cyclise this compound under different conditions (LDA or $\text{BF}_3 \cdot \text{Et}_2\text{O}$) failed and led only to the degradation of **80**.



Scheme D-30: Attempted synthesis of, reaction conditions and yields: i) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{K}_2\text{S}_2\text{O}_8$, DMF, 115°C , 2 h, 35%; ii) 1. LDA (bought), THF, -78°C , 1h, then RT, 1 h – degradation, 2. LDA (freshly prepared), -78°C , 30 min, then 0°C , 1h – degradation, 3. $\text{BF}_3 \cdot \text{Et}_2\text{O}$, THF, RT, oN, degradation

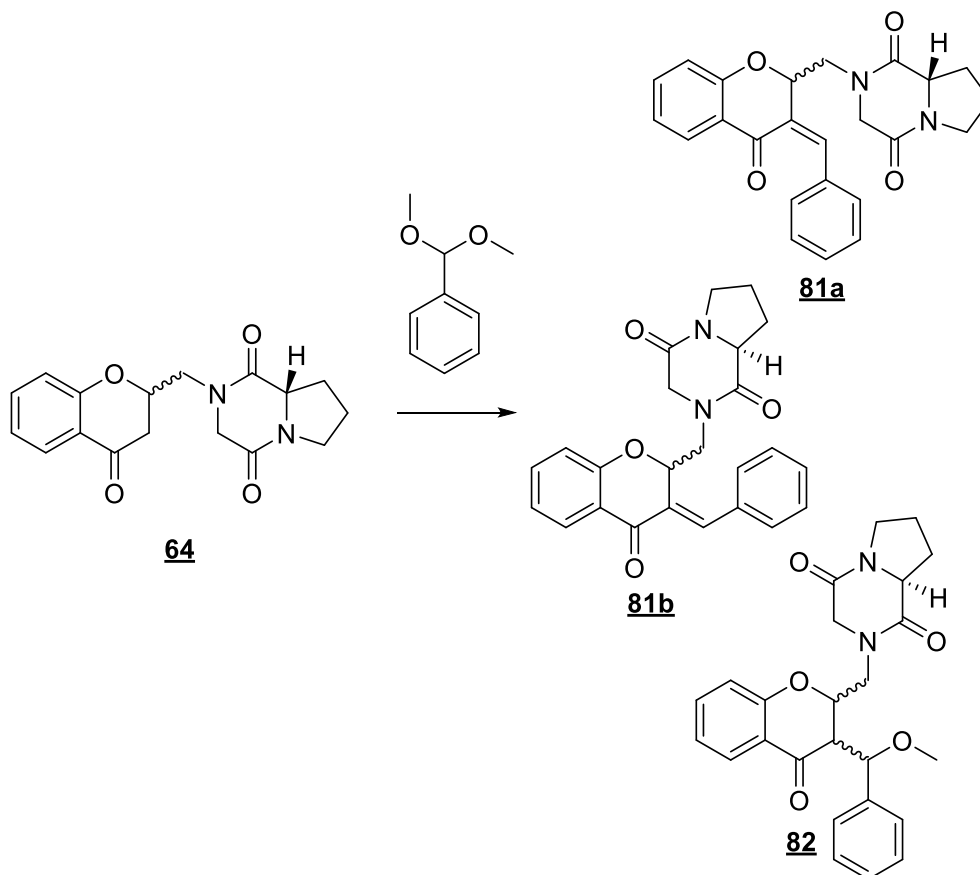
In attempts to achieve the formal condensation with benzaldehyde, various different reaction conditions were examined. The first approach was to investigate if the double bond in **59** could be isomerized using a palladium catalyst (see Scheme D-31). Unfortunately, no conversion occurred under the used reaction conditions ($\text{Pd}(\text{ACN})_2\text{Cl}_2$, MeOH), only the starting material was recovered.



Scheme D-31: Attempted double bond isomerisation of **59**, reaction conditions: $\text{Pd}(\text{ACN})_2\text{Cl}_2$, MeOH, RT, 2 d – no reaction

The second envisioned strategy was the reaction with benzaldehyde dimethyl acetal and a Lewis acid (see Scheme D-32). Boron trifluoride diethyl etherate as a Lewis acid gave contradictory results (see table D-7), once a mixture of starting material, condensation product **81b** and ether **82** (confirmed by MS) was obtained, when the reaction was repeated 4 times, no conversion could be seen. Other, weaker Lewis acids (FeCl_3 , MgBr_2) did not give a reaction, $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in DCM led to degradation of the starting materials, probably due to the

evaporation of the volatile solvent and reagent. The best results were obtained when TMSOTf was used in DCM, under these conditions, solely **81** (mixture of E and Z isomers) was obtained in 52% yield.

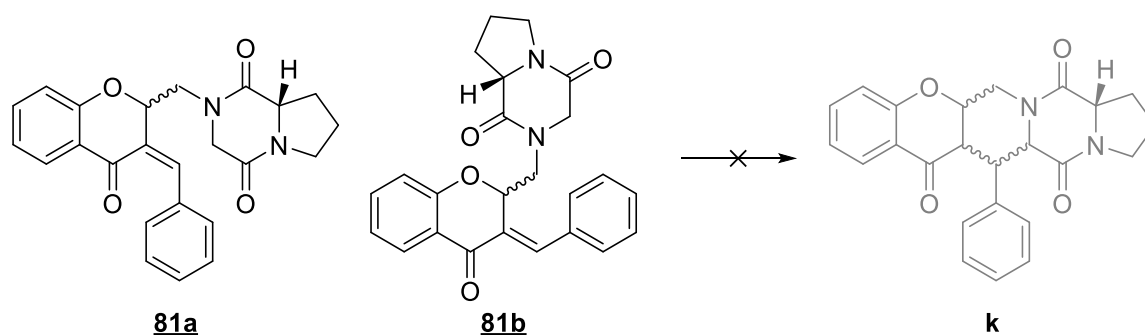


Scheme D-32: Reaction between **64** and benzaldehyde dimethyl acetal, reaction conditions are shown in table C-5

Table D-7: Reaction conditions used for the reaction of **64** and benzaldehyde dimethyl acetal

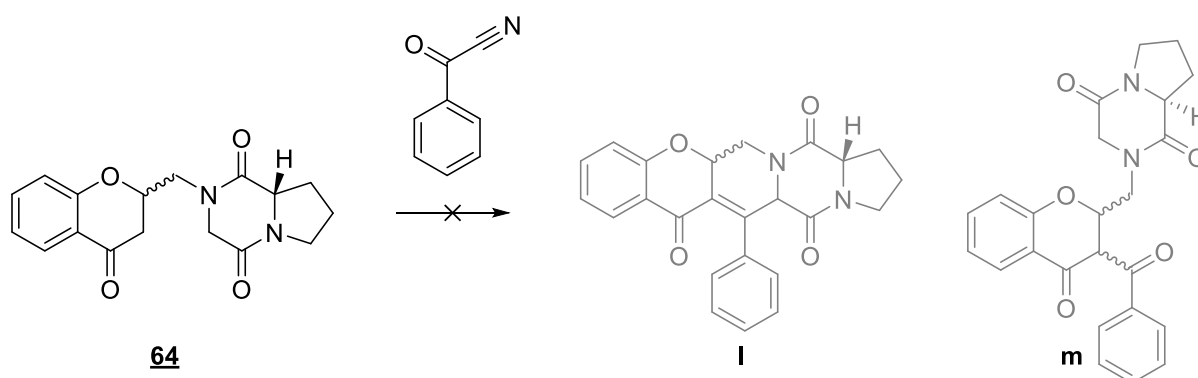
REAGENTS	TEMPERATURE, TIME	OUTCOME
BF ₃ *Et ₂ O, THF	RT, 2 d	5 attempts, once successful – mixture of 64 , 81b & 82 , otherwise no reaction
FeCl ₃ , THF	RT, 4 d	No reaction
MgBr ₂ , THF	RT, 4 d	No reaction
BF ₃ *Et ₂ O, DCM	RT, 3 d	Degradation
TMSOTf, DCM	-78 °C, 1h, 0 °C, 1.5 h, RT, oN	Selective formation of 81a & 81b (52%, mixture of isomers, E1:E2:Z1:Z2=1:0.30:0.92:0.27)

Unfortunately, the cyclisation never occurred directly in the course of the reaction with benzaldehyde dimethyl acetal. An attempt to cyclise the mixture of **81a** and **81b** using LiHMDS in THF only led to degradation products. It seems plausible that the cyclisation of the E product **81b** is difficult because of the conformation, why no cyclisation of **82** or **81a** occurred would need further investigation and is not fully clear.



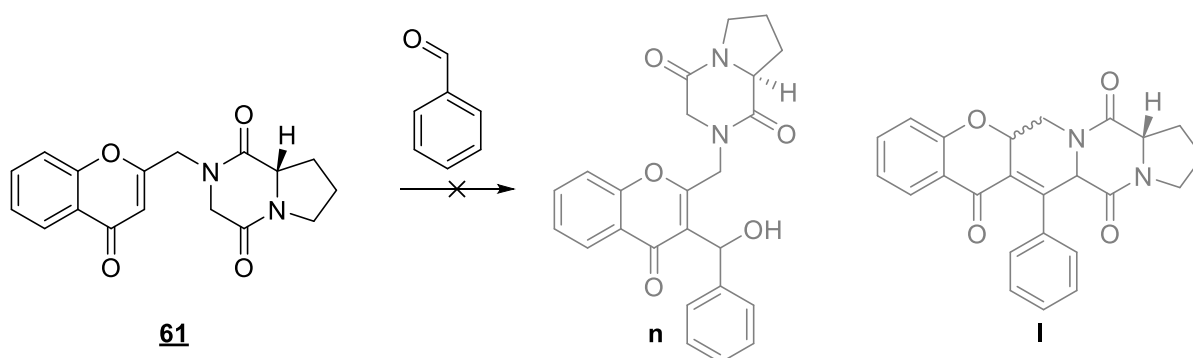
Scheme D-33: Attempted cyclisation of 81, reaction conditions: LiHMDS, THF, -30 °C, 45 min, RT, 1 h – degradation

Another envisioned strategy was working in a different oxidation state and reacting **64** with benzoyl cyanide and LiHMDS following ref ¹⁴⁴; however, also in this case only degradation products were obtained, and no product showing the desired mass could be found.



Scheme D-34: Attempted reaction of 64 and benzoyl cyanide, reaction conditions: LiHMDS, THF, RT, 1.5 h

The last tried synthetic route was a Baylis-Hilman reaction of **61** and benzaldehyde using DABCO (1,4-Diazabicyclo[2.2.2]octane) ¹⁴⁵. Unfortunately, no conversion occurred under the used conditions.



Scheme D-35: Baylis-Hilman reaction, reaction conditions: DABCO, MeOH, RT, 4d – no reaction

In summary, it seems that the cyclisation is difficult to achieve and needs further investigation or a completely different synthetic approach.

Biology and Structure-Activity

Further details of the *in vitro* and *in silico* studies and the SAR analysis can be found in the publication in the appendix. Cell culture experiments were conducted by Anna Cseke, Katrin Wlcek, Simon Decker and Sarah Palme. *In silico* predictions were done by Floriane Montanari.

A.18. *In vitro* studies

A.18.1. General

Inhibitory activity for Pgp and BCRP was tested in an intracellular accumulation assay of daunorubicin or mitoxantrone in cells overexpressing Pgp or BCRP, respectively. Experimental details of the assay can be found in the publication in the appendix. Generally, first an experiment at 100 μ M final concentration was conducted. If compounds showed an inhibitory effect of more than 50% compared to full inhibition by the established inhibitors verapamil (Pgp) or Ko143 (BCRP), IC₅₀ values were determined. To enable comparison to the *in silico* classification models, compounds showing an IC₅₀ below 10 μ M were considered active.

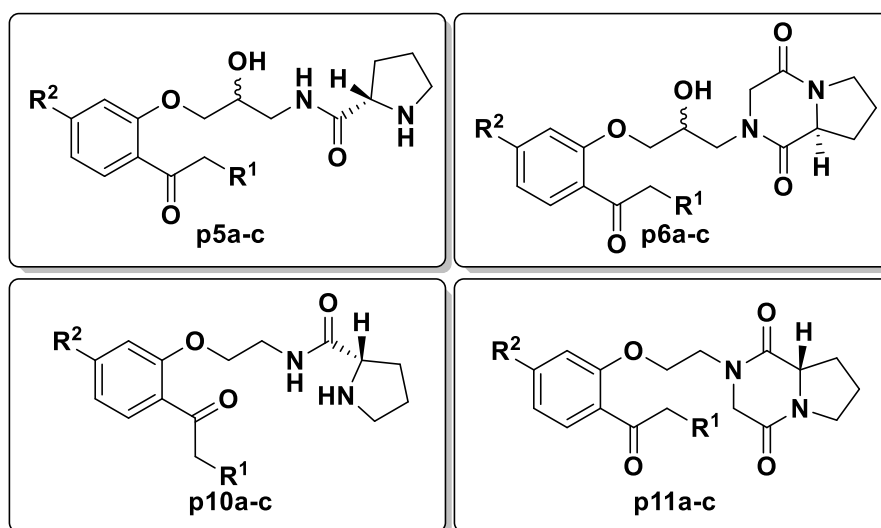
A.18.2. Publication: “Subtle Structural Differences Trigger Inhibitory Activity of Propafenone Analogues at the Two Polyspecific ABC Transporters: P-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)”, proline and diketopiperazine derivatives of propafenones and 2-ethoxyacetophenones

Table E-1 shows the results obtained for the proline and diketopiperazine derivatives of propafenones and 2-ethoxyacetophenones, figure E-1 shows the structures of the synthesized compounds. The results are explained in more detail in the publication in the appendix. In summary, six compounds were found to be inhibitors of BCRP (**p5b**, **p6a**, **p6b**, **p10a**, **p10b**, **p11b**), additionally one compound (**p5a**) showed borderline activity on BCRP with an IC₅₀ value of 16.13 ± 3 μ M. With respect to Pgp, also 6 compounds were classified as inhibitors (**p5a**, **p5b**, **p6a**, **p10a**, **p10b**, **p11b**) and two as borderline inhibitors (**p6b**, **p11a**).

If the inhibitory activities on Pgp and BCRP are compared, the result is that four compounds (**p5a**, **p6a**, **p10a** and **p10b**) are more effective on Pgp than on BCRP, with the most pronounced difference for **p5a**, which shows an about eight-fold higher inhibition of Pgp than of BCRP. **p6a**, **p10a** and **p10b** were more than 2.6-fold better inhibitors for Pgp than for BCRP, and compounds **p5b** and **p11a** exhibited comparable inhibitory activity on both ABC transporters. Only two compounds, namely **p6b** and **p11b**, displayed a higher inhibitory activity on BCRP than on Pgp.

Table E-1: Results of *in silico* predictions and *in vitro* IC₅₀ evaluation of BCRP and Pgp inhibition for the synthesized compounds

Cpd ^a	logP ^c	Substituents		BCRP		Pgp	
		R ¹	R ²	<i>in silico</i> ^e	IC ₅₀ [μ M] ^f	<i>in silico</i> ^e	IC ₅₀ [μ M]
p5a	2.37	Bn ^b	H	0.25	16.1 $\pm$ 3.0	0.97	2.1 $\pm$ 0.4
p5b	2.36	Bn ^b	OMe	0.37	3.1 $\pm$ 0.5	0.92	2.2 $\pm$ 0.6
p5c	0.53	H	H	0.08	- ^d	0.46	58.7 $\pm$ 9.2
p6a	1.93	Bn ^b	H	0.39	5.8 $\pm$ 1.0	0.98	1.6 $\pm$ 0.6
p6b	1.92	Bn ^b	OMe	0.64	3.8 $\pm$ 0.6	0.96	11.0 $\pm$ 1.0
p6c	0.09	H	H	0.15	- ^d	0.78	- ^d
p10a	2.94	Bn ^b	H	0.17	3.3 $\pm$ 0.6	0.88	1.3 $\pm$ 0.1
p10b	2.93	Bn ^b	OMe	0.39	2.9 $\pm$ 0.5	0.83	1.0 $\pm$ 0.2
p10c	1.10	H	H	0.07	- ^d	0.19	34.8 $\pm$ 1.8
p11a	2.50	Bn ^b	H	0.40	21.5 $\pm$ 2.9	0.94	13.9 $\pm$ 1.5
p11b	2.49	Bn ^b	OMe	0.75	2.3 $\pm$ 0.0	0.91	6.2 $\pm$ 0.4
p11c	0.66	H	H	0.19	- ^d	0.42	- ^d
24	0.28	Me	H	0.09	-	0.59	-

^a Compound name^b benzyl^c calculated as logP(o/w) with MOE 2014.09^d IC₅₀ was not determined^e score given by the *in silico* models, between 0 and 1. It roughly corresponds to the probability of being an inhibitor.^f IC₅₀ values are given as mean $\pm$ SD of at least 3 independent experiments with at least duplicates in each experiment and reported in μ M. p11a was measured in 2 independent experiments for inhibition on BCRP.Figure E-1: Structures of tested compounds p5a-c to p11a-c, 24 corresponds to p5a-c, R¹=Me, R²=H

A.18.3. Chromone and chromanone compounds

The results of the biological assays and *in silico* screening for the chromone and chromanone compounds are summarized in table E-2, the structures can be found below in figure E-2. Compounds **76** and **78** have not been tested yet due to purification issues, and compound **62** could not be measured due to insolubility in DMSO. Concerning Pgp inhibitory activity, only one compound (**60**) could be found which is an inhibitor giving an IC₅₀ below the threshold of 10 μ M. All fractions of **75** were found to be borderline inhibitors of roughly the same activity,

indicating that stereochemistry has no discernible effect on Pgp inhibition. Additionally, the mixture of the diastereomers of **77** was identified as a borderline inhibitor with an IC₅₀ of 14.6 μ M.

Table E-2: Results of *in silico* predictions and *in vitro* IC₅₀ evaluation of BCRP and Pgp inhibition for the synthesized chromone and chromanone compounds

Cpd	logP	Substituent		BCRP		Pgp		
		R ¹	R ²	<i>In silico</i>	% inhibition at 100 μ M	<i>In silico</i>	IC ₅₀ [μ M]	% inhibition at 100 μ M
57	0.6	Ph	H	0.11	18.1	0.72	-	11.8
58	0.47	Ph	OMe	0.29	46.3	0.84	12.2 $\pm$ 1.1	42.9
59	1.02	Bn	H	0.09	58.6	0.81	-	32.4
60	0.89	Bn	OMe	0.15	81.89	0.91	8.5 $\pm$ 1.3	67.2
61	-0.9	H	H	0.19	34.4	0.57	-	-5.0
62	-1.1	H	OMe	0.12	n.d. ^a	0.54	n.d. ^a	n.d. ^a
75P1^b	1.22	Ph	H	0.53	57.6	0.78	15.1 $\pm$ 1.0	63.5
75P2^d	1.22	Ph	H	0.53	72.4	0.78	10.9 $\pm$ 1.5	51.8
75P3^d	1.22	Ph	H	0.53	61.6	0.78	11.1 $\pm$ 1.2	47.5
76	1.09	Ph	OMe	0.64	n.d. ^c	0.89	n.d. ^c	n.d.
77	1.64	Bn	H	0.26	77.8	0.78	14.6 $\pm$ 1.7	41.2
78	1.51	Bn	OMe	0.51	n.d. ^c	0.91	n.d. ^c	n.d.
64P2^b	-0.5	H	H	0.33	4.5	0.26	-	-6.8
64P3	-0.5	H	H	0.33	12.1	0.26	-	-0.6
79	-0.7	H	OMe	0.61	75.6	0.42	-	11.8

Unless noted otherwise, IC₅₀ values were determined in three independent experiments in at least duplicates and are expression-corrected (see publication in Appendix A for details of the calculation).

^a not determined because of insolubility in DMSO

^b diastereomers were separated (partially), different fractions were tested separately

^c not tested yet due to purification issues

^d IC₅₀ values determined from two independent experiments

For BCRP inhibition, the percental inhibition at a final concentration of 100 μ M compared to Ko143 was determined. Percental inhibition at 100 μ M was determined in triplicates in two independent experiments, the mean values are given in table E-2. Of the chromone compounds **57-62**, **59** and **60** show some activity, and could be classified in the medium to borderline (**59**) or inhibitor (**60**) range. All other chromone compounds show an inhibition under 50% at 100 μ M and are therefore definitely non-inhibitors. The activity of compound **62** could not be determined because of its insolubility in DMSO. Concerning the chromone compounds **75-79**, all compounds except the isomers of **64** show an inhibition above 50% at 100 μ M. The isomers of **75** show activity in the same range, and, if at all, stereochemistry only has a small effect on activity. **77** and **79** show higher activities and would probably give an

expression-corrected IC_{50} below 10 μ m. Most of the compounds active on BCRP also show at least medium activity on Pgp, thus giving dual inhibitors. An interesting exception is **79**, which shows no inhibition of Pgp, but is an inhibitor of BCRP. In general, reduction of the conjugated double bond seems to be beneficial for both inhibitory activity on BCRP and Pgp. **76** and **78** were not tested yet due to purification issues, however would probably give a performance similar to or slightly more active than their non-methoxylated counterparts.

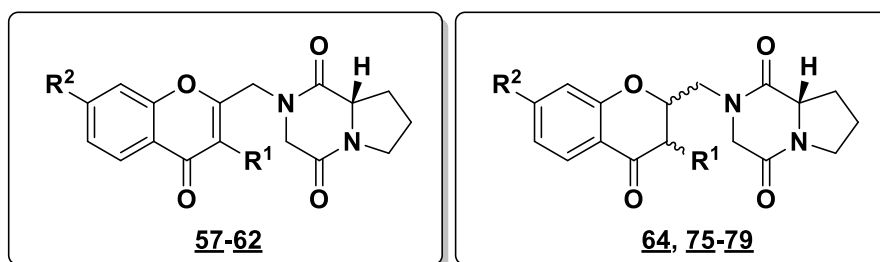


Figure E-2: Structures of tested compounds **57-79**

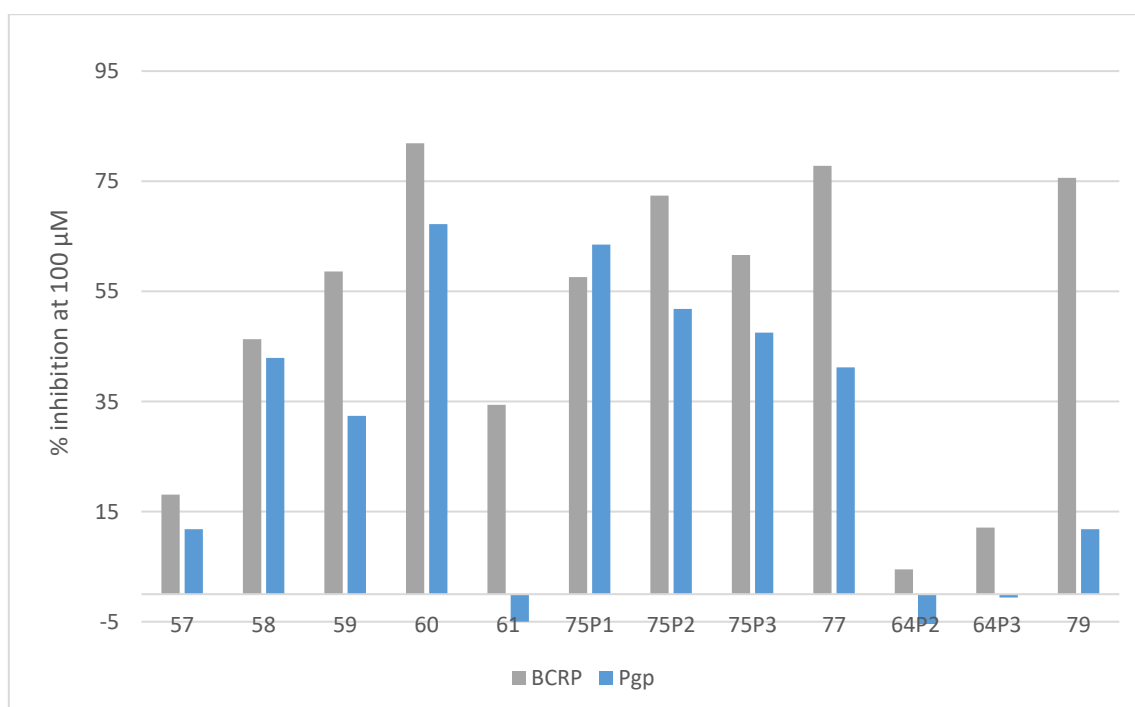


Figure E-3: %inhibition at 100 μ M for BCRP and Pgp for chromone compounds

A.19. *In silico* classification models

A.19.1. General

In addition to biological testing, inhibitory properties of the synthesized compounds were predicted for both Pgp and BCRP. The used models are binary classification models for Pgp and BCRP inhibition built on previously published large datasets¹⁴⁶. Both models are based on ECFP-like (extended-connectivity fingerprint) 1024-bit fingerprints as descriptors and use logistic regression (for BCRP) and support vector machine (for Pgp) as base classifiers. The models are classification models, thus predicting the likelihood of a compound to be an

inhibitor of any of the two transporters. As threshold for active/inactive an IC_{50} of 10 μ M was used.

The scores obtained for the respective compounds are given in table E-1 and E-2.

As outlined in more detail in the publication, the classification model tends to be too optimistic for Pgp inhibition, i.e. predicting too many compounds as inhibitors. This can be explained by propafenones in the training set being all actives. This becomes especially evident for the chromone and chromanone compounds, of which nearly all are predicted as active, while in the accumulation assay, only one inhibitor and two borderline inhibitors are identified.

On the contrary, the BCRP model has a tendency to predict too many compounds as inactive when they are, in fact, active. Again, this can be explained by the propafenone derivatives in the training set, which all are inactive on BCRP. Interestingly, **79** was predicted correctly to be an inhibitor of BCRP and not of Pgp.

A.20. Structure-activity relationship (SAR)

Molecular features that were investigated en route from GPV005 to FTC included

- Basicity of the substituent at the nitrogen atom (proline vs amide)
- Rigidity of the substituent at the nitrogen atom (proline vs fixed conformation in diketopiperazine ring)
- Spacer length (2-hydroxypropylene vs ethylene)
- Lipophilicity variations (benzyl vs hydrogen or phenyl)
- Effect of a methoxy group on the central aromatic ring
- Rigidity at the phenone/linker regions (introduction of a second ring fused to the central aromatic ring)
- Influence of degree of insaturation of this ring.

LogP values were calculated for all compounds in order to be able to assess the logP/ pIC_{50} relationship. Concerning P-glycoprotein inhibition, the SAR pattern of the published propafenone and 2-ethoxyacetophenones is comparable to the observations already established for propafenones, which include

- A significant correlation of pIC_{50} and logP ($r^2=0.71$; ¹⁴⁷),
- Elimination of the phenyl ring of the phenylpropiophenone moiety leads to decreased biological activity (most probably due to too low lipophilicity),
- Shortening of the distance between the central aromatic ring and the nitrogen atom causes a slightly decreased biological activity ¹⁴⁸,
- Increased electron density of the central aromatic ring causes slightly increased activity ¹⁴⁹.

However, this SAR pattern cannot explain all activity differences, for example the compound **p6a** with a diketopiperazine moiety at the nitrogen atom is by a factor of 7 more active than its methoxy analogous **p6b**, and even the ethylene analogue **p11b** is more active.

With regard to the chromone and chromanone compounds, a general lack of high activity-compounds could be observed for Pgp. For the compounds active enough that an IC₅₀ was determined, the trend seems a bit unclear, as the compound with the in comparison lowest logP (**40**) is the most active one. In general, it seems that the added flexibility in the ring by removing the insaturation is beneficial for activity. Judging from the finding that the most active derivative of the chromone series is methoxy substituted, it seems probable that the to date not tested compounds **76** and **78** should be at least mediocely active. Separation of the stereoisomers of **75** seems to validate the hypothesis that Pgp does not discriminate between stereoisomers¹⁵⁰.

Concerning BCRP inhibition, there are no obvious trends, thus giving a more complex picture. There is no correlation between log P and pIC₅₀ values. While among the chromone compounds only two mediocely active compounds can be found, nearly all the chromanone compounds except the unsubstituted derivative **64** show more than 50% inhibition at 100 µM. Again, no high-activity compounds can be expected, as none of them showed more inhibitory activity than Ko143. Still, reduction of the double bond is definitely beneficial, indicating that the very flat structure of the insaturated ring is detrimental. An extreme example of the fact that there is no logP – pIC₅₀ correlation is compound **79** which, in spite of a negative logP, shows some activity on BCRP.

A.21. Selectivity vs multitarget inhibition

For the published compounds, the activity differences between Pgp and BCRP are less than a factor of 10 (selectivity indices (SI) Pgp/BCRP = 0.13-2.82). Nevertheless, some trends become evident in the data. The highest selectivity for Pgp was found for the two 2-hydroxypropylene compounds **p5a** (SI=0.13) and **p6a** (SI=0.27) bearing no methoxy on the central aromatic ring and either proline or diketopiperazine as the nitrogen part. For BCRP, compounds **p6b** and **p11b** showed the highest selectivity (SI values of 2.82 and 2.70), respectively. Both are methoxy-substituted and show a diketopiperazine substituent. The other compounds can be considered as dual transporter inhibitors with SI values between 0.34 and 0.71. Factors contributing most to BCRP selectivity therefore seem to be substitution on the central aromatic ring and switching to the diketopiperazine motif, which might also be related to the reduced basicity. In Pgp inhibition, reduced basicity/H-bond donor properties have been linked to lower activity, which is not seen for BCRP¹⁵¹.

With regard to the chromone and chromanone compounds, no selectivity indices can be calculated as only the inhibition in % compared to Ko143 was determined for BCRP. Still, a trend towards more inhibitory activity on BCRP can be seen (e.g. figure E-3, all compounds except one fraction of **75** show a higher inhibitory activity on BCRP than on Pgp). Although it seems to be the case that the compounds most active on BCRP also inhibit Pgp most, also compounds selective for BCRP can be found (**79**).

Conclusion

A.22.Synthesis

In summary, successful synthetic routes could be developed for six out of the seven synthetic concepts.

For the propafenone derivatives, the achievement with the most practical relevance was the development of an easy, high-yielding procedure for the protection of the ketone as the ketal. In general, the synthesis of the diketopiperazines in one step from primary amines and methyl (2-chloroacetyl)-L-prolinate (**7**) is a very convenient procedure which has not gotten the attention it deserves so far.

For the synthesis of compounds with an ethylene linker, despite initial difficulties, two successful routes could be presented. Synthesis of the trifluoroacetate and subsequent hydrolysis are possible under mild conditions and should be recognized as an elegant way of preparing primary amines from bromides.

With respect to chromones, compounds with $R^1=Ph$ could be prepared in only three steps from the respective hydroxyphenylbenzylketones, which constitutes a fast and appealing procedure. For $R^1=Ph$, one step more had to be included to achieve the cyclisation. This was the first reported synthesis for all intermediates, thus allowing the access of new, useful synthons. Compounds with $R^1=H$ were produced through a newly-developed way, as condensations of hydroxyacetophenones and esters using NaH had not previously been described for amino-substituted compounds. Non-acidic conditions for the cyclisation compatible with the amine protecting group had to be found, which was achieved by using pyrrolidine. In summary, this gave a much faster synthesis of the primary amine intermediate (3 steps) than the syntheses presented in the patent by Rowbottom¹³⁶ (5 or 6 steps).

Chromanone compounds (except unsubstituted **64**) were not accessible by reducing the final chromone diketopiperazines as initially planned, however reduction of the Boc-protected intermediates proceeded smoothly for all derivatives, thus allowing the preparation of all target molecules. However, diketopiperazine formation was of low yield and many side products were formed, therefore some optimization of this step would be beneficial.

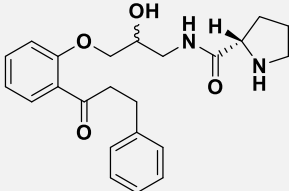
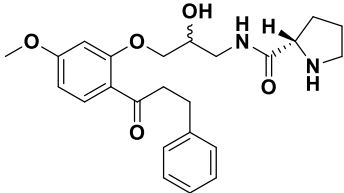
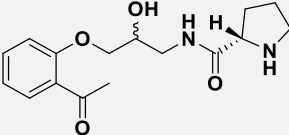
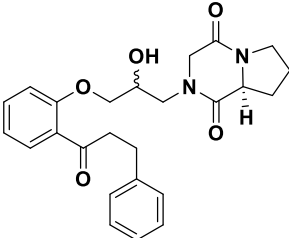
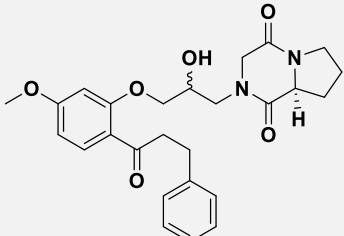
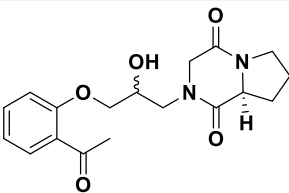
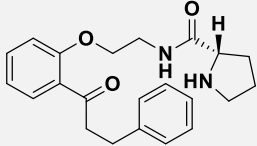
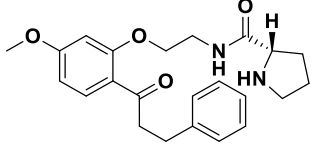
Synthesis of final target molecules **F**, unfortunately, was not successful. A wide variety of reaction conditions was tried, however none of them led to the desired product, indicating that probably a totally different synthetic concept would be needed.

A.23.Biological testing and structure-activity relationship

In general, the assumption that more propafenone-like compounds inhibit Pgp to a greater extent could be confirmed. The chromone compounds were generally less active on both transporters compared to their reduced chromanone counterparts, with no clear preference for Pgp or BCRP inhibition. The chromanones showed borderline inhibitory activity on Pgp and slightly higher activities on BCRP, leading to the conclusion that inclusion of features of FTC leads to at least a tendency towards favoured BCRP inhibition. In table F-1 and F-2, all compounds and their qualitative assessment as inhibitors ($IC_{50}<10\text{ }\mu\text{M}$), borderline inhibitors ($10\text{ }\mu\text{M}<IC_{50}<25\text{ }\mu\text{M}$) or non-inhibitors are summarized. For the BCRP inhibitory activity where

no IC₅₀ values were determined, the threshold was set judging from experience at 40% inhibition for borderline inhibitors and at 70% inhibition for inhibitors.

Table F-1: Summarized inhibitory properties of propafenone and 2-ethoxyacetophenone compounds

COMPOUND NR.	STRUCTURE	BCRP	Pgp
p5a		Borderline inhibitor	Inhibitor
p5b		Inhibitor	Inhibitor
p5c		Non-inhibitor	Non-inhibitor
p6a		Inhibitor	Inhibitor
p6b		Inhibitor	Borderline inhibitor
p6c		Non-inhibitor	Non-inhibitor
p10a		Inhibitor	Inhibitor
p10b		Inhibitor	Inhibitor

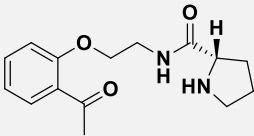
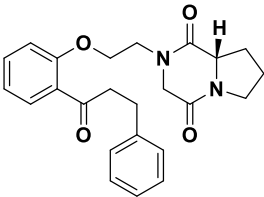
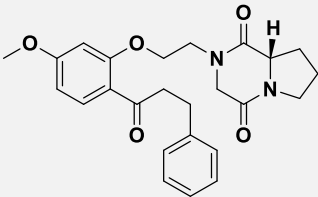
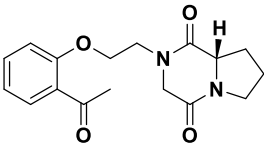
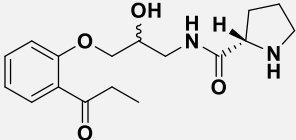
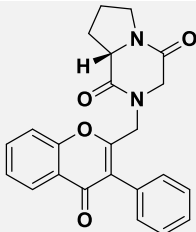
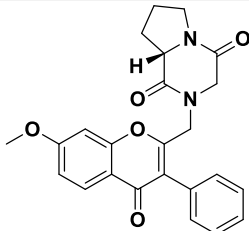
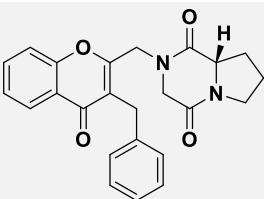
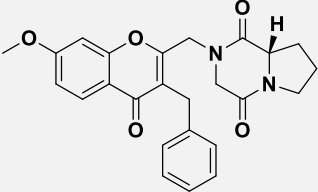
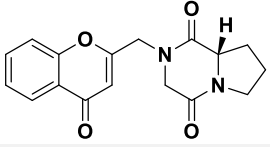
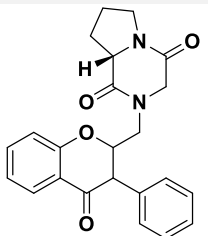
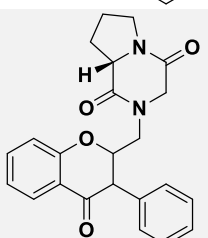
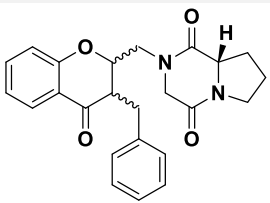
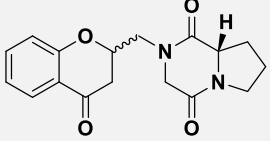
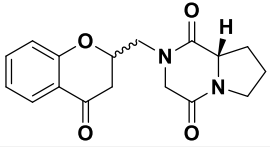
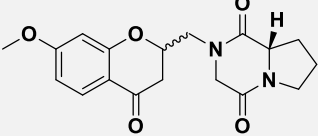
COMPOUND NR.	STRUCTURE	BCRP	Pgp
p10c		Non-inhibitor	Non-inhibitor
p11a		Borderline inhibitor	Borderline inhibitor
p11b		Inhibitor	Inhibitor
p11c		Non-inhibitor	Non-inhibitor
<u>24</u>		Non-inhibitor	Non-inhibitor

Table F-2: Summarized inhibitory properties of chromone and chromanone compounds

COMPOUND NR.	STRUCTURE	BCRP	Pgp
<u>57</u>		Non-inhibitor	Non-inhibitor
<u>58</u>		Borderline inhibitor	Borderline inhibitor
<u>59</u>		Borderline inhibitor	Non-inhibitor

COMPOUND NR.	STRUCTURE	BCRP	Pgp
<u>60</u>		Inhibitor	Inhibitor
<u>61</u>		Non-inhibitor	Non-inhibitor
<u>75P1</u>		Borderline inhibitor	Borderline inhibitor
<u>75P2</u>		Inhibitor	Borderline inhibitor
<u>75P3</u>		Borderline inhibitor	Borderline inhibitor
<u>77</u>		Inhibitor	Borderline inhibitor
<u>64P2</u>		Non-inhibitor	Non-inhibitor
<u>64P3</u>		Non-inhibitor	Non-inhibitor
<u>79</u>		Inhibitor	Non-inhibitor

A.24.Outlook

The most obvious next step would be the development of a synthetic route for the last target molecule **G** and purification or development of more selective routes towards molecules **76** and **78**.

The insights gained by the SAR of the synthesized compounds could be used in new models for predicting transporter activity. Both general and selective inhibitors could be found, this knowledge could be used for the design of new inhibitors with enhanced properties. In general, every new compound series, which explores a new patch of the chemical space, helps the understanding of how the ABC transporters Pgp and BCRP interact with their substrates and inhibitors.

Experimental Part

A.25. General information

General. All moisture-sensitive reactions were conducted in anhydrous solvents (Sigma–Aldrich) under dry argon atmosphere. All solvents and reagents were obtained from Sigma–Aldrich, ABCR, TCI, or Acros and used without further purification. Yields are not optimized.

Chromatography. Reactions were monitored by analytical thin-layer chromatography (TLC) using precoated TLC sheets ALUGRAM Xtra SIL G/UV254 (Macherey–Nagel, 0.20 mm silica gel 60 layer with fluorescent indicator UV254). Visualization was carried out by UV light (254 nm), ninhydrin, and/or Seebach stain. Flash chromatography was performed using Merck silica gel 60m (0.040–0.063 mm) using glass columns, or using a MPLC system consisting of BESTA HD-200 pumps and a Foxy 200 fraction collector. Analytical HPLC was conducted using a Shimadzu system consisting of a LC-20A pump, a DGU-20A5 degasser, a SIL-20A autosampler, a CTO-20AC column oven, a SPD-M20A diode array detector, a CMBM-20A control element and FRC-10A fraction collector. Preparative HPLC was carried out on a Shimadzu system consisting of two LC-8A pumps, an injector for preparative columns, a SPD-10A VP UV-VIS detector, a SCL-10A control element and a FRC-10A fraction collector. For analytical HPLC, the following columns were used: Clarity 5 μ m Oligo-RP 250x4.6 mm (Phenomenex), Hypersil Phenyl-BDS 5 μ m 125x4 mm (ThermoFisher Scientific) and Chiralpak IA 5 μ m 4.6x150 mm. For preparative HPLC, either a Zorbax SB-C18 5 μ m 21.2 mmx25cm (Agilent Technologies) or a Chiralpak IA 5 μ m 20x250 mm (DAICEL) column was used. Water-acetonitrile mixtures (HPLC-grade solvents) were used as mobile phases for both preparative and analytical separations.

NMR spectroscopy. NMR spectra were registered either on a Bruker Avance 200 spectrometer at 200 MHz for first experiments or structure confirmation. Structure elucidation was conducted on a Bruker Avance 500 (UltraShield) spectrometer using a 5 mm switchable probe (PA BBO 500SB BBF-H-D-05-Z, ^1H , BB = 19F and ^{31}P - 15N or TCI Prodigy Kryo-probe head, 5 mm, triple resonance-invers-detection probe head) with z axis gradients and automatic tuning and matching accessory (Bruker BioSpin). The resonance frequency for ^1H NMR was 500.13 MHz and for ^{13}C NMR 125.75 MHz. All measurements were performed for a solution in fully deuterated chloroform or DMSO at 298 K. Standard 1D and gradient-enhanced (ge) 2D experiments, like double quantum filtered (DQF) COSY, NOESY, HSQC, and HMBC, were used as supplied by the manufacturer. Chemical shifts are referenced internally to the residual, non-deuterated solvent signal for chloroform ^1H (δ 7.26 ppm) or DMSO ^1H (δ 2.50 ppm) and to the carbon signal of the solvent for chloroform ^{13}C (δ 77.00 ppm) or DMSO ^{13}C (δ 39.52 ppm). Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS), and coupling constants (J) are given in Hertz (Hz). Multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), septet, or m (multiplet).

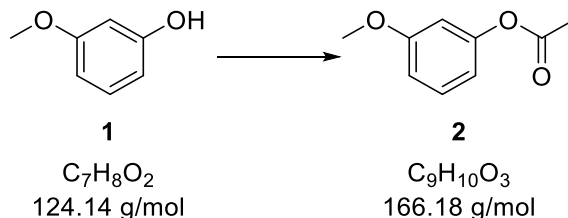
High-resolution electrospray ionization mass spectrometry (HRESI-MS). HR-ESI-MS spectra were obtained on a maXis HD ESI-Qq-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) using direct infusion. The ESI ion source was operated as follows: capillary voltage: 1.0 to 4.0 kV (individually optimized), nebulizer: 0.4 bar (N_2), dry gas flow: 4 Lmin $^{-1}$ (N_2), and dry temperature: 200°C. Mass spectra were recorded in the range of m/z 50–1550 in the positive-ion mode. The sum formulas were determined using Bruker Compass Data Analysis 4.2 based on the mass accuracy ($\Delta m/z$ 2 ppm) and isotopic pattern matching (SmartFormula algorithm).

A.26.Synthesis of compounds

A.26.1. Synthesis of starting materials, reagents and reference compounds

A.26.1.1. Synthesis of 2-hydroxyacetophenone derivatives

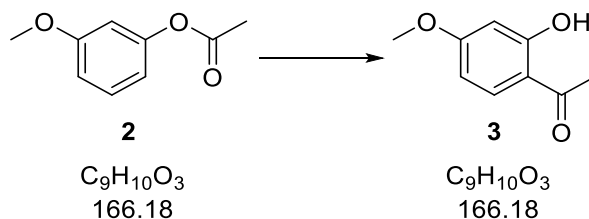
A.26.1.1.1. Synthesis of 3-methoxyphenyl acetate (**2**)



Prepared according to ⁹⁶. 3-Methoxyphenol (**1**, 5g, 1 eq, 40.3 mmol) was added to acetic anhydride (4.52 g, 1.1 eq, 44.3 mmol) and pyridine (3.98 g, 1.25 eq, 50.3 mmol) and heated for 3 h at 100 °C. Then TLC indicated complete conversion, and the reaction mixture was cooled to room temperature, poured on water (150 mL), acidified with HCl (3N) and extracted three times with diethyl ether (150 mL each). The combined organic phases were washed with sat. NaHCO₃ solution, dried over sodium sulphate and the solvent was evaporated. To remove persistent pyridine, the product was re-uptaken in Et₂O and washed three times with CuSO₄ solution (10%, combined 200 mL) and once with water, dried over sodium sulphate and evaporated to yield 5.89 g (88%) of **2** as a yellow oil.

¹H NMR (200 MHz, CDCl₃): δ=2.29 (s, 3H, MeCO₂), 3.80 (s, 3H, OMe), 6.64 (t, J=2.2 Hz, 1H, 2), 6.69 (ddd, J=8.0, 2.1, 0.9 Hz, 1H, 4 or 6), 6.78 (ddd, J=8.3, 2.4, 0.9 Hz, 1H, 4 or 6), 7.27 ppm (t, J=8.2 Hz, 5). NMR data were in good accordance with the literature.

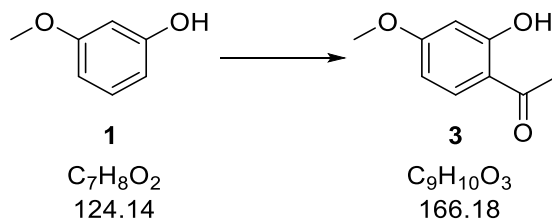
A.26.1.1.2. Synthesis of 1-(2-hydroxy-4-methoxyphenyl)ethan-1-one (**3**) – Method 1



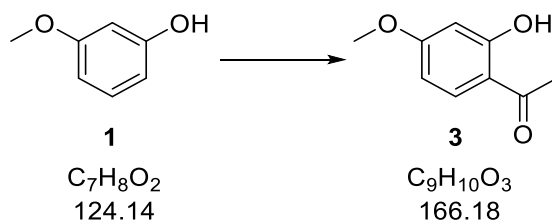
Prepared according to ⁹⁶. Aluminium chloride (5.42 g, 1.5 eq, 40.7 mmol) was placed in a 500 mL three-necked flask, and 3-methoxyphenyl acetate (**2**, 4.5 g, 1 eq, 27.1 mmol) was added dropwise during 15 min. Then the reaction mixture was heated to 130 °C and held for 1 h 30 min at this temperature, which lead to the formation of a red foam. Afterwards the reaction mixture was cooled to room temperature, mixed manually and heated for another 45 min at 130 °C. After cooling, the mixture was then treated with ice and ethyl acetate was added. The phases were separated and the aqueous phase was extracted three more times with EtOAc (100 mL each). The organic phases were dried over sodium sulphate, filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE to EtOAc=6:1 to 4:1) to yield 2.22 g (49%) of **3** as a yellow oil.

¹H NMR (500 MHz, CDCl₃): δ=2.56 (s, 3H, (CO)CH₃), 3.83 (s, 3H, OMe), 6.42 (d, J=2.2 Hz, 1H, 3), 6.44 (dd, J=8.7, 2.4 Hz, 1H, 5), 7.63 (d, J=8.8 Hz, 1H, 6), 12.76 ppm (s, 1H, OH)

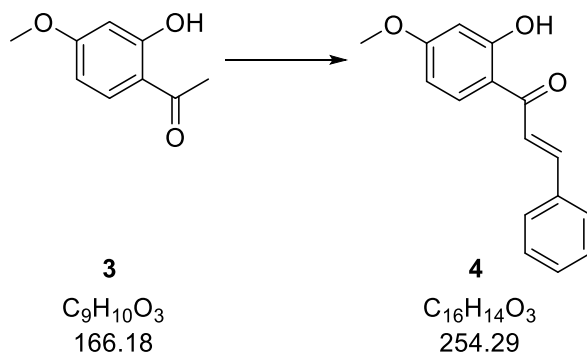
¹³C NMR (125 MHz, CDCl₃): δ=26.18 (CH₃, (CO)CH₃), 55.54 (CH₃, OMe), 100.81 (CH, 3), 107.61 (CH, 5), 113.88 (C_q, 1), 132.26 (CH, 6), 165.25 (C_q, 2), 166.09 (C_q, 4), 202.55 ppm (C_q, ketone)

A.26.1.1.3. Synthesis of 1-(2-hydroxy-4-methoxyphenyl)ethan-1-one (3) – Method 2

Preparation according to ⁹⁷. 3-Methoxyphenol (12.4 g, 1 eq, 100 mmol) was placed in an Argon-flushed 500 mL flask and titanium(IV)chloride (12 mL, 1.1 eq, 110 mmol) was added during 20 min. After 10 more minutes to let the gas formation subside, acetyl chloride (10.7 mL, 1.5 eq, 150 mmol) was added during 30 min, which led to foaming and gas formation. After stirring at room temperature for another 10 min, the mixture was heated to 120 °C, which led to the formation of a dark foam. The foam was manually mixed and ground, and heated for one hour at 120 °C. Afterwards dichloromethane was added and the mixture was carefully hydrolysed with water. The phases were separated, and the aqueous phase was extracted three more times with dichloromethane. The combined organic extracts were washed twice with water (150 mL each), dried over sodium sulphate and the solvent was evaporated. The crude product was purified by column chromatography (PE:EtOAc=7:1 to 1:1) yielding 8.88 g product **3** (64%) as a yellow oil.

A.26.1.1.4. Synthesis of 1-(2-hydroxy-4-methoxyphenyl)ethan-1-one (3) – Method 3

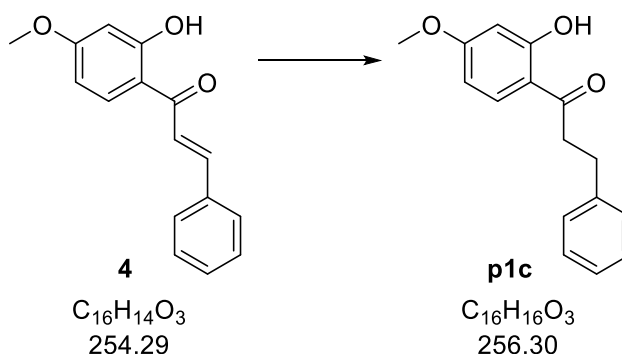
Preparation according to ⁹⁸. 3-Methoxyphenol (5 g, 1 eq, 40.3 mmol) and acetyl chloride (4.74 g, 1.5 eq, 60.45 mmol) were dissolved in toluene (15 mL) and BF₃ etherate (7.7 mL, 1.5 eq, 60.45 mmol) was added slowly during 10 min. The reaction mixture was heated for 3 h at 85 °C, then TLC indicated completion. The orange mixture was poured onto water (100 mL) and extracted four times with EtOAc (100 mL each). The combined organic extracts were washed once with brine (100 mL), dried over sodium sulphate, filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE:EtOAc=6:1 to 3:1) to yield 4.27 g (64%) of **3** as a yellow oil.

A.26.1.1.5. Synthesis of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (4)

Preparation inspired by ^{99a}. 2'-Hydroxy-4'-methoxyacetophenone (**3**, 5 g, 1 eq, 30 mmol) and benzaldehyde (3.19 g, 1 eq, 30 mmol) were dissolved in ethanol (96%, 130 mL) and freshly ground NaOH (3.01 g, 2.5 eq, 75 mmol) was added portion wise. The mixture was stirred for two days at room temperature. Ice was added and the pH was adjusted to 2-3 using 6 N HCl. The formed brown solid was filtered, washed with water and a small amount of EtOH and dried in a desiccator. This first fraction of product was purified by recrystallization (96% EtOH) yielding 2.19 g of **4**. The mother liquor of the filtration and the recrystallization were concentrated and extracted three times with DCM. The organic phases were dried, filtered and evaporated. As this residue consisted mainly of starting material, it was dissolved in EtOH (100 mL) and benzaldehyde (3 mL) and NaOH (3 g) were added. The mixture was stirred at room temperature for 3 days, workup like described above gave a second fraction of 4.73 g product (combined 6.92 g, 91%, yellow solid).

¹H NMR (500 MHz, D₆-DMSO): δ =3.85 (s, 3H, OMe), 6.53 (d, J=2.5 Hz, 1H, Ar3), 6.58 (dd, J=8.8, 2.5 Hz, 1H, Ar5), 7.45-7.50 (m, 3H, 4' & 3',5'), 7.84 (d, J=15.4 Hz, 1H, 2), 7.90-7.95 (m, 2H, 2', 6'), 8.04 (d, J=15.4 Hz, 1H, 3), 8.30 (d, J=9.1 Hz, 1H, Ar6), 13.45 ppm (s, 1H, OH)

¹³C NMR (125 MHz, D₆-DMSO): δ =55.83 (CH₃, OMe), 100.95 (CH, Ar3), 107.51 (CH, Ar5), 113.86 (C_q, Ar1), 121.21 (CH, 3), 128.96 (CH, 2C, 3', 5'), 129.15 (CH, 2C, 2', 6'), 130.86 (CH, 4'), 132.79 (CH, Ar6), 134.55 (C_q, 1'), 144.20 (CH, 2), 165.77 (C_q, Ar2), 166.08 (C_q, Ar4), 191.92 ppm (C_q, ketone 1)

A.26.1.1.6. 1-(2-hydroxy-4-methoxyphenyl)-3-phenylpropan-1-one (p1c)

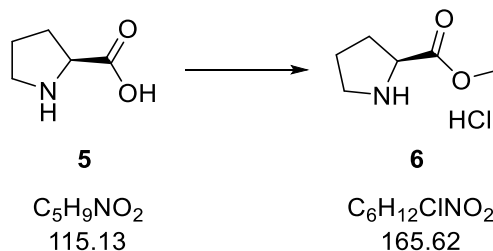
Preparation inspired by ^{99b}. The chalcone **4** (2.19 g, 1 eq, 8.6 mmol) was dissolved in THF (60 mL) and palladium on charcoal (10%, 500 mg) was added. The reaction was stirred under a hydrogen atmosphere (1 atm) for 3 h, then filtered over celite and evaporated. The crude product was purified by recrystallization from 96% EtOH to yield 1.51 g (68%) of **p1c** as a white solid.

^1H NMR (500 MHz, CDCl_3): δ =3.06 (t, J =7.3 Hz, 2H, CH_2Ph), 3.25 (t, J =7.3 Hz, 2H, $\text{CH}_2(\text{CO})$), 3.84 (s, 3H, OMe), 6.39-6.45 (m, 2H, 3&5), 7.19-7.28 (m, 3H, 2', 6' & 4'), 7.31 (t, J =7.1 Hz, 2H, 3', 5'), 7.64 (d, J =8.7 Hz, 1H, 6), 12.81 ppm (s, 1H, OH)

^{13}C NMR (125 MHz, CDCl_3): δ =30.26 (CH_2 , CH_2Ph), 39.66 (CH_2 , $(\text{CO})\text{CH}_2$), 55.55 (CH_3 , OMe), 100.90 (CH, 3), 107.65 (CH, 5), 113.36 (C_q , 1), 126.24 (CH, 4'), 128.36 (CH, 2C, 2', 6'), 128.56 (CH, 2C, 3', 5'), 131.41 (CH, 6), 140.85 (C_q , 1'), 165.35 (C_q , 2), 165.97 (C_q , 4), 203.50 ppm (C_q , ketone)

A.26.1.2. Synthesis of methyl (2-chloroacetyl)-L-prolinate

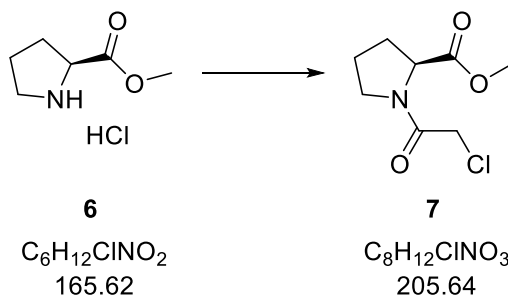
A.26.1.2.1. Synthesis of L-proline methyl ester hydrochloride (6)



Following ¹⁰¹, L-proline (15 g, 1 eq, 130 mmol) was dissolved in methanol (125 mL) and cooled with an ice bath. Thionyl chloride (20.15 g, 1.3 eq, 169 mmol) was added dropwise during 1.25 h. Subsequently the reaction mixture was warmed to room temperature overnight. As TLC indicated complete conversion, the solvent was removed under reduced pressure yielding 22.46 g (96%) of **6** as a viscous light brown oil (ca 80:20 mixture of rotamers).

^1H NMR (200 MHz, CDCl_3): δ =1.93-2.26 (m, 3H), 2.26-2.54 (m, 1H), 3.33-3.71 (m, 2H), 3.80 (s, 3H), 4.39-4.60 (m, 1H), 8.80 (brs, 0.2H), 9.08 (brs, 0.8H), 10.02 (brs, 0.2 H), 10.36 ppm (brs, 0.8H)

A.26.1.2.2. Synthesis of methyl (2-chloroacetyl)-L-prolinate (7)



Preparation according to ¹⁰². Proline methyl ester hydrochloride (**6**, 8.04 g, 1 eq, 43 mmol) was dissolved in DCM (86 mL), cooled to 0 °C and triethyl amine (12 mL 2 eq, 86 mmol) and chloroacetyl chloride (2.6 mL, 1.1 eq, 47 mmol) were added. The mixture was warmed to room temperature and stirred for two days. Then sat. NaHCO_3 solution was added, the phases were separated, and the aqueous phase was extracted three more times with DCM. The combined organic extracts were washed with brine, dried over sodium sulphate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH=30:1 to 15:1) to yield 4.47 g (53%) of **7** as a yellowish oil (ca 80:20 mixture of two mesomeric forms).

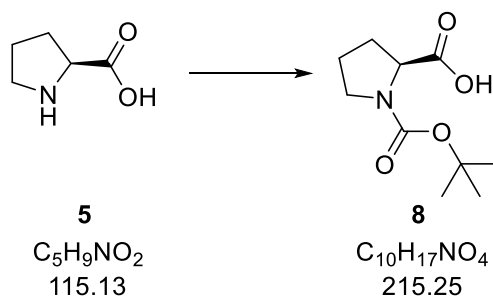
^1H NMR (500 MHz, CDCl_3): δ =1.84-1.95 (m, 0.4H, Pro4*), 1.96-2.13 (m, 2.4H, Pro3-1, Pro4-1 & Pro4-2), 2.14-2.30 (m, 1.4H, Pro3-1 & Pro3*), 3.54-3.72 (m, 2H, 5 & 5*), 3.71 (s, 2.4 H, OMe), 3.75 (s, 0.6 H, OMe*), 3.92 (d, J =12.6 Hz, 0.2 H, 2'*-1), 3.96 (d, J =12.6 Hz, 0.2 H, 2'*-2), 4.03 (d, J =12.6 Hz, 0.8 H, 2'-1), 4.07 (d, J =12.6 Hz, 0.8 H, 2'-2), 4.48 (dd, J =8.8, 3.8 Hz, 0.8 H, Pro2), 4.56 ppm (dd, J =7.7, 3.0 Hz, 0.2 H, Pro2*)

^{13}C NMR (125 MHz, CDCl_3 major isomer): δ =24.78 (CH_2 , Pro4), 29.01 (CH_2 , Pro3), 41.79 (CH_2 , 2'), 46.99 (CH_2 , Pro5), 52.29 (CH_3 , OMe), 59.11 (CH , Pro2), 165.00 (C_q , CON), 172.11 ppm (C_q , ester)

^{13}C NMR (125 MHz, CDCl_3 minor isomer (*)): δ =22.22 (CH_2 , Pro4), 31.21 (CH_2 , Pro3), 41.69 (CH_2 , 2'), 46.99 (CH_2 , Pro5), 52.79 (CH_3 , OMe), 59.24 (CH , Pro2), 165.27 (C_q , CON), 171.96 ppm (C_q , ester)

A.26.1.3. Synthesis of the diketopiperazine (S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione

A.26.1.3.1. Synthesis of N-Boc-D-proline (8)

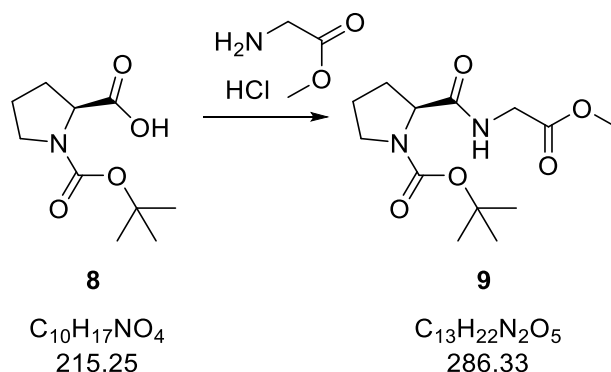


Prepared according to ¹⁰³. L-proline (**5**, 3.45 g, 1 eq, 30 mmol) was dissolved in saturated NaHCO_3 solution (45 mL) and cooled to 0 °C. At this temperature, Boc_2O (7.20 g, 1.1 eq, 33 mmol) dissolved in THF (16 mL) was added during 39 min. After stirring at 0 °C for 30 more min, the reaction mixture was stirred at room temperature for 21 h. Then THF was removed under reduced pressure and the residue was adjusted to pH=2 using 2 N HCl. The aqueous phase was extracted four times with EtOAc (100 mL each), and the combined organic phases were washed once with brine, dried over sodium sulphate, filtered and the solvent was evaporated. The product **8** was obtained as a white solid (6.27 g, 97%).

^1H NMR (200 MHz, CDCl_3): δ =1.42 (s, 3H), 1.48 (s, 6H), 1.81-2.17 (m, 3H), 2.17-2.41 (m, 1H), 3.25-3.65 (m, 2H), 4.17-4.43 (m, 1H), 8.50 ppm (brs, 1H)

NMR data were in good accordance with the literature.

A.26.1.3.2. Synthesis of tert-butyl (S)-2-((2-methoxy-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate (9)

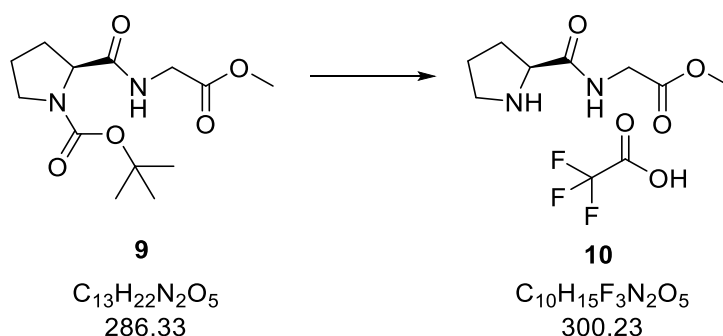


Preparation based loosely on ¹⁰⁴. Glycine methyl ester hydrochloride (1.40 g, 1 eq, 11.1 mmol) was dissolved in a mixture of DCM (20 mL) and DMF (7 mL) and cooled to 0 °C. At this temperature, 4-methylmorpholine (2.37 g, 2.1 eq, 223.4 mmol) was added during 5 min. After stirring for 15 more minutes, first N-Boc-D-proline (**8**, 2.4 g, 1 eq, 11.1 mmol) dissolved in DCM (20 mL) was added during 10 min, then DCC (2.30 g, 1 eq, 11.1 mmol) was added portion wise during 5 min. The mixture was stirred for another 30 min at 0 °C and then warmed to room temperature overnight. The reaction mixture was filtered and washed with chloroform. The organic phase was extracted with sat. NaHCO_3

and brine (75 mL each), dried over sodium sulphate, filtered and the solvent was removed until the precipitation of dicyclohexyl urea started. Et₂O was added, the mixture was filtered and the solvent was evaporated again. This procedure was repeated one more time, to yield 3.20 g **9** (quant.) as a yellowish oil.

¹H NMR (200 MHz, CDCl₃): δ=1.45 (s, 9H), 1.73-2.42 (m, 4H), 3.27-3.54 (m, 2H), 3.73 (s, 3H), 3.98-4.11 (m, 2H), 4.19-4.40 (m, 1H), 6.56 ppm (brs, 1H)

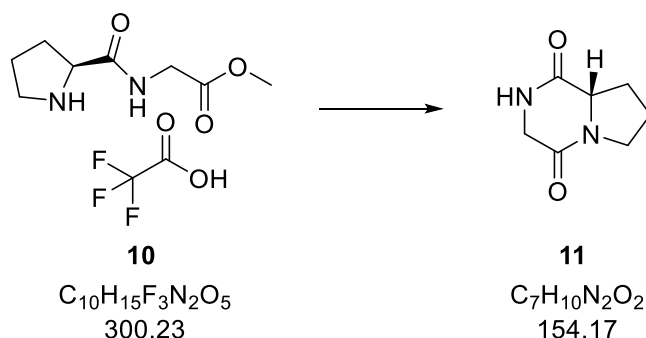
A.26.1.3.3. Synthesis of methyl (S)-prolylglycinate trifluoroacetate (**10**)



Preparation according to ¹⁰⁵. **9** (2.0 g, 1 eq, 7.0 mmol) was dissolved in DCM (10 mL) and trifluoroacetic acid (10 mL) was added, and the mixture was stirred at room temperature for 45 min. The solvent was removed under vacuum. The residue was redissolved in toluene and the solvent was evaporated again (this procedure was repeated twice) to yield 2.69 g of crude product **10**, which was used in the next step without purification.

¹H NMR (200 MHz, CDCl₃): δ=1.94-2.27 (m, 3H), 2.33-2.63 (m, 1H), 3.34-3.60 (m, 2H), 3.73 (s, 3H), 3.89-4.20 (m, 2H), 4.58-4.81 (m, 1H), 7.65 (brs, 1H), 8.10 (t, J=4.4 Hz, 1H), 10.19 ppm (brs, 1H)

A.26.1.3.4. Synthesis of (S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**11**)



Preparation according to ¹⁰⁵. Crude **10** (2.69 g) was dissolved in methanol (50 mL) and triethyl amine (14 mL) was added. The mixture was heated for 16 h under reflux, then the solvent was removed under reduced pressure. The solid residue was treated with isopropanol (10 mL), and the formed precipitate was filtered and dried to yield a first fraction of **11** (0.49 g, white solid). The filtrate was evaporated, isopropanol was added and cooled to obtain a second fraction (354 mg, altogether 844 mg, 78%, white solid).

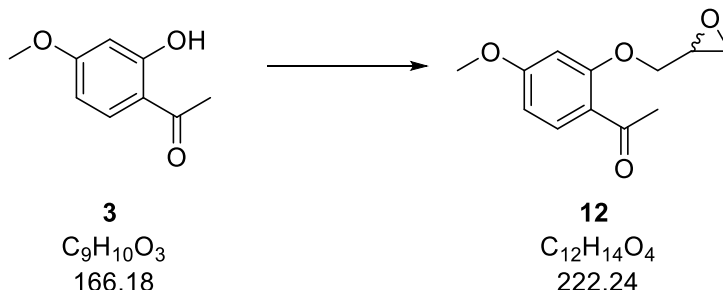
¹H NMR (500 MHz, CDCl₃): δ=1.84-1.97 (m, 1H, 7-1), 1.99-2.12 (m, 2H, 7-2 & 8-1), 2.32-2.41 (m, 1H, 8-2), 3.50-3.59 (m, 1H, 6-1), 3.59-3.67 (m, 1H, 6-Dik-2), 3.89 (dd, J=16.6, 4.3 Hz, 1H, 3-1), 4.05-4.13 (m, 2H, 8a & 3-2), 6.72 ppm (brs, 1H, NH)

¹³C NMR (125 MHz, CDCl₃): δ=22.39 (CH₂, 7), 28.42 (CH₂, 8), 45.32 (CH₂, 6), 46.64 (CH₂, 3), 58.51 (CH, 8a), 163.43 (C_q, 4), 169.80 ppm (C_q, 1)

A.26.2. Synthesis of proline derivatives of propafenone derivatives and 2-ethoxyacetophenones

A.26.2.1. First attempts at the synthesis

A.26.2.1.1. Synthesis of 1-(4-methoxy-2-(oxiran-2-ylmethoxy)phenyl)ethan-1-one (**12**)

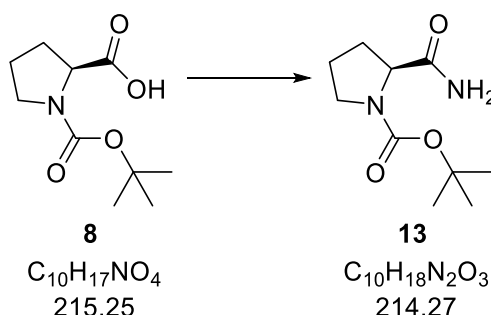


Preparation according to ¹⁰⁶. 2-Hydroxy-4-methoxyacetophenone (**3**, 1 g, 1 eq, 6 mmol) was dissolved in epichlorohydrin (14 mL, 30 eq, 179 mmol) and freshly ground NaOH (262 mg, 1.1 eq, 6.6 mmol) was added. The mixture was heated under reflux for 24 h, then the mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc and water, the phases were separated and the organic phase was extracted two more times with water. The organic phase was dried over sodium sulphate, filtered and the solvent was evaporated yielding 1.31 g (98%) of **12** as a yellow solid.

¹H NMR (500 MHz, CDCl₃): δ =2.61 (s, 3H, (CO)CH₃), 2.77 (dd, J=4.7, 2.5 Hz, 1H, oxiraneCH₂-1), 2.94 (t, J=4.4 Hz, 1H, oxiraneCH₂-2), 3.38-3.43 (m, 1H, oxiraneCH), 3.83 (s, 3H, OMe), 3.97 (dd, J=10.9, 6.1 Hz, 1H, OCH₂-1), 4.34 (dd, J=11.0, 2.8 Hz, 1H, OCH₂-2), 6.43 (d, J=2.2 Hz, 1H, 3), 6.54 (dd, J=8.7, 2.0 Hz, 1H, 5), 7.83 ppm (d, J=8.8 Hz, 1H, 6)

¹³C NMR (125 MHz, CDCl₃): δ =31.90 (CH₃, (CO)CH₃), 44.58 (CH₂, oxiraneCH₂), 49.81 (CH, oxiraneCH), 55.51 (CH₃, OMe), 69.53 (CH₂, OCH₂), 99.28 (CH, 3), 105.74 (CH, 5), 121.34 (C_q, 1), 132.73 (CH, 6), 159.71 (C_q, 2), 164.37 (C_q, 4), 197.51 ppm (C_q, ketone)

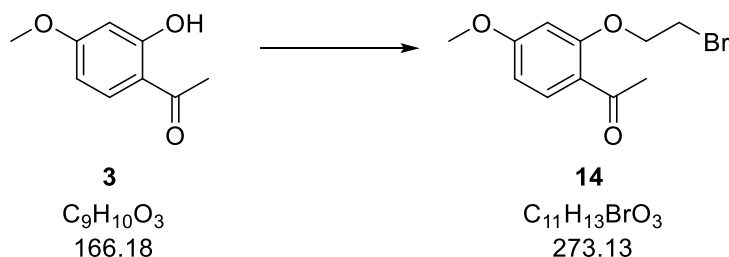
A.26.2.1.2. Synthesis of N-Boc-D-prolineamide (**13**)



Following ¹⁰⁸, N-Boc-D-proline (**8**, 3 g, 1 eq, 13.9 mmol) and triethyl amine (1.93 mL, 1 eq, 13.9 mmol) were dissolved in THF (200 mL) and cooled to -12 °C. At this temperature, ethyl chloroformate (3.47 g, 2.3 eq, 32.0 mmol) was added, which led to the precipitation of a white solid. After stirring for 30 more min at -12 °C, aqueous ammonia (25%, 8.6 mL, 4 eq, 55.6 mmol) was added dropwise during 20 min. The mixture was stirred for 20 min at -20 °C and then warmed to room temperature. After stirring at room temperature for 19 h, TLC indicated completion and the mixture was concentrated under reduced pressure. The residue was taken up in DCM (200 mL) and extracted with sat. NaHCO₃ solution, water and brine (100 mL each). The organic phase was dried over sodium sulphate, filtered and the solvent was evaporated. The crude product was purified by recrystallization from Et₂O to yield 1.79 g (60%) of **13** as a white solid.

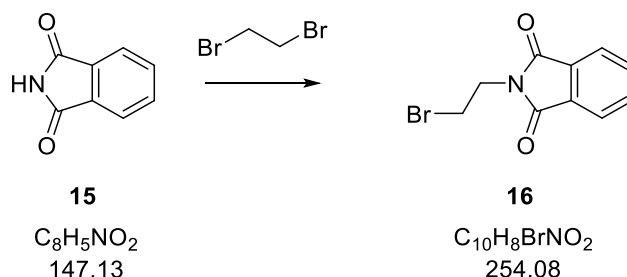
^1H NMR (200 MHz, CDCl_3): δ =1.47 (s, 9H), 1.79-2.43 (m, 4H), 3.27-3.59 (m, 2H), 4.15-4.38 (m, 1H), 5.44 (brs, 1H), 6.00 (brs, 0.5H), 6.85 ppm (brs, 0.5H)

A.26.2.1.3. Synthesis of 1-(2-(2-bromoethoxy)-4-methoxyphenyl)ethan-1-one (**14**)

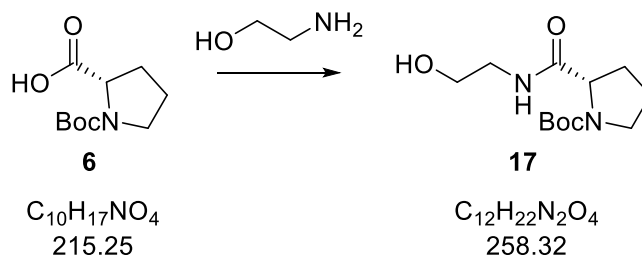


Following ¹⁰⁹, 2-hydroxy-4-methoxyacetophenone (1.0 g, 1 eq, 6.0 mmol), 1,2-dibromoethane (6.78 g, 6 eq, 36.1 mmol), potassium carbonate (1.66 g, 2 eq, 12.0 mmol) and tetrabutylammonium bromide (193 mg, 0.1 eq, 0.6 mmol) were dissolved in toluene (3 mL) and water (6 mL) and heated at reflux for 2 days. Then the phases were separated and the organic phase was extracted with 1 N NaOH and brine (50 mL each). The combined aqueous phases were extracted once with toluene (50 mL), and the organic phases were dried over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography (PE:EtOAc=10:1 to 1:1) to yield 1.06 g (65%) of **14** as a beige solid. ^1H NMR (500 MHz, CDCl_3): δ =2.65 (s, 3H, (CO)CH₃), 3.72 (t, J=6.3 Hz, 2H, CH₂Br), 3.85 (s, 3H, OMe), 4.38 (t, J=6.6 Hz, 2H, OCH₂), 6.39 (s, 1H, 3), 6.56 (d, J=8.5 Hz, 1H, 5), 7.87 ppm (d, J=8.5 Hz, 1H, 6) ^{13}C NMR (125 MHz, CDCl_3): δ =28.78 (CH₂, CH₂Br), 32.36 (CH₃, (CO)CH₃), 55.58 (CH₃, OMe), 68.18 (CH₂, OCH₂), 98.99 (CH, 3), 105.54 (CH, 5), 121.24 (C_q, 1), 132.92 (CH, 6), 159.28 (C_q, 2), 164.37 (C_q, 4), 197.58 ppm (C_q, ketone)

A.26.2.1.4. Synthesis of 2-(2-bromoethyl)isoindoline-1,3-dione (**16**)

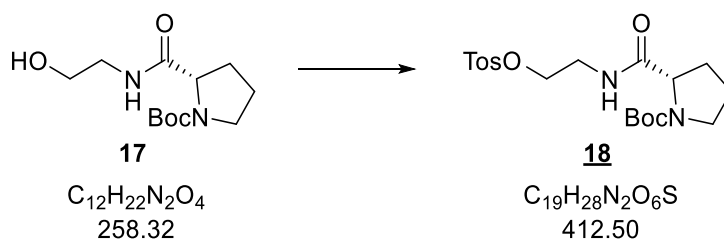


Preparation according to ¹²¹. 1,2-dibromoethane (6 g, 1.5 eq, 31.9 mmol) and potassium carbonate (5.89 g, 2 eq, 42.6 mmol) were dissolved/suspended in DMF (40 mL) and phthalimide (3.13 g, 1 eq, 21.3 mmol) was added portion wise during 15 min. The reaction was stirred for 3 h at room temperature, diluted with cold water (80 mL) and extracted three times with DCM (100 mL each). The combined organic extracts were washed twice with sat. NH_4Cl solution (70 mL each), dried over sodium sulphate, filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE:EtOAc=6:1) to yield 4.67 g (86%) of **16** as a white solid. ^1H NMR (200 MHz, CDCl_3): δ =3.61 (t, J=6.7 Hz, 2H), 4.11 (t, J=6.7 Hz, 2H), 7.68-7.81 (m, 2H), 7.82-7.93 ppm (m, 2H)

A.26.2.1.5. Synthesis of tert-butyl (S)-2-((2-hydroxyethyl)carbamoyl)pyrrolidine-1-carboxylate (17**)**

Preparation according to ¹²³. N-Boc-L-proline (4 g, 1 eq, 18.6 mmol) and N-hydroxysuccinimide (2.30 g, 1.08 eq, 20.0 mmol) were dissolved in DCM (70 mL) and cooled to 0 °C. DCC (4.13 g, 1.08 eq, 20.0 mmol) dissolved in DCM (70 mL) was added dropwise during 20 min, then the reaction was warmed to room temperature and stirred for 2.5 hrs at room temperature. The mixture was filtered, washed with DCM (15 mL) and 2-aminoethanol (3.3 mL, 3 eq, 55.7 mmol) was added, which led to the precipitation of a white solid, and stirred at RT overnight. Then the mixture was filtered again and washed with DCM. The DCM-phase was extracted with water (150 mL), and the aqueous phase was extracted twice with DCM (50 mL each). The combined organic extracts were washed with water and brine (100 mL each), dried over sodium sulphate, filtered and the solvent was evaporated yielding a first fraction of **17** (2.7 g). To increase the yield, the aqueous phases were extracted 8 more times with EtOAc (combined 1000 mL), dried over sodium sulphate and evaporated to yield a second fraction (2.09 g, 4.70 g (99%) combined, both white solids).

¹H NMR (200 MHz, CDCl_3): δ =1.45 (s, 9H), 1.76-2.37 (m, 4H), 3.25-3.60 (m, 4H), 3.69 (t, J =4.9 Hz, 2H), 4.16-4.30 (m, 1H), 6.54 (brs, 0.4 H), 6.96 ppm (brs, 0.6 H)

A.26.2.1.6. Synthesis of tert-butyl (S)-2-((2-(tosyloxy)ethyl)carbamoyl)pyrrolidine-1-carboxylate (18**)**

Preparation following ¹²⁶. **17** (2 g, 1 eq, 7.74 mmol) and pyridine (4.6 mL) were dissolved in DCM (15 mL) and cooled to 0 °C. At this temperature, tosyl chloride (1.85 g, 1.25 eq, 9.68 mmol) was added portion wise during 5 min. After stirring for another 5 min at 0 °C, the reaction mixture was warmed to room temperature and stirred overnight. Afterwards TLC indicated that there was still starting material present, therefore more tosyl chloride (1.85 g, 1.25 eq, 9.68 mmol) was added and stirred for 3 hrs. Then TLC showed complete consumption of starting material, and the reaction mixture was diluted with DCM (50 mL) and extracted with water (twice), 2 N HCl and 10% CuSO_4 solution (70 mL each). The combined aqueous extracts were re-extracted once with DCM, the combined organic extracts were washed once with brine, dried over sodium sulphate and the solvent was evaporated. The crude product was purified by column chromatography (PE:EtOAc=4:1 to pure EtOAc) to yield 432 mg **18** (14%, colourless oil, 1/3 to 2/3 mixture of rotamers, minor rotamer=*) next to the di-tosylated product (1.12 g).

¹H NMR (500 MHz, CDCl_3): δ =1.36 (s 3H, Boc*), 1.45 (s, 6H, Boc), 1.79-1.99 (m, 3H, Pro4 & Pro3-1), 2.11-2.22 (m, 1H, Pro3-2), 2.40 (s, 2H, Tos- CH_3), 2.41 (s, 1H, Tos- CH_3^*), 3.15-3.27 (m, 2H, 1'), 3.35-3.54

(m, 2H, Pro5), 4.09-4.27 (m, 2H, Pro2 & 2'), 4.99 (t, J=5.5 Hz, 0.33 H, NH*), 5.77 (t, J=5.8 Hz, 0.66 H, NH), 7.27 (d, J=8.5 Hz, 1.33 H, Ar3, 5), 7.30 (d, J=7.9 Hz, 0.66 H, Ar3,5*), 7.71-7.76 ppm (m, 2H, Ar2,6)
Major isomer:

¹³C NMR (125 MHz, CDCl₃): δ=21.44 (CH₃, TosCH₃), 24.51 (CH₂, Pro4), 28.42 (CH₃, 3C, Boc), 29.85 (CH₂, Pro3), 42.07 (CH₂, 1'), 46.76 (CH₂, Pro5), 58.96 (CH, Pro2), 63.44 (CH₂, 2'), 80.46 (C_q, Boc), 126.98 (CH, Ar2, 6), 129.59 (CH, Ar3,5), 137.39 (C_q, Ar1), 143.10 (C_q, Ar4), 154.78 (C_q, carbamate), 172.74 ppm (C_q, CON)

Minor isomer (*):

¹³C NMR (125 MHz, CDCl₃): δ=21.44 (CH₃, TosCH₃), 23.56 (CH₂, Pro4), 28.26 (CH₃, 3C, Boc), 30.84 (CH₂, Pro3), 42.12 (CH₂, 1'), 46.29 (CH₂, Pro5), 58.91 (CH, Pro2), 63.27 (CH₂, 2'), 79-98 (C_q, Boc), 126.98 (CH, Ar2, 6), 129.77 (CH, Ar3,5), 136.82 (C_q, Ar1), 143.61 (C_q, Ar4), 153.64 (C_q, carbamate), 173.01 ppm (C_q, CON)

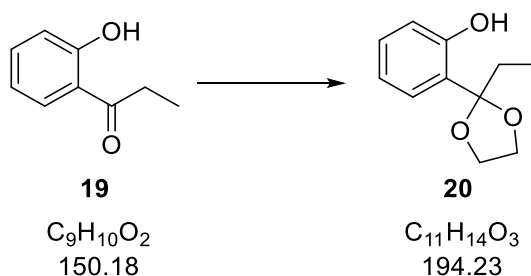
HRMS (ESI): calcd for C₁₉H₂₈N₂NaO₆S m/z 435.1560 [M+Na]⁺, found m/z 435.1564 [M+Na]⁺.

A.26.2.2. Publication "Subtle Structural Differences Trigger Inhibitory Activity of Propafenone Analogues at the Two Polyspecific ABC Transporters: P-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)"

The syntheses described in the publication can be found in the reprint in the appendix.

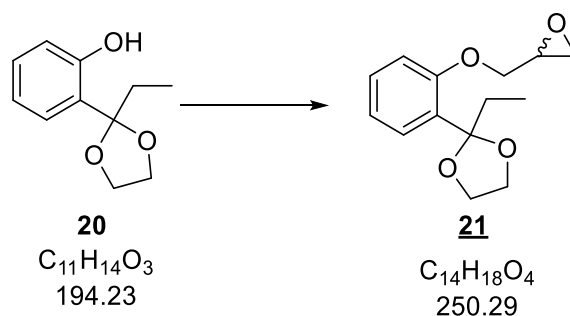
A.26.2.3. Synthesis of the ethyl derivatives

A.26.2.3.1. Synthesis of 2-(2-ethyl-1,3-dioxolan-2-yl)phenol (20)



Preparation loosely following ¹⁵². 2'-Hydroxypropiophenone (19, 12.1 mL, 1 eq, 88 mmol), ethylene glycol (2.0 mL, 1.5 eq, 36 mmol) and triisopropyl orthoformate (8.04 mL, 1.5 eq, 36 mmol) were dissolved/suspended in hexane (140 mL) giving a 2-phasic mixture. Trifluoromethanesulfonic acid (36 mg, 0.01 eq, 0.24 mmol) was added and the mixture was heated at 40 °C for 3.5 days. Then Et₃N (6 mL) was added, followed by sat. NaHCO₃ solution (150 mL). The mixture was extracted four times with Et₂O (4x 150 mL, a few drops Et₃N added), and the combined organic extracts were dried over sodium sulphate, filtered and evaporated. The crude product **20** (17.05 g, quant., white solid) was used without further purification.

¹H NMR (200 MHz, CDCl₃): δ=0.92 (t, J=7.5 Hz, 3H), 1.95 (q, J=7.4 Hz, 2H), 3.82-3.98 (m, 2H), 3.99-4.19 (m, 2H), 6.78-6.96 (m, 2H), 7.12-7.33 (m, 2H), 8.27 ppm (s, 1H)

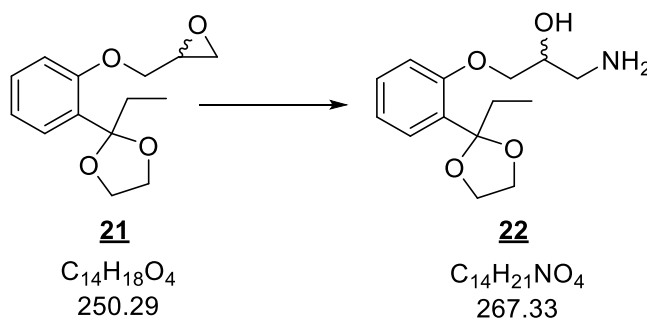
A.26.2.3.2. Synthesis of 2-ethyl-2-(2-(oxiran-2-ylmethoxy)phenyl)-1,3-dioxolane (21)

Preparation loosely following ¹⁵³. The ketal **20** (3.22 g, 1 eq, 16.6 mmol) was dissolved in DMF (35 mL) and heated to 75 °C (oil bath temperature). At this temperature, sodium hydride (60% dispersion, 998 mg, 1.5 eq, 25.0 mmol) was added portion wise during 30 min. After stirring for another hour at 75 °C, epichlorohydrin (3.9 mL, 3 eq, 49.9 mmol) was added and the reaction was heated at 75 °C overnight. Subsequently the reaction mixture was concentrated, redissolved in Et₂O (100 mL) and extracted with sat. NaHCO₃ solution and water (100 mL each). The combined aqueous phases were re-extracted once with diethyl ether, and the combined organic phases were dried over sodium sulphate, filtered and evaporated. The crude product was suspended in hexane, the solvent was decanted and the product was dried again yielding 3.65 g (88%) of **21** as a colourless oil.

¹H NMR (500 MHz, CDCl₃): δ=0.84 (t, J=7.6 Hz, 3H, CH₂CH₃), 2.16 (q, J=7.6 Hz, 2H, CH₂CH₃), 2.89 (d, J=3.5 Hz, 2H, oxirane-CH₂), 3.37-3.41 (m, 1H, oxirane-CH), 3.80-3.88 (m, 2H, OCH₂CH₂O-1), 3.98-4.04 (m, 2H, OCH₂CH₂O-2), 4.07 (dd, J=11.3, 5.0 Hz, 1H, OCH₂-1), 4.29 (dd, J=11.3, 2.8 Hz, 1H, OCH₂-2), 6.92 (d, J=8.2 Hz, 1H, 3), 6.94 (t, J=7.6 Hz, 1H, 5), 7.26 (td, J=7.7, 1.3 Hz, 1H, 4), 7.47 ppm (dd, J=7.7, 1.4 Hz, 1H, 6)

¹³C NMR (125 MHz, CDCl₃): δ=8.13 (CH₃, CH₂CH₃), 30.75 (CH₂, CH₂CH₃), 44.72 (CH₂, oxirane-CH₂), 50.33 (CH, oxirane-CH), 64.74 (CH₂, 2C, OCH₂CH₂O), 110.70 (C_q, ketal), 113.71 (CH, 3), 120.69 (CH, 5), 127.72 (CH, 6), 129.28 (CH, 4), 130.18 (C_q, 1), 156.07 ppm (C_q, 2)

HRMS (ESI): calcd for C₁₄H₁₈NaO₄ m/z 273.1097 [M+Na]⁺, found m/z 273.1102 [M+Na]⁺.

A.26.2.3.3. Synthesis of 1-amino-3-(2-(2-ethyl-1,3-dioxolan-2-yl)phenoxy)propan-2-ol (22)

Preparation following ¹⁰⁷. The epoxide **21** (2.6 g, 1 eq, 10.4 mmol) was dissolved in methanol (100 mL) and NH₃ in methanol (7 M, 39 mL, 26.4 eq, 274 mmol) was added and stirred for 4 d at room temperature in a closed flask. Then the solvent was evaporated, and the crude product (mixture with dimer) was purified by column chromatography (DCM:MeOH:aq NH₄OH conc.=100:0:1 to 100:10:1) yielding 1.12 g (40%) of **22** as an orange oil.

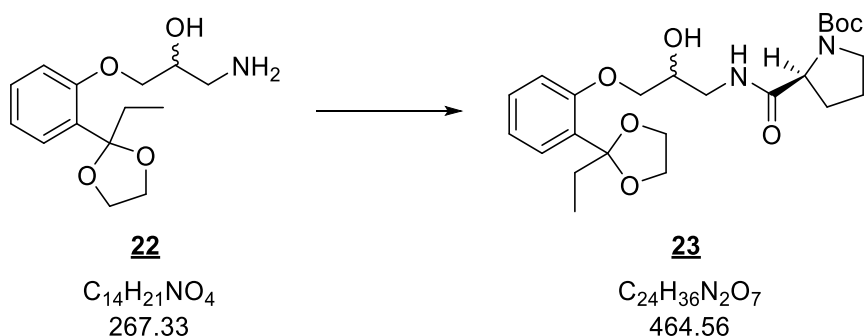
¹H NMR (500 MHz, CDCl₃): δ=0.84 (t, J=7.6 Hz, 3H, CH₂CH₃), 2.12 (q, J=7.5 Hz, 2H, CH₂CH₃), 2.80-3.12 (m, 4H, CH₂N & NH₂), 3.80-3.88 (m, 2H, OCH₂CH₂O-1), 3.94-4.07 (m, 4H, OCH₂CH₂O-2, CHOH & OCH₂-

1), 4.20 (dd, $J=8.8, 2.5$ Hz, 1H, OCH₂-2), 6.89 (d, $J=8.2$ Hz, 1H, 6), 6.94 (t, $J=7.4$ Hz, 1H, 4), 7.26 (td, $J=7.8, 1.7$ Hz, 1H, 5), 7.43 ppm (dd, $J=7.7, 1.7$ Hz, 1H, 3)

¹³C NMR (125 MHz, CDCl₃): $\delta=7.94$ (CH₃, CH₂CH₃), 31.19 (CH₂, CH₂CH₃), 44.33 (CH₂, CH₂N), 64.63 (CH₂, OCH₂CH₂O), 64.71 (CH₂, OCH₂CH₂O), 71.22 (CH, CHOH), 72.39 (CH₂, OCH₂), 111.06 (C_q, ketal), 114.45 (CH, 6), 120.86 (CH, 4), 127.23 (CH, 3), 129.43 (CH, 5), 130.12 (C_q, 2), 156.4 ppm (C_q, 1)

HRMS (ESI): calcd for C₁₄H₂₂NO₄ m/z 268.1543 [M+H]⁺, found m/z 268.1547 [M+H]⁺.

A.26.2.3.4. Synthesis of tert-butyl (2S)-2-((3-(2-(2-ethyl-1,3-dioxolan-2-yl)phenoxy)-2-hydroxypropyl)carbamoyl)pyrrolidine-1-carboxylate (**23**)



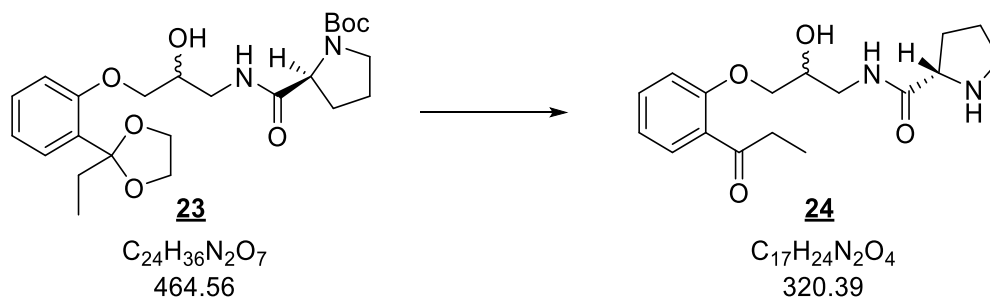
The amine **22** (889 mg, 1 eq, 3.3 mmol) and Boc-proline (787 mg, 1.1 eq, 3.7 mmol) were dissolved in DCM (20 mL) and first DMAP (20 mg, 0.05 eq, 0.2 mmol) and then DCC (755 mg, 1.1 eq, 3.7 mmol) were added portion wise. The reaction was stirred at room temperature overnight. The reaction mixture was filtered, washed with sat. NaHCO₃ solution and water, and the combined aqueous extracts were washed twice with DCM. The combined organic fractions were washed once with brine, dried over sodium sulphate and evaporated. The crude product was purified by column chromatography (twice, first DCM to DCM:MeOH=40:1, then PE:EtOAc=2:1 to pure EtOAc) yielding 613 mg (35%, mixture of diastereomers) of **23** as a white solid.

¹H NMR (500 MHz, CDCl₃): $\delta=0.83/0.84$ (t, $J=7.4$ Hz/t, $J=7.4$ Hz, 3H, CH₂CH₃), 1.38 (s, 4.5 H, Boc), 1.43 (s, 4.5 H, Boc), 1.81-1.99 (m, 2H, Pro4), 2.11 (q, $J=7.3$ Hz, 2H, CH₂CH₃), 2.08-2.29 (m, 2H, Pro3), 3.28-3.51 (m, 3H, Pro5 & CH₂N-1), 3.52-3.60 (m 1H, CH₂N-2), 3.78-3.90 (m, 3H, OCH₂CH₂O-1 & OCH₂-1), 3.97-4.10 (m, 3H, OCH₂CH₂O-2 & CHOH), 4.17 (dd, $J=9.5, 3.5$ Hz, 1H, OCH₂-2), 4.18-4.31 (m, 1H, Pro2), 6.89 (d, $J=8.2$ Hz, 1H, 6), 6.94 (t, $J=7.4$ Hz, 1H, 4), 7.26 (td, $J=7.8, 1.1$ Hz, 1H, 5), 7.42 ppm (d, $J=7.3$ Hz, 1H, 3)

¹³C NMR (125 MHz, CDCl₃): $\delta=7.94/7.99$ (CH₃, CH₂CH₃), 23.47/24.45 (CH₂, Pro4), 28.23 (CH₃, 3C, Boc), 30.70 (CH₂, Pro3), 31.23 (CH₂, CH₂CH₃), 41.50 (CH₂, CH₂N), 46.95 (CH₂, Pro5), 61.13 (CH, Pro2), 64.66 (CH₂, OCH₂CH₂O), 64.74 (CH₂, OCH₂CH₂O), 68.91 (CH, CHOH), 71.73 (CH₂, OCH₂), 80.06/80.65 (C_q, Boc), 111.10 (C_q, ketal), 114.54/114.57 (CH, 6), 120.93 (CH, 4), 127.19/127.12 (CH, 3), 129.53 (CH, 5), 130.17 (C_q, 2), 156.38 (C_q, 1), 172.06 ppm (C_q, CON)

HRMS (ESI): calcd for C₂₄H₃₆N₂NaO₇ m/z 487.2415 [M+Na]⁺, found m/z 487.2417 [M+Na]⁺.

A.26.2.3.5. Synthesis of (2S)-N-(2-hydroxy-3-(2-propionylphenoxy)propyl)pyrrolidine-2-carboxamide (**24**)



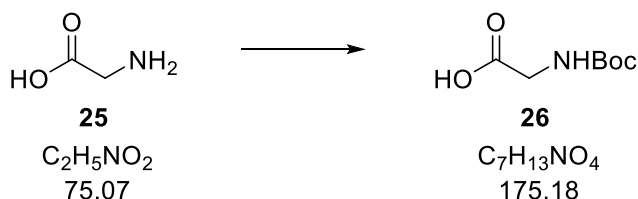
The protected product **23** (500 mg, 1 eq, 1.1 mmol) was dissolved in dioxane (5.8 mL) and aqueous HCl (4 M, 6.5 mL) and was stirred for 6 h at room temperature. Subsequently, the mixture was rendered basic with 2 N NaOH and extracted four times with EtOAc. The combined organic phases were dried over sodium sulphate, filtered and evaporated yielding 340 mg (99%) of **24** as a yellow honey.

^1H NMR (500 MHz, $CDCl_3$): δ =1.17 (t, J =7.3 Hz, 3H, CH_2CH_3), 1.65-1.75 (m, 2H, Pro4), 1.85-1.94 (m, 1H, Pro3-1), 2.11-2.20 (m, 1H, Pro3-2), 2.84-2.93 (m, 1H, Pro5-1), 2.96 (q, J =7.5 Hz, 2H, CH_2CH_3), 2.98-3.04 (m, 1H, Pro5-2), 3.36-3.46 (m, 2H, CH_2N-1 & NH), 3.60 (ddd, J =14.0, 6.3, 3.0 Hz, 1H, CH_2N-2), 3.79 (td, J =8.8, 5.6 Hz, 1H, Pro2), 3.98-4.15 (m, 3H, CHOH & OCH_2), 6.96 (d, J =8.5 Hz, 1H, 6), 7.01 (t, J =7.4 Hz, 1H, 4), 7.43 (td, J =7.7, 1.3 Hz, 1H, 5), 7.63 ppm (dt, J =7.7, 1.5 Hz, 1H, 3)

^{13}C NMR (125 MHz, $CDCl_3$): δ =8.44 (CH_3 , CH_2CH_3), 26.09/26.14 (CH_2 , Pro4), 30.75/30.77 (CH_2 , Pro3), 36.20/36.23 (CH_2 , CH_2CH_3), 42.67/42.71 (CH_2 , CH_2N), 47.13/47.18 (CH_2 , Pro5), 60.28/60.32 (CH, Pro2), 69.66/69.79 (CH, CHOH), 70.72/70.79 (CH_2 , OCH_2), 113.46/113.51 (CH, 6), 121.08/121.11 (CH, 4), 128.58 (C_q , 2), 129.92 (CH, 3), 133.23 (CH, 5), 157.25 (C_q , 1), 177.04 (C_q , CON), 203.39 ppm (C_q , ketone)
 HRMS (ESI): calcd for $C_{17}H_{25}N_2O_4$ m/z 321.1809 $[M+H]^+$, found m/z 321.1807 $[M+H]^+$.

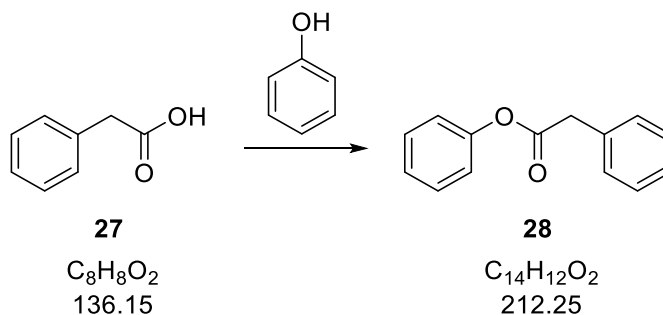
Hydrochloride formation: The amine **24** (242 mg, 1 eq, 0.8 mmol) was dissolved in dioxane (5.7 mL) and aq. HCl (2 N, 4.6 mL) was added and stirred for 5 h. The solvent was evaporated, giving 265 mg of the hydrochloride (99%, brown honey).

HRMS (ESI): calcd for $C_{17}H_{25}N_2O_4$ m/z 321.1809 $[M+H]^+$, found m/z 321.1810 $[M+H]^+$.

A.26.3. Synthesis of chromone derivatives**A.26.3.1. Synthesis of chromone derivatives with R¹=Ph****A.26.3.1.1. Synthesis of Boc-glycine (26)**

Glycine (**25**, 3 g, 1 eq, 40 mmol) was dissolved in a dioxane-water mixture (50:50, 100 mL), and first triethyl amine (5.5 mL, 1 eq, 40 mmol) and then Boc₂O (8.72 g, 1 eq, 40 mmol) dissolved in dioxane (10 mL) was added dropwise during 20 min. The reaction was stirred at room temperature overnight and then concentrated. The mixture was washed once with MTBE and subsequently rendered acidic using 2 N HCl. The aqueous phase was extracted three times with EtOAc, the combined organic phases were washed once with brine, dried over sodium sulphate and evaporated. The product **26** was obtained as a white solid (7.0 g, quant.).

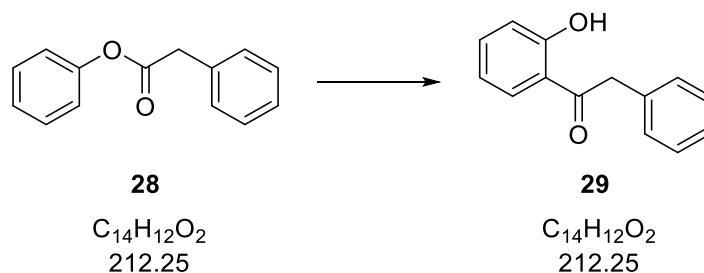
¹H NMR (200 MHz, CDCl₃): δ=1.46 (s, 9 H), 3.84-4.12 (m, 2H), 5.07 (brs, 0.6 H), 6.78 (brs, 0.3 H), 10.87 ppm (brs, 1H)

A.26.3.1.2. Synthesis of phenyl 2-phenylacetate (28)

Preparation according to ¹³¹. Phenylacetic acid (**27**, 10 g, 1 eq, 73.4 mmol) was dissolved in chloroform (150 mL) and thionyl chloride (5.33 mL, 1 eq, 73.4 mmol) was added. The mixture was heated for 4 h under reflux, then the solvent was reduced under reduced pressure. The residue was dissolved in chloroform (150 mL) and phenol (6.91 g, 1 eq, 73.4 mmol) was added and stirred overnight at room temperature. As TLC showed remaining phenol on the next day, another batch of phenylacetic acid (5 g) was heated with SOCl₂ (5.3 mL) in chloroform (100 mL) under reflux for 3 h, evaporated, added to the reaction mixture and stirred again overnight. This led to complete consumption of phenol, and the reaction mixture was evaporated. The crude product was purified by column chromatography (PE:EtOAc=15:1-10:1) yielding 15.40 g (99%) of **28** as a white solid.

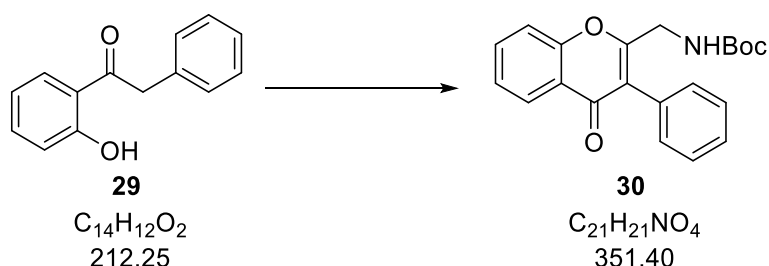
¹H NMR (500 MHz, CDCl₃): δ=3.88 (s, 2H), 7.08 (d, J=7.9 Hz, 2H), 7.23 (t, J=7.4 Hz, 1H), 7.33 (t, J=6.9 Hz, 1H), 7.35-7.44 ppm (m, 6H)

¹³C NMR (125 MHz, CDCl₃): δ=41.39 (CH₂), 121.41 (CH, 2C), 125.83 (CH), 127.31 (CH), 128.69 (CH, 2C), 129.27 (CH, 2C), 129.35 (CH, 2C), 133.42 (C_q), 150.68 (C_q), 169.99 ppm (C_q)

A.26.3.1.3. Synthesis of 1-(2-hydroxyphenyl)-2-phenylethan-1-one (29)

Preparation according to ¹³¹. $AlCl_3$ (11.78 g, 1.25 eq, 88.3 mmol) was added to the ester **28** (15 g, 1 eq, 70.7 mmol), flushed with argon and stirred for 10 min at RT as a violent reaction set in. Then the mixture was heated at 100 °C for 1 h. After cooling to room temperature, conc. HCl (10 mL) was added portion wise followed by ice and water. After cooling again to room temperature, DCM was added, the phases were separated and the aqueous phase was extracted four more times with DCM. The combined organic phases were washed once with brine, dried over sodium sulphate, filtered and evaporated. To remove some of the also formed para product, the crude product was reuptaken in DCM and extracted once with aq. NaOH (0.5%, 250 mL). The NaOH-phase was extracted once with DCM, the combined DCM-phases were washed once with water, dried over sodium sulphate, filtered and evaporated. The crude product was then purified by column chromatography (PE:EtOAc=20:1 to 10:1) yielding 5.44 g (36%) of **29** as a colourless oil.

¹H NMR (200 MHz, $CDCl_3$): δ =4.27 (s, 2H), 6.89 (t, J =7.6 Hz, 1H), 6.96 (dd, J =8.4, 1.0 Hz, 1H), 7.19-7.39 (m, 5H), 7.44 (ddd, J =8.4, 7.2, 1.4 Hz, 1H), 7.83 (dd, J =8.0, 1.3 Hz, 1H), 12.19 ppm (s, 1H)

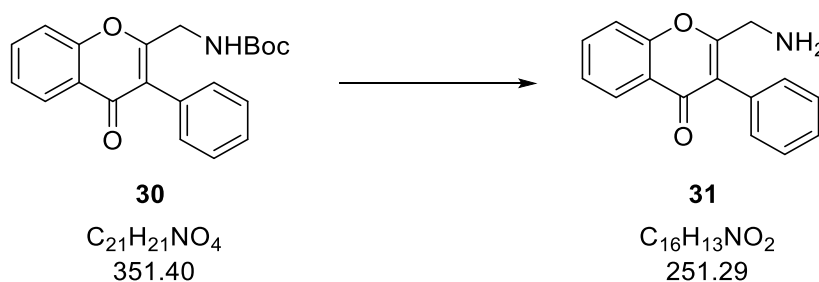
A.26.3.1.4. Synthesis of tert-butyl ((4-oxo-3-phenyl-4H-chromen-2-yl)methyl)carbamate (30)

Preparation according to ¹³⁰. 2-Hydroxyphenylbenzylketone (**29**, 1.49 g, 1 eq, 7.02 mmol) was dissolved in DCM (50 mL) and triethyl amine (7.3 mL, 7.5 eq, 52.65 mmol), Boc-glycine (1.36 g, 1.1 mmol, 7.72 mmol) and HATU (4.76 g, 1.7 eq, 12.5 mmol) were added. The mixture was stirred for 42 h, then water was added, the phases were separated and the aqueous phase was extracted three more times with DCM. The combined organic phases were dried over Na_2SO_4 , filtered and evaporated. The crude product was purified by column chromatography (petrol ether:EtOAc=6:1 to 3:1) to yield 1.64 g (66%) of **30** as a cream solid.

¹H NMR (500 MHz, $CDCl_3$): δ =1.45 (s, 9H, Boc- CH_3), 3.76 (s, 2H, CH_2N), 4.28 (brs, 1H, NH), 7.29 (d, J =7.3 Hz, 2H, 2', 6'), 7.36-7.43 (m, 2H, 6 & 4'), 7.45 (t, J =7.6 Hz, 2H, 3', 5'), 7.48 (d, J =8.2 Hz, 1H, 8), 7.69 (t, J =7.9 Hz, 1H, 7), 8.24 ppm (d, J =7.9 Hz, 1H, 5)

¹³C NMR (125 MHz, $CDCl_3$): δ =28.29 (CH_3 , 3C, Boc), 41.25 (CH_2 , CH_2N), 80.13 (C_q , $C(CH_3)_3$), 117.86 (CH, 8), 123.49 (C_q , 4a), 123.58 (C_q , 3), 125.16 (CH, 6), 126.32 (CH, 5), 127.26 (CH, 4'), 128.61 (CH, 2C, 3', 5'), 130.27 (CH, 2C, 2', 6'), 131.47 (C_q , 1'), 133.66 (CH, 7), 155.42 (C_q , O(CO)N), 155.80 (C_q , 8a), 160.81 (C_q , 2), 176.89 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{21}H_{21}NNaO_4$ m/z 374.1363 $[M+Na]^+$, found m/z 374.1362 $[M+Na]^+$.

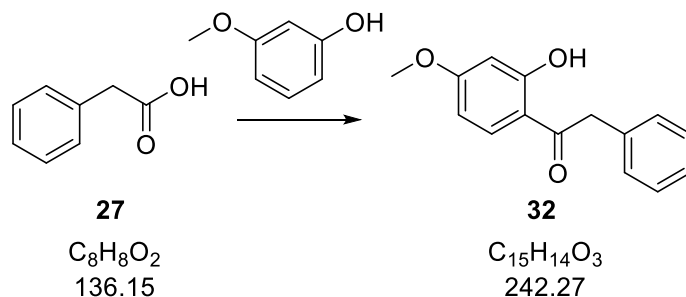
A.26.3.1.5. Synthesis of 2-(aminomethyl)-3-phenyl-4H-chromen-4-one (31)

Preparation according to ¹³⁰. The Boc-protected product **30** (792 mg, 1 eq, 2.25 mmol) was dissolved in DCM (10 mL) and TFA (6.7 mL) was added and stirred at RT for 2 h. Then the reaction mixture was concentrated under reduced pressure and taken up in sat. $NaHCO_3$ solution and EtOAc. The phases were separated and the aqueous phase was extracted three more times with EtOAc. The organic phases were dried over Na_2SO_4 , filtered and evaporated. The crude product was purified by column chromatography (DCM to DCM:MeOH=30:1) to yield 198 mg (35%) of **31** as an orange solid.

^1H NMR (500 MHz, $CDCl_3$): δ =3.53 (brs, 2H, NH_2), 3.76 (s, 2H, CH_2N), 7.28 (d, J =7.0 Hz, 2H, 2', 6'), 7.36-7.42 (m, 2H, 6 & 4'), 7.44 (t, J =7.4 Hz, 2H, 3', 5'), 7.50 (d, J =8.2 Hz, 1H, 8), 7.70 (ddd, J =8.2, 7.4, 1.4 Hz, 1H, 7), 8.24 ppm (dd, J =8.1, 1.4 Hz, 1H, 5)

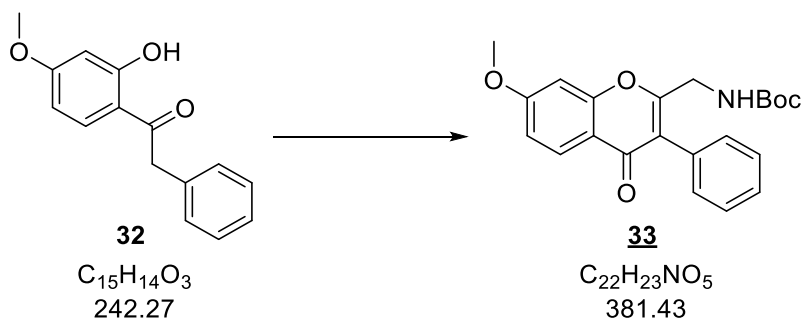
^{13}C NMR (125 MHz, $CDCl_3$): δ =42.65 (CH_2 , CH_2N), 117.74 (CH, 8), 122.92 (C_q , 3), 123.41 (C_q , 4a), 125.12 (CH, 6), 126.32 (CH, 5), 128.17 (CH, 4'), 128.58 (CH, 2C, 3', 5'), 130.28 (CH, 2C, 2', 6'), 131.85 (C_q , 1'), 133.71 (CH, 7), 155.85 (C_q , 8a), 164.19 (C_q , 2), 177.18 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{16}H_{14}NO_2$ m/z 252.1019 $[M+H]^+$, found m/z 252.1016 $[M+H]^+$.

A.26.3.1.6. Synthesis of 1-(2-hydroxy-4-methoxyphenyl)-2-phenylethan-1-one (32)

Preparation according to ¹³². 3-Methoxyphenol (5 g, 1 eq, 40.3 mmol) and phenylacetic acid (**27**, 5.76 g, 1.05 eq, 42.3 mmol) were treated with $BF_3 \cdot Et_2O$ and heated for 5 h at 85 °C. The mixture was stirred overnight at room temperature and then water was added and it was extracted three times with EtOAc. The combined organic extracts were washed once with brine, dried over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography (PE:EtOAc=20:1 to 5:1) yielding 2.17 g (22%) of **32** as a white solid.

^1H NMR (200 MHz, $CDCl_3$): δ =3.83 (s, 3H), 4.22 (s, 2H), 6.40-6.50 (m, 2H), 7.21-7.41 (m, 5H), 7.76 (dd, J =7.9, 2.0 Hz, 1H), 12.72 ppm (s, 1H)

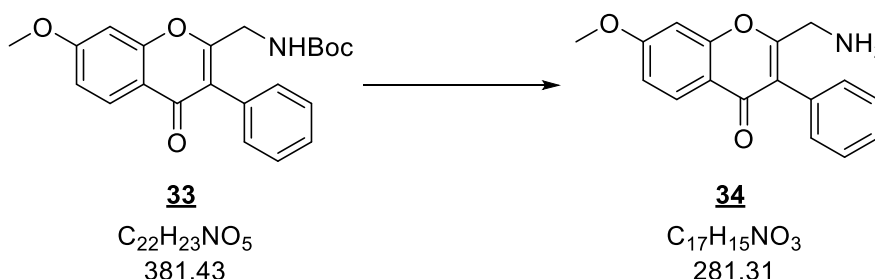
A.26.3.1.7. Synthesis of tert-butyl ((7-methoxy-4-oxo-3-phenyl-4H-chromen-2-yl)methyl)carbamate (33**)**

Following the same procedure as for **30**, **33** was prepared from 2-hydroxy-4-methoxybenzoine (**32**, 1.85 g, 1 eq, 7.6 mmol) to yield 1.82 g (63%, white solid) after purification (PE:EtOAc=6:1 to 2:1).

^1H NMR (500 MHz, $CDCl_3$): δ =1.45 (s, 9H, Boc- CH_3), 3.91 (s, 3H, OMe), 4.24 (brs, 2H, CH_2N), 4.85 (brs, 1H, NH), 6.87 (s, 1H, 8), 6.97 (d, J =8.9 Hz, 1H, 6), 7.27 (d, J =8.1 Hz, 2H, 2', 6'), 7.38 (t, J =6.9 Hz, 1H, 4'), 7.43 (t, J =7.2 Hz, 2H, 3', 5'), 8.13 ppm (d, J =8.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =28.30 (CH_3 , 3C, Boc), 41.21 (CH_2 , CH_2N), 55.83 (C_q , OMe), 80.11 (C_q , $C(CH_3)_3$), 100.05 (CH, 8), 114.52 (CH, 6), 117.33 (C_q , 4a), 123.54 (C_q , 3), 127.70 (CH, 5), 128.18 (CH, 4'), 128.55 (CH, 2C, 3', 5'), 130.30 (CH, 2C, 2', 6'), 131.59 (C_q , 1'), 155.42 (C_q , O(CO)N), 157.54 (C_q , 8a), 160.25 (C_q , 2), 160.10 (C_q , 7), 177.31 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{22}H_{23}NNaO_5$ m/z 404.1468 $[M+Na]^+$, found m/z 404.1468 $[M+Na]^+$.

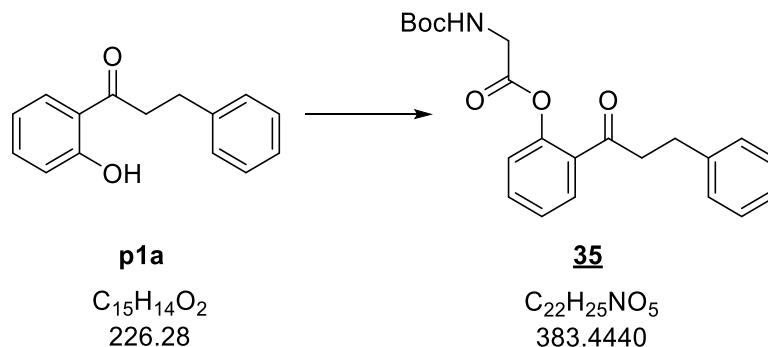
A.26.3.1.8. Synthesis of 2-(aminomethyl)-7-methoxy-3-phenyl-4H-chromen-4-one (34**)**

Following the same procedure as for **31**, **34** was obtained as a dark solid after purification by column chromatography (DCM to DCM:MeOH=30:1) (181 mg, 45%).

^1H NMR (500 MHz, $CDCl_3$): δ =1.94 (brs, 2H, NH_2), 3.91 (s, 3H, OMe), 4.24 (brs, 2H, CH_2N), 6.90 (d, J =2.1 Hz, 1H, 8), 6.98 (dd, J =8.9, 2.3 Hz, 1H, 6), 7.25-7.29 (m, 2H, 2', 6'), 7.38 (t, J =7.4 Hz, 1H, 4'), 7.44 (t, J =7.5 Hz, 2H, 3', 5'), 8.14 ppm (d, J =9.0 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =41.21 (CH_2 , CH_2N), 55.83 (C_q , OMe), 99.90 (CH, 8), 114.40 (CH, 6), 117.30 (C_q , 4a), 123.64 (C_q , 3), 127.72 (CH, 5), 128.03 (CH, 4'), 128.50 (CH, 2C, 3', 5'), 130.34 (CH, 2C, 2', 6'), 132.08 (C_q , 1'), 157.60 (C_q , 8a), 159.33 (C_q , 2), 164.05 (C_q , 7), 176.58 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{17}H_{16}NO_3$ m/z 282.1125 $[M+H]^+$, found m/z 282.1125 $[M+H]^+$.

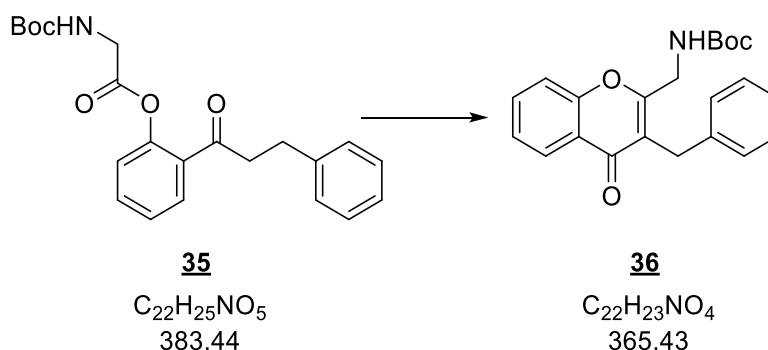
A.26.3.2. Synthesis of chromone compounds, R¹=Bn**A.26.3.2.1. Synthesis of 2-(3-phenylpropanoyl)phenyl 2-((tert-butoxycarbonyl)amino)acetate (**35**)**

Preparation according to ¹³³. The phenol **p1a** (1g, 1 eq, 4.4 mmol) and Boc-glycine (929 mg, 1.2 eq, 5.3 mmol) were dissolved in DCM (15 mL) and triethyl amine (1.84 mL, 3 eq, 13.3 mmol) and subsequently HATU (2.02 g, 1.2 eq, 5.3 mmol) was added and the mixture was stirred for two days at room temperature. The mixture was treated with semi-saturated NaHCO₃ solution, the phases were separated and the aqueous phase was extracted three times with DCM. The combined organic phases were washed once with brine, dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (PE:EtOAc= 10:1 to 5:1) yielding 1.05 g (62%) of **35** as a colourless oil.

¹H NMR (500 MHz, CDCl₃): δ=1.48 (s, 9H, Boc-CH₃), 3.00 (t, J=7.6 Hz, CH₂Ph), 3.22 (t, J=7.7 Hz, 2H, (CO)CH₂), 4.20 (d, J=5.7 Hz, 2H, CH₂N), 5.14 (brs, NH), 7.16 (d, J=8.2 Hz, 1H, 6), 7.16-7.24 (m, 3H, 2', 6' and 4'), 7.30 (t, J=7.4 Hz, 2H, 3', 5'), 7.32 (t, J=7.4 Hz, 1H, 4), 7.54 (t, J=7.7 Hz, 1H, 5), 7.77 (d, J=7.9 Hz, 1H, 3) ppm

¹³C NMR (125 MHz, CDCl₃): δ=28.29 (CH₃, Boc-CH₃), 29.89 (CH₂, CH₂Ph), 42.70 (CH₂, 2C, CH₂N & (CO)CH₂), 80.11 (C_q, C(CH₃)₃), 123.91 (CH, 6), 126.16 (CH, 4'), 126.33 (CH, 4), 128.40 (CH, 2C, 2', 6'), 128.51 (CH, 2C, 3', 5'), 129.80 (CH, 3), 130.03 (C_q, 2), 133.50 (CH, 5), 140.90 (C_q, 1'), 148.55 (C_q, 1), 155.71 (C_q, O(CO)N), 169.30 (C_q, ester), 198.91 (C_q, ketone) ppm

HRMS (ESI): calcd for C₂₂H₂₅NNaO₅ m/z 406.1625 [M+Na]⁺, found m/z 406.1624 [M+Na]⁺.

A.26.3.2.2. Synthesis of tert-butyl ((3-benzyl-4-oxo-4H-chromen-2-yl)methyl)carbamate (36**)**

Following ¹³³, the ester **35** (1.80 g, 1 eq, 4.7 mmol) was dissolved in DMF (60 mL), potassium carbonate (1.30 g, 2 eq, 9.4 mmol) was added and the mixture was immediately placed in a 130-135 °C oil bath and heated for 20 min. Then water (10 mL) was added, the mixture was left standing for 20 min, then it was diluted with water and MTBE and extracted three times with MTBE. The combined organic phases were washed once with brine, dried over Na₂SO₄ and evaporated. The crude product was

purified by column chromatography (PE:EtOAc=10:1 to 2:1) to yield 472 mg (27%) of **36** as a slightly yellowish solid.

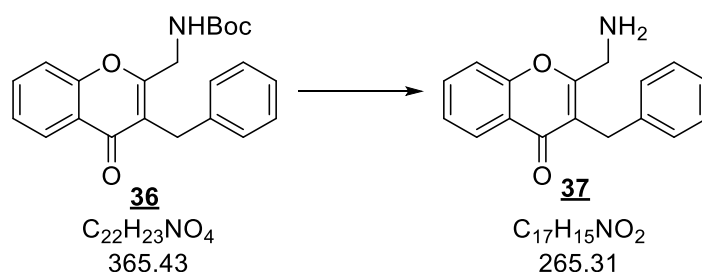
^1H NMR (500 MHz, CDCl_3): δ =1.45 (s, 9H, Boc- CH_3), 4.04 (s, 2H, CH_2Ph), 4.38 (d, J =6.1 Hz, 2H, CH_2N), 4.98 (brs, 1H, NH), 7.16 (t, J =7.3 Hz, 1H, 4'), 7.24 (t, J =7.8 Hz, 2H, 3', 5'), 7.28 (d, J =7.8 Hz, 2H, 2', 6'), 7.38 (t, J =7.5 Hz, 1H, 6), 7.41 (d, J =8.5 Hz, 1H, 8), 7.64 (t, J =8.0 Hz, 1H, 7), 8.22 ppm (dd, J =7.9, 1.4 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =28.30 (CH_3 , 3C, Boc), 29.24 (CH_2 , CH_2Ph), 40.73 (CH_2 , CH_2N), 80.25 (C_q , C(CH_3)₃), 117.73 (CH, 8), 121.13 (C_q , 3), 122.98 (C_q , 4a), 124.98 (CH, 6), 126.16 (CH, 5), 126.20 (CH, 4'), 128.26 (CH, 2C, 2', 6'), 128.55 (CH, 2C, 3', 5'), 133.49 (CH, 7), 139.68 (C_q , 1'), 155.49 (C_q , O(CO)N), 155.78 (C_q , 8a), 161.06 (C_q , 2), 177.72 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{23}\text{NNO}_4$ m/z 388.1519 $[\text{M}+\text{Na}]^+$, found m/z 388.1516 $[\text{M}+\text{Na}]^+$.

Coumarin: HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{23}\text{NNO}_4$ m/z 388.1519 $[\text{M}+\text{Na}]^+$, found m/z 388.1519 $[\text{M}+\text{Na}]^+$.

A.26.3.2.3. Synthesis of 2-(aminomethyl)-3-benzyl-4H-chromen-4-one (**37**)



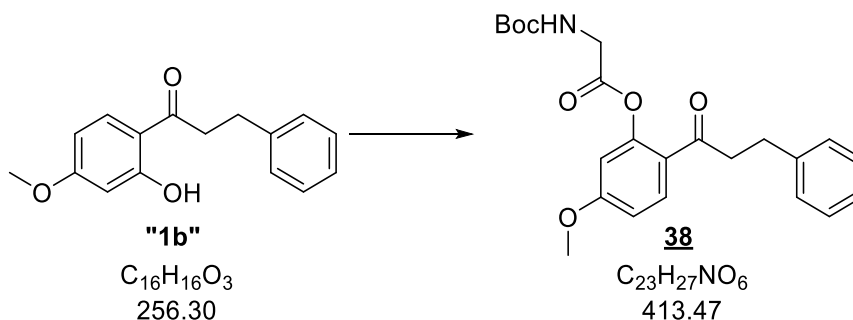
The protected product **36** (420 mg, 1 eq, 1.15 mmol) was dissolved in DCM (6 mL) and TFA (3.4 mL) was added. The mixture was stirred at room temperature for 2.5 h and then evaporated. The residue was dissolved in EtOAc and sat. NaHCO_3 solution and the phases were separated. The aqueous phase was extracted 3 more times with EtOAc. The combined organic fractions were dried over Na_2SO_4 , filtered, evaporated and purified by column chromatography (DCM:MeOH: NH_4OH conc. =100:0:2 to 100:2:1) to yield 208 mg (68%) **37** as a brown amorphous solid.

^1H NMR (500 MHz, CDCl_3): δ =3.88 (s, 2H, CH_2N), 3.99 (s, 2H, CH_2Ph), 7.14-7.20 (m, 1H, 4'), 7.24-7.27 (m, 4H, 2', 6' & 3', 5'), 7.39 (t, J =7.6 Hz, 1H, 6), 7.44 (d, J =8.4 Hz, 1H, 8), 7.65 (ddd, J =8.5, 7.2, 1.4 Hz, 1H, 7), 8.24 ppm (dd, J =8.1, 1.5 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =29.17 (CH_2 , CH_2Ph), 42.59 (CH_2 , CH_2N), 117.69 (CH, 8), 119.94 (C_q , 3), 122.94 (C_q , 4a), 124.90 (CH, 6), 126.14 (CH, 5), 126.26 (CH, 4'), 128.11 (CH, 2C, 2', 6'), 128.62 (CH, 2C, 3', 5'), 133.45 (CH, 7), 139.99 (C_q , 1'), 155.86 (C_q , 8a), 165.05 (C_q , 2), 177.93 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{17}\text{H}_{16}\text{NO}_2$ m/z 266.1176 $[\text{M}+\text{H}]^+$, found m/z 266.1175 $[\text{M}+\text{H}]^+$.

A.26.3.2.4. Synthesis of 5-methoxy-2-(3-phenylpropanoyl)phenyl 2-((tert-butoxycarbonyl)amino)acetate (**38**)

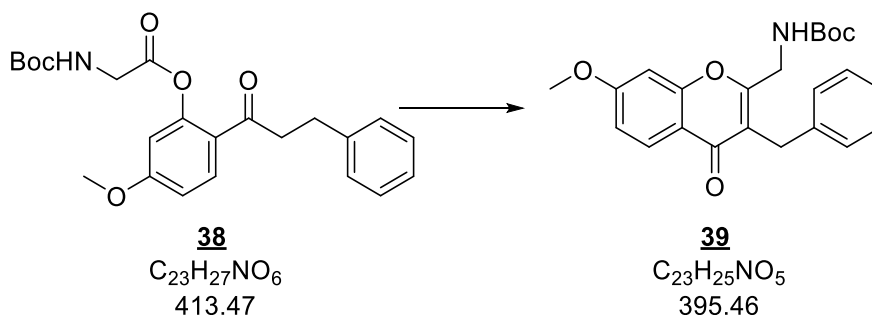


Following the same procedure as for **35**, **38** was prepared from 1-(2-hydroxy-4-methoxyphenyl)-3-phenylpropan-1-one (2.26 g, 1 eq, 8.8 mmol), Boc-glycine (1.86 g, 1.2 eq, 10.6 mmol), HATU (4.03 g, 1.2 eq, 10.6 mmol) and Et₃N (3.68 mL, 3 eq, 26.6 mmol) to yield 3.10 g (85%) as a white solid after column chromatography (PE:EtOAc=20:1 to 2:1).

¹H NMR (500 MHz, CDCl₃): δ=1.47 (s, 9H, Boc-CH₃), 2.97 (t, J=7.0 Hz, CH₂Ph), 3.18 (t, J=7.0 Hz, 2H, (CO)CH₂), 3.85 (s, 3H, OMe), 4.25 (d, J=4.4 Hz, 2H, CH₂N), 5.14 (brs, NH), 6.66 (s, 1H, 6), 6.81 (d, J=8.6 Hz, 1H, 4), 7.18-7.24 (m, 1H, 4'), 7.21 (d, J=6.9 Hz, 2H, 2', 6'), 7.30 (t, J=6.9 Hz, 2H, 3', 5'), 7.80 (d, J=8.6 Hz, 1H, 3) ppm

¹³C NMR (125 MHz, CDCl₃): δ=28.30 (CH₃, Boc-CH₃), 30.09 (CH₂, CH₂Ph), 42.15 (CH₂, (CO)CH₂), 42.78 (CH₂, CH₂N), 55.78 (CH₃, OMe), 80.08 (C_q, C(CH₃)₃), 109.28 (CH, 6), 112.10 (CH, 4), 121.84 (C_q, 2), 126.12 (CH, 4'), 128.37 (CH, 2C, 2', 6'), 128.51 (CH, 2C, 3', 5'), 131.95 (CH, 3), 141.09 (C_q, 1'), 151.03 (C_q, 1), 155.78 (C_q, O(CO)N), 163.75 (C_q, 5), 169.31 (C_q, ester), 198.95 (C_q, ketone) ppm

A.26.3.2.5. Synthesis of tert-butyl ((3-benzyl-7-methoxy-4-oxo-4H-chromen-2-yl)methyl)carbamate (**39**)



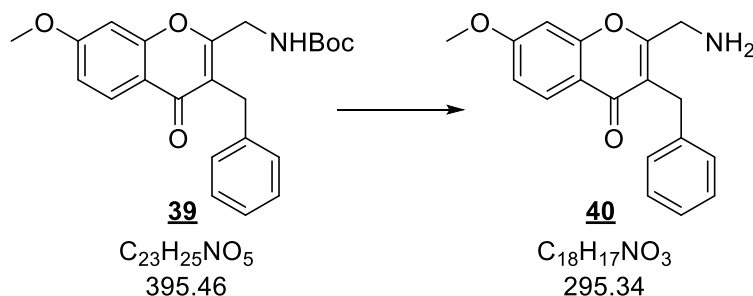
Following the same procedure as for **36**, **39** was obtained as a slightly yellowish solid after purification by column chromatography (PE:EtOAc=20:1 to pure EtOAc) (293 mg, 20%).

¹H NMR (500 MHz, CDCl₃): δ=1.45 (s, 9H, Boc-CH₃), 3.88 (s, 3H, OMe), 4.01 (s, 2H, CH₂Ph), 4.36 (brs, 2H, CH₂N), 4.95 (brs, 1H, NH), 6.79 (s, 1H, 8), 6.94 (d, J=8.4 Hz, 1H, 6), 7.13-7.19 (m, 1H, 4'), 7.21-7.32 (m, 4H, 3', 5' and 2', 6'), 8.11 ppm (d, J=8.9 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=28.30 (CH₃, 3C, Boc), 29.15 (CH₂, CH₂Ph), 40.66 (CH₂, CH₂N), 55.78 (CH₃, OMe), 80.20 (C_q, C(CH₃)₃), 99.80 (CH, 8), 114.39 (CH, 6), 116.87 (C_q, 4a), 120.95 (C_q, 3), 126.15 (CH, 4'), 127.52 (CH, 5), 128.26 (CH, 2C, 2', 6'), 128.52 (CH, 2C, 3', 5'), 139.84 (C_q, 1'), 155.48 (C_q, O(CO)N), 157.47 (C_q, 8a), 160.47 (C_q, 2), 163.9 (C_q, 7), 177.10 ppm (C_q, ketone 4)

HRMS (ESI): calcd for C₂₃H₂₆NO₅ m/z 396.1805 [M+H]⁺, found m/z 396.1803 [M+H]⁺.

Coumarin: HRMS (ESI): calcd for C₂₃H₂₅NNaO₅ m/z 418.1625 [M+Na]⁺, found m/z 418.1626 [M+Na]⁺.

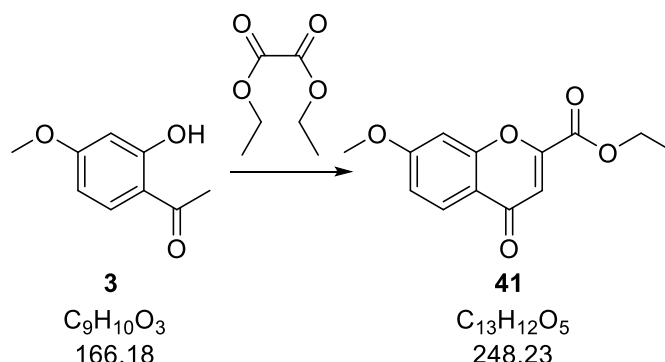
A.26.3.2.6. Synthesis of 2-(aminomethyl)-3-benzyl-7-methoxy-4H-chromen-4-one (40)

Following the same procedure as for **37**, **40** was obtained after purification by column chromatography (DCM to DCM:MeOH=40:1) (146 mg, 58%) as a brown solid.

^1H NMR (500 MHz, CDCl_3): δ =3.85 (s, 2H, CH_2N), 3.90 (s, 3H, OMe), 3.97 (s, 2H, CH_2Ph), 6.84 (d, J =2.3 Hz, 1H, 8), 6.96 (dd, J =8.8, 2.4 Hz, 1H, 6), 7.15-7.20 (m, 1H, 4'), 7.24-7.27 (m, 4H, 3', 5' and 2', 6'), 8.13 ppm (d, J =8.8 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =29.07 (CH_2 , CH_2Ph), 42.53 (CH_2 , CH_2N), 55.77 (CH_3 , OMe), 99.77 (CH, 8), 114.33 (CH, 6), 116.84 (C_q , 4a), 119.66 (C_q , 3), 126.19 (CH, 4'), 127.50 (CH, 5), 128.11 (CH, 2C, 2', 6'), 128.59 (CH, 2C, 3', 5'), 140.16 (C_q , 1'), 157.57 (C_q , 8a), 163.88 (C_q , 7), 164.51 (C_q , 2), 177.29 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{18}\text{H}_{18}\text{NO}_3$ m/z 296.1281 $[\text{M}+\text{Na}]^+$, found m/z 296.1286 $[\text{M}+\text{Na}]^+$.

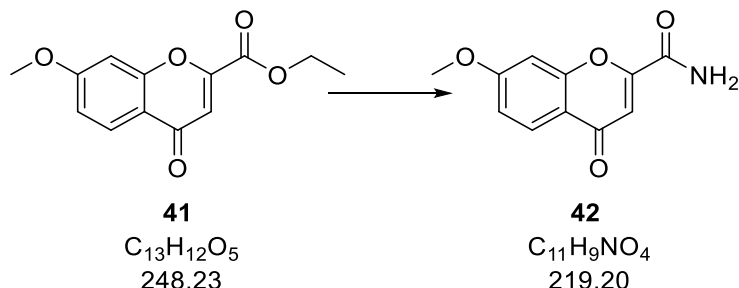
A.26.3.2.7. Synthesis of ethyl 7-methoxy-4-oxo-4H-chromene-2-carboxylate (41)

Prepared following ¹⁵⁴. Sodium (3.58 g, 4.3 eq, 156 mmol) was cut into small pieces and dissolved during 45 min in ethanol (240 mL). Then diethyl oxalate (11.4 mL, 2.3 eq, 84 mmol) and 2-hydroxy-4-methoxyacetophenone (6 g, 1 eq, 36.1 mmol) dissolved in EtOH (24 mL) was added dropwise during 30 min, which led to a dark orange colour. The reaction mixture was heated under reflux for 1 hour and then left standing at room temperature overnight. Then it was acidified with 6 N HCl, filtered and evaporated. The residue was dissolved in EtOH (200 mL), conc. H_2SO_4 (3 mL) was added and the mixture was stirred at 80 °C for 1 hour. After concentration under reduced pressure, the residue was reuptaken in EtOAc and sat. NaHCO_3 solution. The phases were separated and the aqueous phase was extracted three more times with EtOAc. The combined organic phases were dried over sodium sulphate, filtered and the solvent was removed under reduced pressure. The crude product was recrystallized from a MeOH-diisopropyl ether mixture (MeOH:diisopropyl ether=4:1) to yield 6.17 g (69%) of **41** as a white solid.

^1H NMR (500 MHz, CDCl_3): δ =1.43 (t, J =7.1 Hz, 3H, CH_3), 3.92 (s, 3H, OCH₃), 4.46 (q, J =7.1 Hz, 2H, CH_2), 7.00 (s, 1H, 8), 7.01 (dd, J =7.6, 2.5 Hz, 1H, 6), 7.07 (s, 1H, 3), 8.09 ppm (dd, J =7.4, 2.4 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =14.10 (CH_3 , CH_3), 55.97 (CH_3 , OCH_3), 62.94 (CH_2 , CH_2), 100.53 (CH , 8), 115.08 (CH , 3), 115.70 (CH , 6), 118.39 (C_q , 4a), 127.07 (CH , 5), 151.80 (C_q , 2), 157.89 (C_q , 8a), 160.61 (COO), 164.88 (C_q , 7), 177.62 ppm (C_q , ketone 4)

A.26.3.2.8. Synthesis of 7-methoxy-4-oxo-4H-chromene-2-carboxamide (**42**)

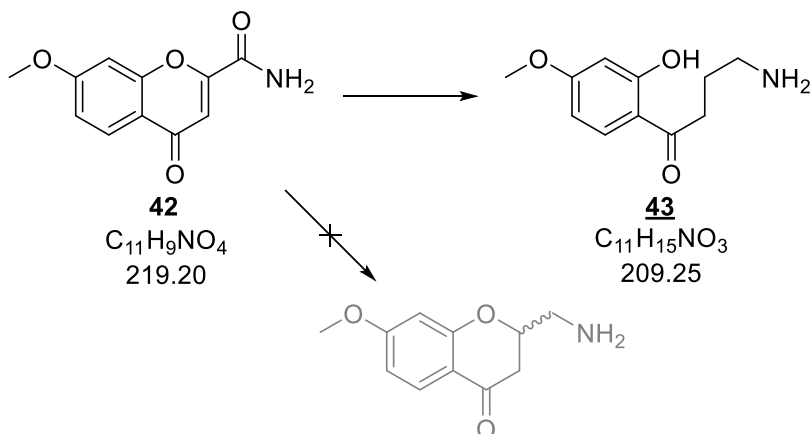


The ethyl ester (2 g, 1 eq, 8.05 mmol) was dissolved in methanol (10 mL), cooled to 0 °C and NH_3 in MeOH (7 M, 23 mL, 20 eq, 161 mmol) was added dropwise during 30 min, which lead to the precipitation of a white solid. The reaction mixture was warmed to room temperature and stirred overnight. The solid was separated by filtration, washed with 96% EtOH and dried under vacuum yielding 1.62 g (92%) of **42** as a white solid.

^1H NMR (500 MHz, D_6 -DMSO): δ =3.91 (s, 3H, OCH_3), 6.76 (s, 1H, 3), 7.11 (dd, J =9.0, 2.4 Hz, 1H, 6), 7.20 (d, J =2.2 Hz, 1H, 8), 7.95 (d, J =8.8 Hz, 1H, 5), 8.16 (brs, 1H, NH), 8.51 ppm (brs, 1H, NH)

^{13}C NMR (125 MHz, D_6 -DMSO): δ =56.07 (CH_3 , OCH_3), 100.99 (CH , 8), 110.55 (CH , 3), 115.27 (CH , 6), 117.46 (C_q , 4a), 126.39 (CH , 5), 155.47 (C_q , 2), 157.01 (C_q , 8a), 160.76 (CON), 164.23 (C_q , 7), 176.57 ppm (C_q , ketone 4)

A.26.3.2.9. Attempted synthesis of 2-(aminomethyl)-7-methoxychroman-4-one, synthesis of 4-amino-1-(2-hydroxy-4-methoxyphenyl)butan-1-one (**43**)

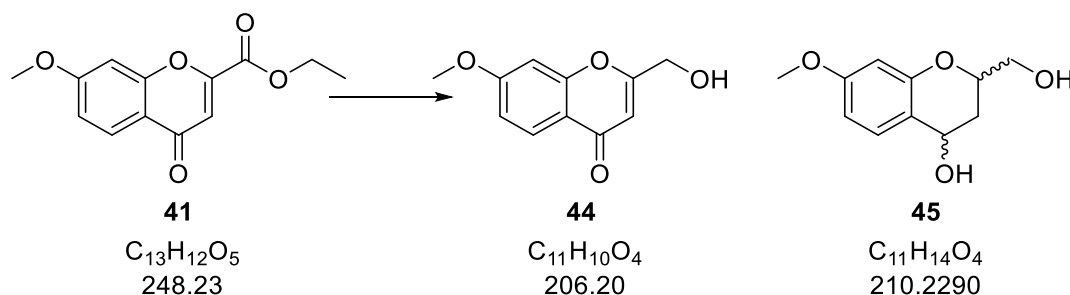


The amide **42** (200 mg, 1 eq, 0.91 mmol) was suspended in THF (50 mL) and LiAlH_4 (208 mg, 6 eq, 5.47 mmol) was added portion wise. The reaction was refluxed for 4 h and subsequently stirred at room temperature overnight. Then the mixture was cooled to 0 °C, 0.2 mL water, then 0.2 mL 15% NaOH, and then 0.6 mL water were added dropwise and stirred at room temperature for 2 h. It was filtered through celite, diluted with EtOAc and water, the phases were separated and the aqueous phase was extracted three more times with EtOAc, filtered and evaporated. After purification by column chromatography (EtOAc to EtOAc:MeOH=7:1) a product (15 mg), which was determined to be **43**, was obtained.

^1H NMR (500 MHz, D_6 -DMSO): δ =2.05 (quin, J =7.9 Hz, 2H, 3), 3.02 (t, J =8.5 Hz, 2H, 4), 3.81 (s, 3H, OMe), 4.03 (t, J =7.4 Hz, 2H, 2), 6.37 (dd, J =8.7, 2.4 Hz, 1H, Ph5), 6.52 (s, 1H, Ph3), 7.25 ppm (d, J =7.3 Hz, 1H, Ph6)

^{13}C NMR (125 MHz, D_6 -DMSO): δ =20.92 (CH_2 , 3), 34.01 (CH_2 , 4), 55.33 (CH_3 , OMe), 57.49 (CH_2 , 2), 101.19 (CH, Ph3), 106.19 (CH, Ph5), 109.94 (C_q , Ph1), 130.64 (CH, Ph6), 163.86 (C_q , Ph4), 165.40 (C_q , Ph2), 176.42 ppm (C_q , ketone1)

A.26.3.2.10. Synthesis of 2-(hydroxymethyl)-7-methoxy-4H-chromen-4-one (**44**) and 2-(hydroxymethyl)-7-methoxychroman-4-ol (**45**)



Preparation following ¹³⁷. The ester **41** (2 g, 1 eq, 8.1 mmol) was suspended in MeOH (50 mL) and NaBH_4 (1.46 g, 4.8 eq, 38.7 mmol) was added portion wise during 30 min, which led to a vigorous reaction. After stirring for 3.5 h, water and EtOAc were added, the phases were separated and the aqueous phase was extracted three more times with EtOAc. The combined organic phases were dried over sodium sulphate, filtered and the solvent was evaporated yielding 1.68 g (quant.) of a 1:1 mixture of and (both white solids). The two products were separated by column chromatography (solid deposit, PE:EtOAc=2:3 to pure EtOAc).

2-(hydroxymethyl)-7-methoxy-4H-chromen-4-one (**44**):

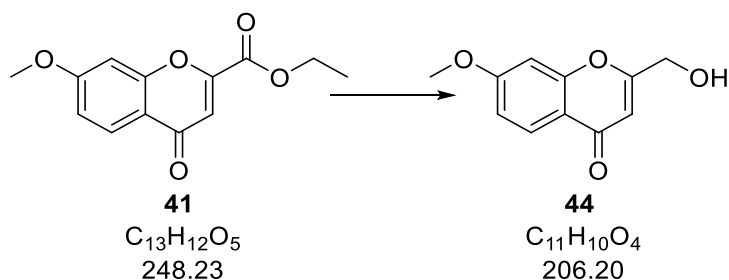
^1H NMR (500 MHz, D_6 -DMSO): δ =3.88 (s, 3H, OCH_3), 4.41 (d, J =6.0 Hz, 2H, OCH_2), 5.79 (t, J =6.1 Hz, 1H, OH), 6.25 (s, 1H, 3), 7.04 (dd, J =8.7, 2.4 Hz, 1H, 6), 7.10 (d, J =2.2 Hz, 1H, 8), 7.92 ppm (d, J =8.8 Hz, 1H, 5)

^{13}C NMR (125 MHz, D_6 -DMSO): δ =56.09 (CH_3 , OCH_3), 59.77 (CH_2 , OCH_2), 100.71 (CH, 8), 107.21 (CH, 3), 114.47 (CH, 6), 117.19 (C_q , 4a), 126.37 (CH, 5), 157.51 (C_q , 8a), 163.77 (C_q , 7), 169.16 (C_q , 2), 176.21 ppm (C_q , ketone 4)

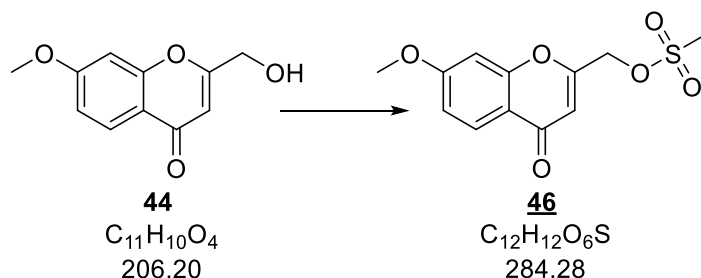
2-(hydroxymethyl)-7-methoxychroman-4-ol (**45**):

^1H NMR (500 MHz, D_6 -DMSO): δ =1.59 (dt, J =12.0, 11.5 Hz, 1H, 3-1), 2.12 (ddd, J =12.8, 6.1, 1.8 Hz, 1H, 3-2), 3.54 (dd, J =11.8, 5.5 Hz, 1H, CH_2O -1), 3.59 (dd, J =11.7, 5.7 Hz, 1H, CH_2O -2), 3.68 (s, 3H, OMe), 4.09 (dtd, J =11.2, 5.0, 1.5 Hz, 1H, 2), 4.70 (dt, J =11.3, 5.7 Hz, 1H, 4), 4.88 (t, J =5.7 Hz, 1H, CH_2OH), 5.31 (d, J =6.6 Hz, 1H, C^4HOH), 6.26 (d, J =2.5 Hz, 1H, 8), 6.46 (dd, J =8.5, 2.5 Hz, 1H, 6), 7.27 ppm (d, J =8.5 Hz, 1H, 5)

^{13}C NMR (125 MHz, D_6 -DMSO): δ =34.08 (CH_2 , 3), 55.04 (CH_3 , OMe), 63.13 (CH, 4), 63.74 (CH_2 , CH_2OH), 76.08 (CH, 2), 100.43 (CH, 8), 106.61 (CH, 6), 119.99 (C_q , 4a), 128.08 (CH, 5), 154.90 (C_q , 8a), 159.26 ppm (C_q , 7)

A.26.3.2.11. Synthesis of 2-(hydroxymethyl)-7-methoxy-4H-chromen-4-one (44**)**

Preparation loosely following ¹³⁷. The ester (**2 g**, 1 eq, 8.1 mmol) was suspended in MeOH (50 mL), cooled with an ice-salt bath and NaBH₄ (1.46 g, 4.8 eq, 38.7 mmol) was added portion wise during 45 min. After stirring for 1.5 h, water and EtOAc were added, the phases were separated and the aqueous phase was extracted three more times with EtOAc. The combined organic phases were dried over sodium sulphate, filtered and the solvent was evaporated yielding 1.60 g (96%) of **44** exclusively.

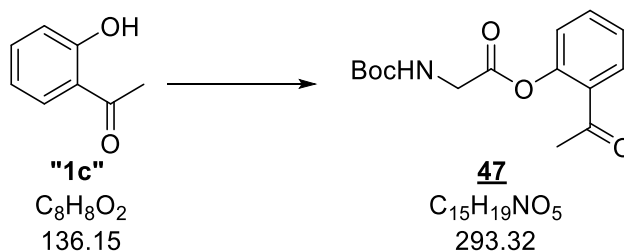
A.26.3.2.12. Synthesis of 2-((mesityloxy)methyl)-7-methoxy-4H-chromen-4-one (46**)**

Preparation according to ¹⁵⁵. The alcohol **44** (500 mg, 1 eq, 2.4 mmol) was suspended in DCM (50 mL), cooled with an ice bath and first triethyl amine (1.0 mL, 3 eq, 7.3 mmol) and then methanesulfonyl chloride (556 mg, 2 eq, 4.9 mmol) was added. The mixture was stirred for an additional 30 min at 0 °C, warmed to room temperature and stirred for 2 h at this temperature. Then water was added, the phases were separated and the aqueous phase was extracted three more times with DCM. The combined organic phases were dried over sodium sulphate, filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE:EtOAc=3:2 to 1:10) yielding 550 mg (80%) of **46** as a yellowish oil.

¹H NMR (500 MHz, CDCl₃): δ =3.14 (s, 3H, Mes), 3.90 (s, 3H, OCH₃), 5.07 (s, 2H, OCH₂), 6.39 (s, 1H, 3), 6.85 (s, 1H, 8), 6.98 (d, J=8.7 Hz, 1H, 6), 8.07 ppm (d, J=8.7 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ =38.35 (CH₃, Mes), 55.90 (CH₃, OCH₃), 65.90 (CH₂, OCH₂), 100.27 (CH, 8), 111.73 (CH, 3), 114.93 (CH, 6), 117.62 (C_q, 4a), 127.19 (CH, 5), 157.93 (C_q, 8a), 159.05 (C_q, 2), 164.49 (C_q, 7), 176.91 ppm (C_q, ketone 4)

HRMS (ESI): calcd for C₁₂H₁₂NaO₆S m/z 307.0247 [M+Na]⁺, found m/z 307.0247 [M+Na]⁺.

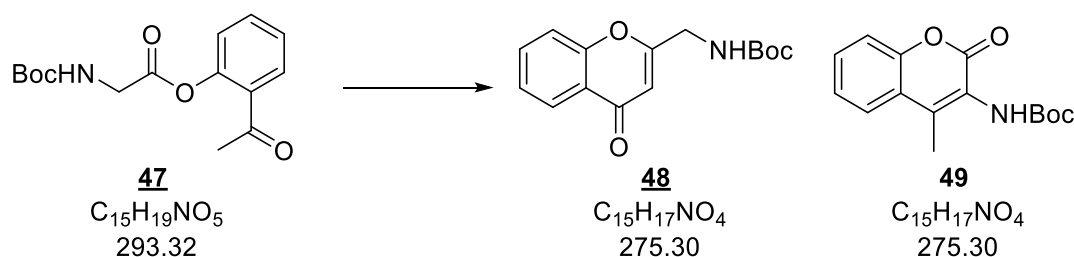
A.26.3.2.13. Synthesis of 2-acetylphenyl (tert-butoxycarbonyl)glycinate (47)

Following the same procedure as for 35, 47 was prepared from 2'-hydroxyacetophenone (1 g, 1 eq, 7.3 mmol), Boc-glycine (1.54 g, 1.2 eq, 8.8 mmol), HATU (3.35 g, 1.2 eq, 8.8 mmol) and Et₃N (3.1 mL, 3 eq, 22.0 mmol) to yield 1.97 g (92%) of 47 as a white solid after column chromatography (PE:EtOAc=20:1 to 2:1).

¹H NMR (500 MHz, CDCl₃): δ=1.46 (s, 9H, Boc-CH₃), 2.56 (s, 3H, CH₃), 4.24 (d, J=4.9 Hz, 2H, CH₂N), 5.13 (brs, NH), 7.16 (d, J=7.9 Hz, 1H, 6), 7.36 (t, J=7.4 Hz, 1H, 4), 7.56 (t, J=7.6 Hz, 1H, 5), 7.85 (d, J=7.5 Hz, 1H, 3) ppm

¹³C NMR (125 MHz, CDCl₃): δ=28.28 (CH₃, Boc-CH₃), 29.14 (CH₃), 42.79 (CH₂, CH₂N), 80.16 (C_q, C(CH₃)₃), 123.90 (CH, 6), 126.37 (CH, 4), 129.87 (C_q, 2), 130.64 (CH, 3), 133.80 (CH, 5), 148.76 (C_q, 1), 155.73 (C_q, O(CO)N), 169.31 (C_q, ester), 197.40 (C_q, ketone) ppm

HRMS (ESI): calcd for C₁₅H₁₉NNaO₅ m/z 316.1155 [M+Na]⁺, found m/z 316.1157 [M+Na]⁺.

A.26.3.2.14. Synthesis of tert-butyl ((4-oxo-4H-chromen-2-yl)methyl)carbamate (48) and tert-butyl (4-methyl-2-oxo-2H-chromen-3-yl)carbamate (49)

Preparation following ¹⁵⁶. The ester (610 mg, 1 eq, 2.07 mmol) was dissolved in pyridine (10 mL) and DBU (0.31 mL, 1 eq, 2.07 mmol) was added. The reaction was stirred for 1 h at 85 °C, then TLC showed that no starting material was present. After cooling, ice was added and the pH was adjusted to 3 using 3 N HCl. The mixture was extracted three times with EtOAc, the combined organic phases were washed once with brine, dried over sodium sulphate and evaporated. The crude product was purified by column chromatography yielding 140 mg of the coumarin 49, 49 mg of the chromone 48 and 47 mg of **p1c**.

Chromone 48:

¹H NMR (500 MHz, CDCl₃): δ=1.47 (s, 9H, Boc-CH₃), 4.29 (d, J=6.3 Hz, 2H, CH₂N), 5.04 (brs, t, J=6.3 Hz, 1H, NH), 6.33 (s, 1H, 3), 7.39 (t, J=7.6 Hz, 1H, 6), 7.43 (d, J=8.4 Hz, 1H, 8), 7.66 (t, J=7.7 Hz, 1H, 7), 8.17 (d, J=7.9 Hz, 1H, 5) ppm

¹³C NMR (125 MHz, CDCl₃): δ=28.29 (CH₃, 3C, Boc-CH₃), 42.17 (CH₂, CH₂N), 80.61 (C_q, C(CH₃)₃), 108.48 (CH, 3), 117.87 (CH, 8), 123.83 (C_q, 4a), 125.22 (CH, 6), 125.74 (CH, 5), 133.76 (CH, 7), 155.44 (C_q, O(CO)N), 156.22 (C_q, 8a), 165.88 (C_q, 2), 178.18 (C_q, ketone 4) ppm

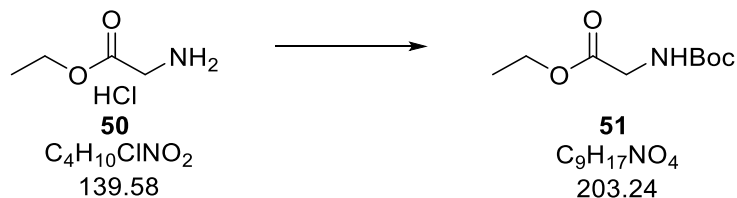
HRMS (ESI): calcd for C₁₅H₁₉NNaO₄ m/z 298.1050 [M+Na]⁺, found m/z 298.1055 [M+Na]⁺.

Coumarin 49:

¹H NMR (500 MHz, CDCl₃): δ=1.50 (s, 9 H, Boc), 2.40 (s, 3H, Me), 6.59 (brs, 1H, NH), 7.30-7.35 (m, 2H, 6&8), 7.49 (t, J=7.8 Hz, 1H, 7), 7.65 ppm (d, J=7.9 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =15.49 (CH_3 , Me), 28.13 (CH_3 , 3C, Boc), 81.26 (C_q , Boc), 116.68 (CH , 8), 120.85 (C_q , 4a), 121.04 (C_q , 3), 124.66 (CH , 6), 124.93 (CH , 5), 130.56 (CH , 7), 141.65 (C_q , 4), 150.79 (C_q , 8a), 153.05 (C_q , Boc), 159.89 ppm (C_q , ester)

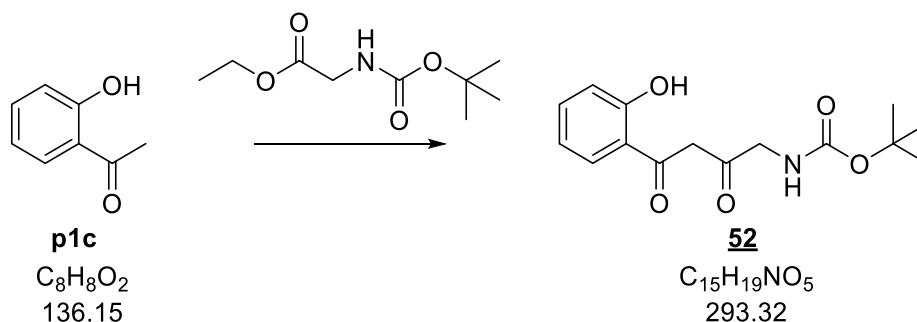
A.26.3.2.15. Synthesis of ethyl (tert-butoxycarbonyl)glycinate (**51**)



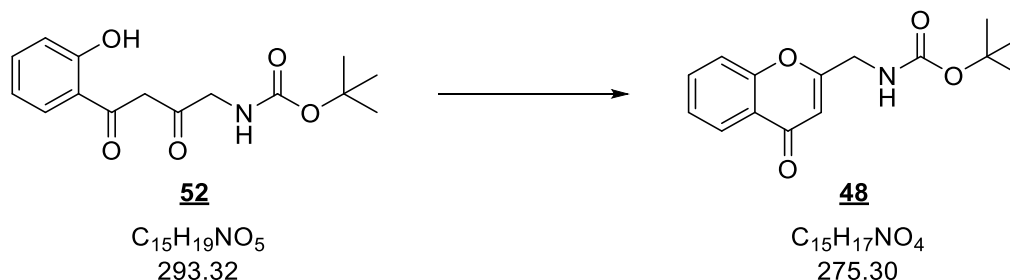
Preparation according to ¹⁵⁷. Glycine ethyl ester hydrochloride (**50**, 11 g, 1 eq, 78.8 mmol) was suspended in THF (200 mL), triethyl amine (21.9 mmol, 2 eq, 157.6 mmol) was added and the mixture was cooled with an ice bath. Boc_2O (17.2 g, 78.8 mmol) was dissolved in THF (30 mL) and added during 40 min. The reaction mixture was warmed to room temperature overnight, then filtered and the solvent was evaporated. The residue was taken up in EtOAc and 1 N HCl. The phases were separated, and the aqueous phase was extracted two more times with EtOAc. The combined organic phases were washed once with brine, dried over sodium sulphate and evaporated. The product **51** (15.36 g, 96%) was obtained as a yellow oil.

^1H NMR (200 MHz, CDCl_3): δ =1.26 (t, J =7.1 Hz, 3H), 1.44 (s, 9 H), 3.88 (d, J =5.4 Hz, 2H), 4.19 (q, J =7.2 Hz, 2H), 5.04 ppm (brs, 1H)

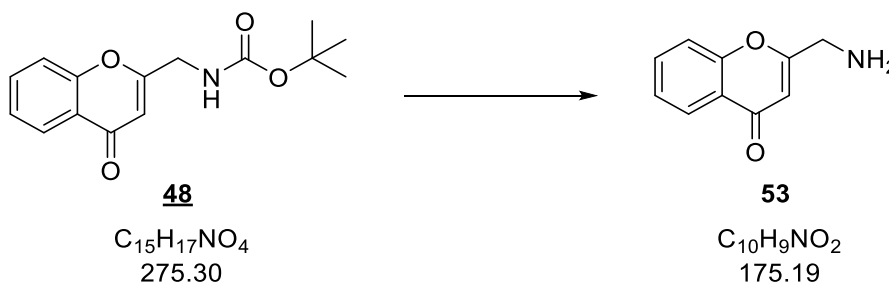
A.26.3.2.16. Synthesis of tert-butyl (4-(2-hydroxyphenyl)-2,4-dioxobutyl)carbamate (**52**)



Prepared according to ¹³⁹. NaH (60% dispersion in mineral oil, 1.77 g, 4 eq, 44 mmol) was washed and then suspended in THF (9 mL). 2'-Hydroxyacetophenone (**p1c**, 1.33 mL, 1 eq, 11 mmol) and Boc-glycine ethyl ester (4.16 g, 2 eq, 22 mmol) were dissolved in THF (2.5 mL) and added dropwise during 15 min, which lead to a yellow colour and warming of the reaction mixture. After complete addition, the reaction mixture was stirred at room temperature for 2 hours and then quenched by the addition of ice. The pH was adjusted to 7 by addition of 2 N HCl. After dilution with deionized water and MTBE the phases were separated, and the aqueous phase was extracted two more times with MTBE. The combined organic phases were washed once with sat. NaHCO_3 solution and once with brine, dried over sodium sulphate and evaporated. The crude product **52** (2.88 g, white solid, 1:0.2:0.2 mixture of diketone:starting material:cyclized product) was used without further purification in the next step. HRMS (ESI): calcd for $\text{C}_{15}\text{H}_{19}\text{NNaO}_5$ m/z 316.1155 [$\text{M}+\text{Na}$] $^+$, found m/z 316.1158 [$\text{M}+\text{Na}$] $^+$.

A.26.3.2.17. Synthesis of tert-butyl ((4-oxo-4H-chromen-2-yl)methyl)carbamate, method 2 (48**)**

Prepared according to ¹³⁸. The crude diketone (3.65 g) was dissolved in methanol (120 mL) and pyrrolidine (0.20 mL, 0.2 eq, 2.49 mmol) and water (22 μ L, 0.1 eq, 1.24 mmol) were added. The mixture was stirred at 55°C for 19 h, then complete consumption of starting material was indicated by TLC. The reaction mixture was concentrated and then diluted with a 1:1:1:1 mixture of MTBE, EtOAc, sat. $NaHCO_3$ solution and deionized water and the phases were separated. The aqueous phase was extracted two more times with EtOAc. The combined organic phases were extracted one with brine, dried over sodium sulphate and evaporated. The crude product was purified by column chromatography (petrol ether:EtOAc=6:1 to 1:1) to yield 1.57 g of **48** (41%, over 2 steps) as a beige solid.

A.26.3.2.18. Synthesis of 2-(aminomethyl)-4H-chromen-4-one (53**)**

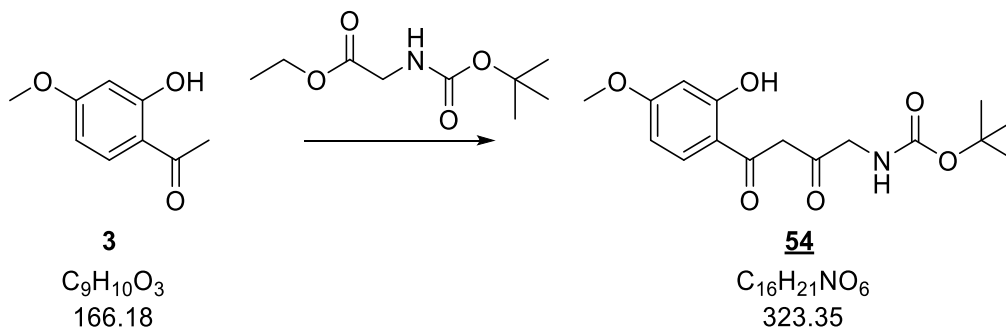
Preparation according to ¹³⁰. The protected product **48** (704 mg, 1 eq, 2.56 mmol) was dissolved in DCM (13 mL) and TFA (7.5 mL) was added. The mixture was stirred at room temperature for 2.5 h and then evaporated. The residue was dissolved in a 1:1 mixture of DCM and MTBE and sat. $NaHCO_3$ solution and the phases were separated. This first fraction was dried with sodium sulphate, evaporated separately and discarded after inspection. The aqueous phase was extracted 20 more times with DCM. The combined organic fractions were dried over Na_2SO_4 , filtered and evaporated to yield 335 mg (75%) product **53** as a yellowish solid.

^1H NMR (500 MHz, $CDCl_3$): δ =1.67 (brs, NH), 3.82 (s, 2H, CH_2N), 6.37 (s, 1H, 3), 7.39 (t, J =7.5 Hz, 1H, 6), 7.44 (d, J =8.4 Hz, 1H, 8), 7.66 (ddd, J =8.2, 7.3, 1.4 Hz, 1H, 7), 8.18 ppm (dd, J =7.9, 1.4 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =44.33 (CH_2 , CH_2N), 108.21 (CH, 3), 117.84 (CH, 8), 123.80 (C_q , 4a), 125.08 (CH, 6), 125.71 (CH, 5), 133.68 (CH, 7), 156.27 (C_q , 8a), 169.44 (C_q , 2), 178.43 (C_q , ketone 4) ppm

HRMS (ESI): calcd for $C_{10}H_{10}NO_2$ m/z 176.0706 $[M+H]^+$, found m/z 176.0705 $[M+H]^+$.

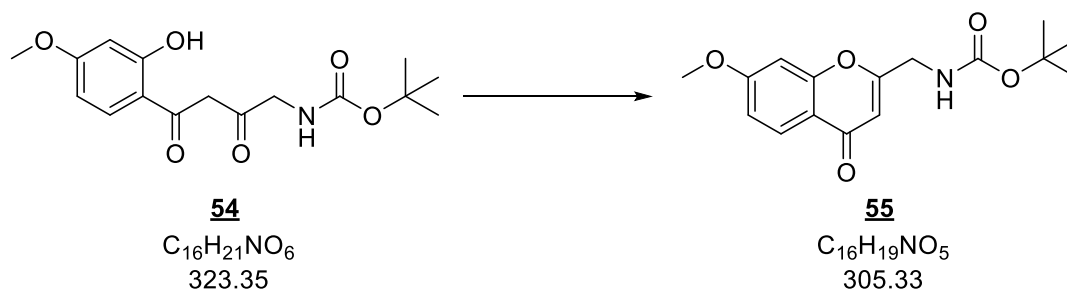
A.26.3.2.19. Synthesis of tert-butyl (4-(2-hydroxy-4-methoxyphenyl)-2,4-dioxobutyl)carbamate (**54**)



Following the same procedure as for **52**, **54** was prepared from 2'-hydroxy-4'-methoxyacetophenone (**3**, 1.83 g, 1 eq, 11 mmol), Boc-glycine ethyl ester (3.15 g, 1.5 eq, 16.5 mmol) and sodium hydride (washed, 2.7 g, 10 eq, 112 mmol). The reaction mixture was stirred overnight prior to workup yielding 2.37g of a crude mixture (diketone:starting material=1:0.2, light brown solid) which was used in the next step without further purification.

HRMS (ESI): calcd for $C_{16}H_{21}NNaO_6$ m/z 346.1261 $[M+Na]^+$, found m/z 346.1257 $[M+Na]^+$.

A.26.3.2.20. Synthesis of tert-butyl ((7-methoxy-4-oxo-4H-chromen-2-yl)methyl)carbamate (**55**)



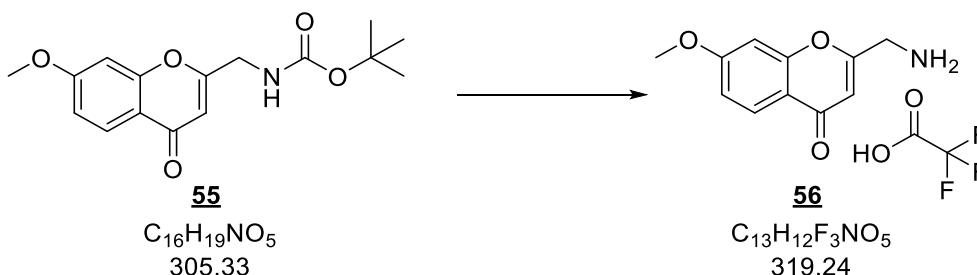
Following the same procedure as for **48**, method 2, starting from crude diketone **54** (2.28 g), **55** was obtained after purification (solid deposit, PE:EtOAc=10:1 to 1:2, 1.29 g, 40% yield over 2 steps) as a cream solid.

^1H NMR (500 MHz, $CDCl_3$): δ =1.47 (s, 9H, Boc- CH_3), 3.90 (s, 3H, OCH_3), 4.27 (d, J =5.8 Hz, 2H, CH_2N), 5.04 (brs, 1H, NH), 6.33 (s, 1H, 3), 6.84 (d, J =2.3 Hz, 1H, 8), 6.97 (dd, J =9.0, 2.1 Hz, 1H, 6), 8.08 (d, J =8.7 Hz, 1H, 5) ppm

^{13}C NMR (125 MHz, $CDCl_3$): δ =28.29 (CH_3 , 3C, Boc- CH_3), 42.17 (CH_2 , CH_2N), 55.84 (CH_3 , OCH_3), 80.60 (C_q , $C(CH_3)_3$), 100.20 (CH, 8), 108.25 (CH, 3), 114.51 (CH, 6), 117.45 (C_q , 4a), 127.10 (CH, 5), 155.43 (C_q , O(CO)N), 158.04 (C_q , 8a), 164.19 (C_q , 7), 165.66 (C_q , 2) 177.61 (C_q , ketone 4) ppm

HRMS (ESI): calcd for $C_{16}H_{19}NNaO_5$ m/z 328.1155 $[M+Na]^+$, found m/z 328.1160 $[M+Na]^+$.

A.26.3.2.21. Synthesis of 2-(aminomethyl)-7-methoxy-4H-chromen-4-one 2,2,2-trifluoroacetate (**56**)



Preparation loosely following ¹³⁰. The Boc-protected product **55** (280 mg, 1 eq, 0.92 mmol) was dissolved in DCM (4.6 mL), TFA (2.6 mL) was added and the mixture was stirred at room temperature for 2 hours. Evaporation yielded the TFA salt **56** (285 mg, 97%, brown solid) which was used in the next step without further purification.

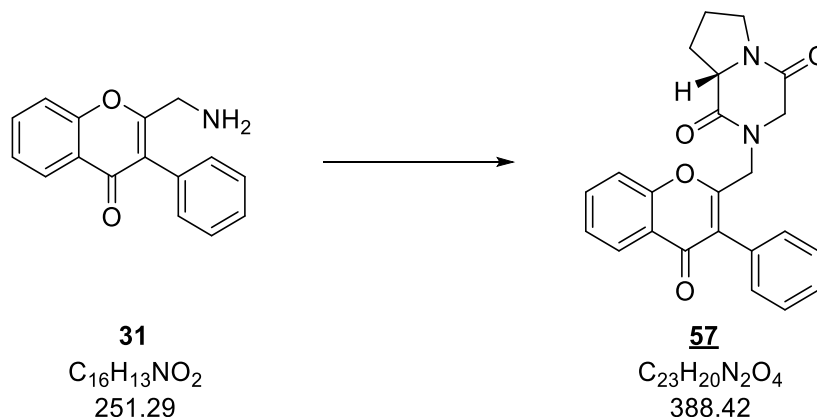
¹H NMR (500 MHz, D₆-DMSO): δ =3.91 (s, 3H, OCH₃), 4.15 (s, 2H, CH₂N), 6.44 (s, 1H, 3), 7.04 (s, 1H, 8), 7.11 (d, J=8.9 Hz, 1H, 6), 7.95 (d, J=8.9 Hz, 1H, 5), 8.53 (brs, 3H, NH₃⁺) ppm

¹³C NMR (125 MHz, D₆-DMSO): δ =39.87 (CH₂, CH₂N), 56.16 (CH₃, OCH₃), 100.63 (CH, 8), 110.71 (CH, 3), 114.87 (CH, 6), 115.83 (C_q, q, J=305.2 Hz, TFA-CF₃), 116.93 (C_q, 4a), 126.60 (CH, 5), 157.44 (C_q, 8a), 158.23 (C_q, q, J=35.4 Hz, TFA-(CO)), 160.81 (C_q, 2), 164.05 (C_q, 7), 175.81 (C_q, ketone 4) ppm

HRMS (ESI): calcd for C₁₁H₁₂NO₃ m/z 206.0812 [M+H]⁺, found m/z 206.0811 [M+H]⁺.

A.26.3.3. Synthesis of diketopiperazine derivatives of chromone compounds

A.26.3.3.1. Synthesis of (S)-2-((4-oxo-3-phenyl-4H-chromen-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**57**)



Preparation according to ^{100b}. The amine (170 mg, 1 eq, 0.68 mmol), methyl (2-chloroacetyl)prolinate (140 mg, 1 eq, 0.68 mmol) and triethylamine (0.12 mL, 1.3 eq, 0.88 mmol) were dissolved in 2-ethoxyethanol (5 mL) and heated at reflux for three days. Then the volatiles were evaporated and the residue was redissolved in deionized water/ sat. NaHCO₃ mixture and DCM. After separation of the phases the aqueous phase was extracted four more times with DCM, the combined organic phases were dried over Na₂SO₄, filtered and evaporated. The crude product was purified by column chromatography (twice, PE:EtOAc=1:1 to pure EtOAc to EtOAc:MeOH=100:1, then DCM to DCM:MeOH=50:1) yielding 79 mg (28%) of **57** as a white foam.

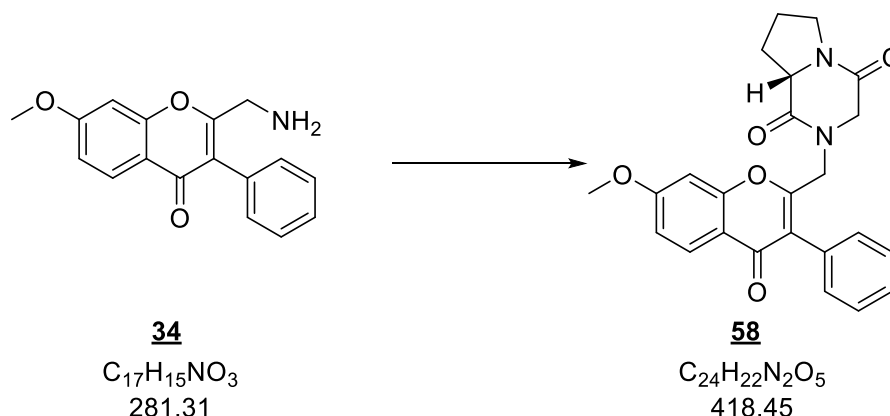
¹H NMR (500 MHz, CDCl₃): δ =1.81-1.97 (m, 1H, 7Dik-1), 1.99-2.10 (m, 2H, 7Dik-2 & 8Dik-1), 2.33-2.43 (m, 1H, 8Dik-2), 3.47-3.62 (m, 2H, 6Dik), 3.79 (d, J=16.4 Hz, 1H, 3Dik-1), 4.05 (t, J=7.9 Hz, 1H, 8aDik), 4.11 (dd, J=13.4, 3.8 Hz, 1H, 3Dik-2), 4.51 (d, J=15.7 Hz, 1H, CH₂N-1), 4.68 (d, J=15.9 Hz, 1H, CH₂N-2),

7.30 (d, $J=7.2$ Hz, 2H, 2',6'), 7.37-7.45 (m, 3H, 4', 6 & 8), 7.46 (t, $J=7.5$ Hz, 2H, 3', 5'), 7.68 (t, $J=7.8$ Hz, 1H, 7), 8.23 ppm (d, $J=8.1$ Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): $\delta=22.63$ (CH_2 , 7Dik), 28.65 (CH_2 , 8Dik), 45.27 (CH_2 , 6Dik), 46.06 (CH_2 , CH_2N), 52.28 (CH_2 , 3Dik), 58.71 (CH, 8aDik), 117.83 (CH, 8), 123.40 (C_q , 4a), 124.95 (C_q , 3), 125.43 (CH, 6), 126.38 (CH, 5), 128.46 (CH, 4'), 128.72 (CH, 2C, 3', 5'), 130.13 (CH, 2C, 2', 6'), 130.98 (C_q , 1'), 133.91 (CH, 7), 155.68 (C_q , 8a), 158.25 (C_q , 2), 162.60 (C_q , 4Dik), 167.46 (C_q , 1Dik), 176.58 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{NaO}_4$ m/z 411.1315 $[\text{M}+\text{Na}]^+$, found m/z 411.1321 $[\text{M}+\text{Na}]^+$.

A.26.3.3.2. Synthesis of (S)-2-((7-methoxy-4-oxo-3-phenyl-4H-chromen-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**58**)



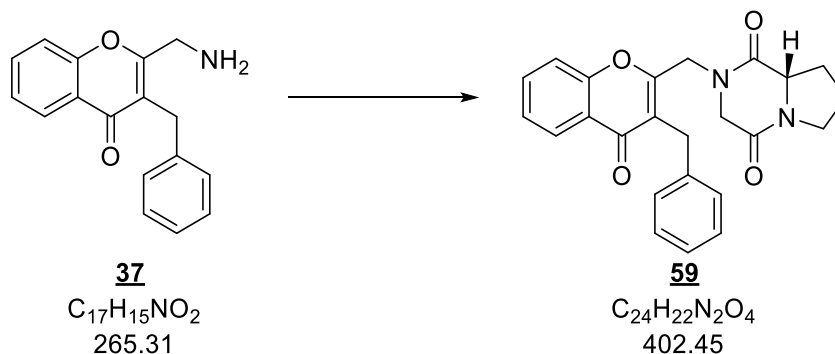
Following the same procedure as for **57**, **58** was prepared from **34** (128 mg, 1 eq, 0.45 mmol). The crude product was purified by column chromatography (PE:EtOAc=1.7:1 to EtOAc, EtOAc:MeOH=100:1 to 30:1) yielding 68 mg (36%) of **58** as a slightly yellowish solid.

^1H NMR (500 MHz, CDCl_3): $\delta=1.85$ -1.96 (m, 1H, 7Dik-1), 1.99-2.10 (m, 2H, 7Dik-2 & 8Dik-1), 2.34-2.43 (m, 1H, 8Dik-2), 3.48-3.64 (m, 2H, 6Dik), 3.77 (d, $J=16.2$ Hz, 1H, 3Dik-1), 3.91 (s, 3H, OMe), 4.05 (t, $J=7.8$ Hz, 1H, 8aDik), 4.09 (d, $J=16.8$ Hz, 1H, 3Dik-2), 4.44 (d, $J=15.6$ Hz, 1H, CH_2N -1), 4.71 (d, $J=15.9$ Hz, 1H, CH_2N -2), 6.90 (d, $J=1.9$ Hz, 1H, 8), 6.98 (dd, $J=9.0, 2.3$ Hz, 1H, 6), 7.28 (d, $J=7.8$ Hz, 2H, 2', 6'), 7.39 (t, $J=7.3$ Hz, 1H, 4'), 7.45 (t, $J=7.6$ Hz, 2H, 3', 5'), 8.12 ppm (d, $J=8.8$ Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): $\delta=22.62$ (CH_2 , 7Dik), 28.69 (CH_2 , 8Dik), 45.28 (CH_2 , 6Dik), 45.91 (CH_2 , CH_2N), 52.14 (CH_2 , 3Dik), 55.94 (CH_3 , OMe), 58.74 (CH, 8aDik), 99.96 (CH, 8), 114.83 (CH, 6), 117.24 (C_q , 4a), 125.02 (C_q , 3), 127.77 (CH, 5), 128.40 (CH, 4'), 128.67 (CH, 2C, 3', 5'), 130.18 (CH, 2C, 2', 6'), 131.11 (C_q , 1'), 157.46 (C_q , 8a), 158.63 (C_q , 2), 162.60 (C_q , 4Dik), 164.25 (C_q , 7), 167.40 (C_q , 1Dik), 175.95 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{22}\text{N}_2\text{NaO}_5$ m/z 441.1421 $[\text{M}+\text{Na}]^+$, found m/z 441.1423 $[\text{M}+\text{Na}]^+$.

A.26.3.3.3. Synthesis of (S)-2-((3-benzyl-4-oxo-4H-chromen-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**59**)



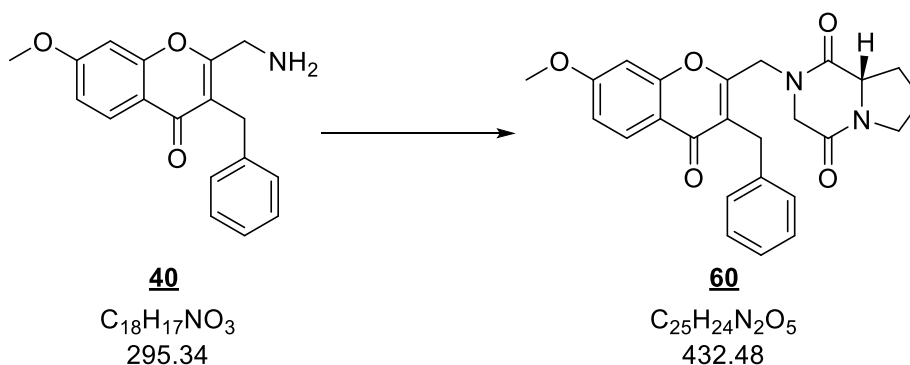
Following the same procedure as for **57**, **59** was prepared from **37** (180 mg, 1 eq, 0.68 mmol). The crude product was purified by column chromatography (twice, first DCM to DCM:MeOH=40:1, then PE:EtOAc=3:1 to pure EtOAc) yielding 86 mg (31%) of **59** as a white solid.

¹H NMR (500 MHz, CDCl₃): δ=1.82-1.92 (m, 1H, 7Dik-1), 1.92-2.03 (m, 2H, 7Dik-2 & 8Dik-1), 2.30-2.39 (m, 1H, 8Dik-2), 3.47-3.58 (m, 2H, 6Dik), 3.87 (t, J=8.1 Hz, 1H, 8aDik), 3.88 (d, J=16.2 Hz, 1H, 3Dik-1), 4.04 (d, J=15.6 Hz, 1H, CH₂Ph-1), 4.09 (d, J=15.3 Hz, 1H, CH₂Ph-2), 4.11 (d, J=15.9 Hz, 1H, 3Dik-2), 4.52 (d, J=15.4 Hz, 1H, CH₂N-1), 4.79 (d, J=15.4 Hz, 1H, CH₂N-2), 7.16 (t, J=7.0 Hz, 1H, 4'), 7.19 (d, J=7.6 Hz, 2H, 2', 6'), 7.25 (t, J=7.2 Hz, 2H, 3', 5'), 7.40 (d, J=8.1 Hz, 1H, 8), 7.42 (t, J=7.4 Hz, 1H, 6), 7.68 (ddd, J=8.3, 7.2, 1.3 Hz, 1H, 7), 8.23 ppm (dd, J=8.0, 1.4 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=22.55 (CH₂, 7Dik), 28.53 (CH₂, 8Dik), 29.06 (CH₂, CH₂Ph), 45.23 (CH₂, 6Dik), 45.77 (CH₂, CH₂N), 52.26 (CH₂, 3Dik), 58.64 (CH, 8aDik), 117.79 (CH, 8), 122.47 (C_q, 3), 122.87 (C_q, 4a), 125.36 (CH, 6), 126.24 (CH, 2C, 5 & 4'), 127.88 (CH, 2C, 2', 6'), 128.59 (CH, 2C, 3', 5'), 133.84 (CH, 7), 139.25 (C_q, 1'), 155.68 (C_q, 8a), 159.14 (C_q, 2), 162.51 (C_q, 4Dik), 167.26 (C_q, 1Dik), 177.53 ppm (C_q, ketone 4)

HRMS (ESI): calcd for C₂₄H₂₂N₂NaO₄ m/z 425.1472 [M+Na]⁺, found m/z 425.1472 [M+Na]⁺.

A.26.3.3.4. Synthesis of (S)-2-((3-benzyl-7-methoxy-4-oxo-4H-chromen-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**60**)



Following the same procedure as for **57**, **60** was prepared from **40** (126 mg, 1 eq, 0.43 mmol). The crude product was purified by column chromatography (PE:EtOAc=1:1 to EtOAc, EtOAc:MeOH=100:1 to 20:1) yielding 58 mg (31%) of **60** as a white solid.

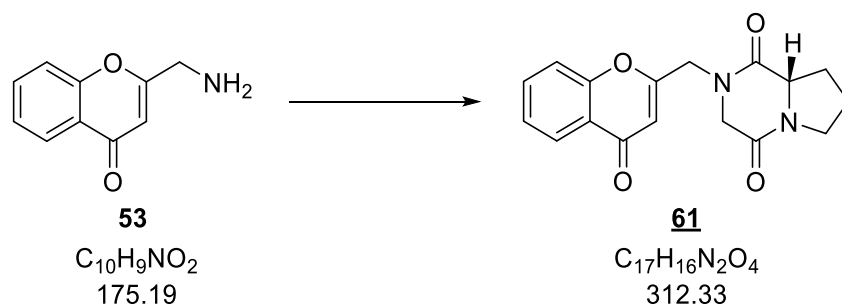
¹H NMR (500 MHz, CDCl₃): δ=1.82-1.92 (m, 1H, 7Dik-1), 1.92-2.05 (m, 2H, 7Dik-2 & 8Dik-1), 2.30-2.39 (m, 1H, 8Dik-2), 3.47-3.58 (m, 2H, 6Dik), 3.86 (t, J=7.7 Hz, 1H, 8aDik), 3.88 (d, J=16.5 Hz, 1H, 3Dik-1), 3.90 (s, 3H, OMe), 3.99-4.14 (m, 2H, CH₂Ph), 4.09 (d, J=15.9 Hz, 1H, 3Dik-2), 4.45 (d, J=15.7 Hz, 1H, CH₂N-1), 4.80 (d, J=15.1 Hz, 1H, CH₂N-2), 6.77 (d, J=2.1 Hz, 1H, 8), 6.97 (dd, J=8.9, 2.4 Hz, 1H, 6), 7.15

(t, J=7.1 Hz, 1H, 4'), 7.19 (d, J=7.3 Hz, 2H, 2', 6'), 7.24 (t, J=7.6 Hz, 2H, 3', 5'), 8.12 ppm (d, J=8.9 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=22.67 (CH₂, 7DiK), 28.55 (CH₂, 8DiK), 28.98 (CH₂, CH₂Ph), 45.22 (CH₂, 6DiK), 47.67 (CH₂, CH₂N), 52.15 (CH₂, 3DiK), 55.89 (CH₃, OMe), 58.64 (CH, 8aDiK), 99.75 (CH, 8), 114.90 (CH, 6), 116.78 (C_q, 4a), 122.42 (C_q, 3), 126.17 (CH, 4'), 127.62 (CH, 5), 127.90 (CH, 2C, 2', 6'), 128.55 (CH, 2C, 3', 5'), 139.44 (C_q, 1'), 157.46 (C_q, 8a), 158.24 (C_q, 2), 162.53 (C_q, 4DiK), 164.15 (C_q, 7), 167.16 (C_q, 1DiK), 176.84 ppm (C_q, ketone 4)

HRMS (ESI): calcd for $C_{25}H_{24}N_2NaO_5$ m/z 455.1577 $[M+Na]^+$, found m/z 455.1582 $[M+Na]^+$.

A.26.3.3.5. Synthesis of (S)-2-((4-oxo-4H-chromen-2-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (61)



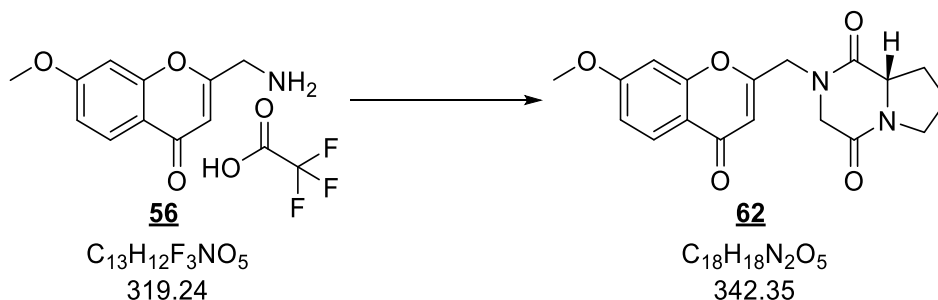
Preparation according to ^{100b}. The amine **53** (280 mg, 1 eq, 1.60 mmol), methyl (2-chloroacetyl)prolinat (334 mg, 1 eq, 1.60 mmol) and triethyl amine (0.32 mL, 1.3 eq, 2.1 mmol) were dissolved in 2-ethoxyethanol (15 mL) and heated at reflux for 43 h. Then the volatiles were evaporated and the residue was redissolved in deionized water/ sat. NaHCO₃ mixture and DCM. After separation of the phases the aqueous phase was extracted four more times with DCM, the combined organic phases were dried over Na₂SO₄, filtered and evaporated. The crude product was purified by column chromatography (petrol ether:EtOAc=1:1 to pure EtOAc, EtOAc:MeOH = 100:1 to 20:1) to yield 322 mg **61** (64%) as a slightly brown foam.

¹H NMR (500 MHz, CDCl₃): δ=1.89-2.01 (m, 1H, 7DiK-1), 2.03-2.18 (m, 2H, 7DiK-2 & 8DiK-1), 2.40-2.50 (m, 1H, 8DiK-2), 3.55-3.63 (m, 1H, 6DiK-1), 3.63-3.73 (m, 1H, 6-DiK-2), 3.97 (d, J=16.7 Hz, 1H, 3DiK-1), 4.20 (t, J=8.1 Hz, 1H, 8aDiK), 4.31 (d, J=16.3 Hz, 1H, 3DiK-2), 4.44 (d, J=16.2 Hz, 1H, CH₂N-1), 4.67 (d, J=16.2 Hz, 1H, CH₂N-2), 6.25 (s, 1H, 3), 7.38-7.46 (m, 2H, 6 & 8), 7.68 (ddd, J=8.8, 6.7, 1.5 Hz, 1H, 7), 8.17 ppm (d, J=8.1 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=22.60 (CH₂, 7Dik), 28.78 (CH₂, 8Dik), 45.38 (CH₂, 6Dik), 47.57 (CH₂, CH₂N), 52.53 (CH₂, 3Dik), 58.89 (CH, 8aDik), 110.18 (CH, 3), 117.94 (CH, 8), 123.73 (C_q, 4a), 125.54 (CH, 6), 125.80 (CH, 5), 134.03 (CH, 7), 156.19 (C_q, 8a), 162.18 (C_q, 2), 162.35 (C_q, 4Dik), 167.64 (C_q, 1Dik), 177.74 ppm (C_q, ketone 4)

HRMS (ESI): calcd for $C_{17}H_{16}N_2NaO_4$ m/z 335.1002 $[M+Na]^+$, found m/z 335.1005 $[M+Na]^+$.

A.26.3.3.6. Synthesis of 2-((7-methoxy-4-oxo-4H-chromen-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**62**)



Following the same procedure as for **57**, **62** was prepared from **56** (280 mg, 1 eq, 0.88 mmol). The amount of Et_3N was doubled (0.35 mL, 2.6 eq, 2.3 mmol) to account for the fact that the trifluoroacetate was used. The crude product was purified by column chromatography (DCM to DCM:MeOH=20:1) yielding 82 mg (27%) of **62** as a yellowish solid.

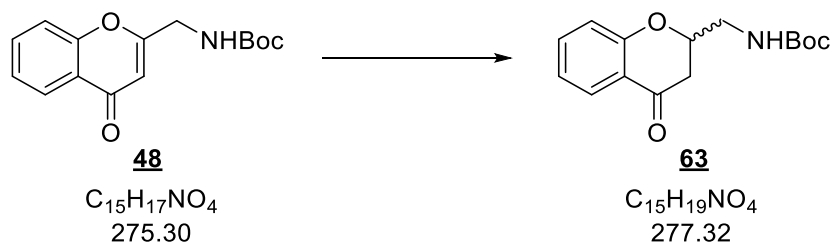
^1H NMR (500 MHz, $CDCl_3$): δ =1.88-2.01 (m, 1H, 7Dik-1), 2.03-2.19 (m, 2H, 7Dik-2 & 8Dik-1), 2.40-2.49 (m, 1H, 8Dik-2), 3.58 (t, J =10.5 Hz, 1H, 6Dik-1), 3.66 (t, J =8.9 Hz, 1H, 6-Dik-2), 3.90 (s, 3H, OMe), 3.96 (d, J =16.7 Hz, 1H, 3Dik-1), 4.19 (t, J =8.1 Hz, 1H, 8aDik), 4.29 (d, J =16.2 Hz, 1H, 3Dik-2), 4.38 (d, J =16.0 Hz, 1H, CH_2N -1), 4.66 (d, J =16.3 Hz, 1H, CH_2N -2), 6.18 (s, 1H, 3), 6.81 (s, 1H, 8), 6.97 (d, J =8.9 Hz, 1H, 6), 8.07 ppm (d, J =9.0 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =22.61 (CH_2 , 7Dik), 28.80 (CH_2 , 8Dik), 45.38 (CH_2 , 6Dik), 47.53 (CH_2 , CH_2N), 52.46 (CH_2 , 3Dik), 55.89 (CH_3 , OMe), 58.92 (CH, 8aDik), 100.27 (CH, 8), 110.40 (CH, 3), 114.74 (CH, 6), 117.61 (C_q , 4a), 127.17 (CH, 5), 158.00 (C_q , 8a), 161.52 (C_q , 2), 162.38 (C_q , 4Dik), 164.29 (C_q , 7), 167.60 (C_q , 1Dik), 177.09 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{18}H_{18}N_2NaO_5$ m/z 365.1108 $[M+Na]^+$, found m/z 365.1112 $[M+Na]^+$.

A.26.4. Synthesis of chromanone compounds

A.26.4.1. Synthesis of tert-butyl ((4-oxochroman-2-yl)methyl)carbamate (**63**)



Preparation according to ¹⁴¹. The chromone **48** (100 mg, 1 eq, 0.36 mmol) was dissolved in THF (5 mL) and cooled to -78 °C (dry ice-acetone bath). L-selectride (1 M in THF, 0.4 mL, 1.1 eq, 0.40 mmol) was added via syringe during 10 min. The solution was kept at -78 °C for one hour, then TLC indicated complete conversion and the reaction was quenched by the addition of sat. NH_4Cl solution (1 mL) and warmed to room temperature. The mixture was diluted with water and ethyl acetate, the phases were separated and the aqueous phase was extracted 2 more times with EtOAc. The combined organic phases were washed once with brine, dried over Na_2SO_4 , filtered and evaporated. The crude product was purified by column chromatography (petrol ether:EtOAc=5:1 to 1:3) to yield 55 mg (55%) of **63** as a white solid.

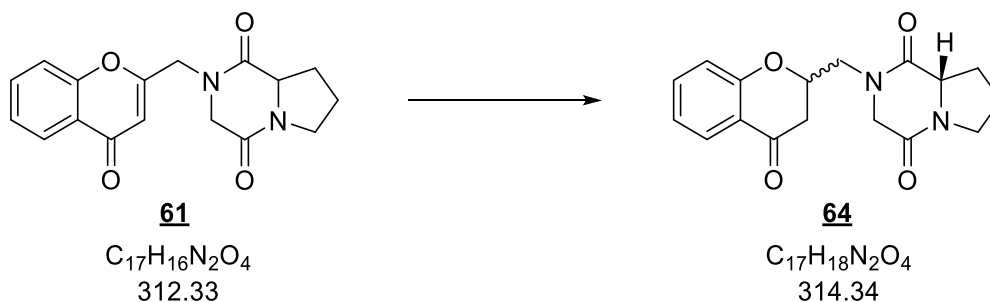
^1H NMR (500 MHz, $CDCl_3$): δ =1.45 (s, 9 H, Boc- CH_3), 2.67 (dd, J =16.9, 2.6 Hz, 1H, 3-1), 2.77 (dd, J =16.9, 13.2 Hz, 1H, 3-2), 3.48 (dt, J =13.4, 6.7 Hz, 1H, CH_2N -1), 3.60 (ddd, J =14.4, 6.1, 3.3 Hz, 1H, CH_2N -2), 4.48-

4.58 (m, 1H, 2), 5.02 (brs, 1H, NH), 6.98 (d, J=8.4 Hz, 1H, 8), 7.03 (t, J=7.5 Hz, 1H, 6), 7.49 (ddd, J=8.6, 7.1, 1.4 Hz, 1H, 7), 7.88 ppm (dd, J=7.8, 1.5 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =28.32 (CH_3 , 3C, Boc), 40.1 (CH_2 , 3), 43.95 (CH_2 , CH_2N), 77.07 (CH, 2), 79.91 (C_q , $\text{C}(\text{CH}_3)_3$), 117.76 (CH, 8), 120.84 (C_q , 4a), 121.64 (CH, 6), 127.03 (CH, 5), 136.08 (CH, 7), 155.80 (C_q , $\text{O}(\text{CO})\text{N}$), 160.97 (C_q , 8a), 191.65 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{15}\text{H}_{19}\text{NNaO}_4$ m/z 300.1206 $[\text{M}+\text{Na}]^+$, found m/z 300.1211 $[\text{M}+\text{Na}]^+$.

A.26.4.2. Synthesis of 2-((4-oxochroman-2-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**64**)



The chromone **61** (150 mg, 1 eq, 0.48 mmol) was dissolved/suspended in THF, cooled to -78°C (dry ice/acetone bath) and L-selectride (1 M in THF, 0.53 mL, 1.1 eq, 0.53 mmol) was added during 10 min, which led to complete dissolution of the solid. The mixture was stirred at -78°C for 1.5 h, then it was quenched by the addition of sat. NH_4Cl solution (1 mL) and subsequently warmed to room temperature. The reaction mixture was diluted with water and EtOAc and the phases were separated. The aqueous phase was extracted four more times with EtOAc. The combined organic phases were washed once with brine, dried over Na_2SO_4 , filtered and evaporated. The crude product was purified by column chromatography (EtOAc to EtOAc:MeOH = 100:1 to 20:1) to yield 89 mg (59%) of **64** as a white solid. The isomers were separated by preparative chiral column chromatography.

Isomer 1:

^1H NMR (500 MHz, CDCl_3): δ =1.87-1.99 (m, 1H, 7Dik-1), 2.01-2.17 (m, 2H, 7Dik-2 & 8Dik-1), 2.35-2.45 (m, 1H, 8Dik-2), 2.64-2.79 (m, 2H, 3), 3.53-3.68 (m, 2H, 6Dik), 3.76 (dd, J=14.4, 6.3 Hz, 1H, $\text{CH}_2\text{N}-1$), 3.83 (d, J=13.9 Hz, 1H, $\text{CH}_2\text{N}-2$), 4.19 (d, J=16.6 Hz, 1H, 3Dik-1), 4.32 (d, J=16.5 Hz, 1H, 3Dik-2), 4.14 (t, J=8.0 Hz, 1H, 8aDik), 4.65-4.73 (m, 1H, 2), 7.00 (d, J=8.4 Hz, 1H, 8), 7.04 (t, J=6.6 Hz, 1H, 6), 7.49 (t, J=7.6 Hz, 1H, 7), 7.87 ppm (d, J=7.6 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.72 (CH_2 , 7Dik), 28.60 (CH_2 , 8Dik), 40.31 (CH_2 , 3), 45.25 (CH_2 , 6Dik), 49.59 (CH_2 , CH_2N), 53.99 (CH_2 , 3Dik), 58.83 (CH, 8aDik), 76.83 (CH, 2), 118.03 (CH, 8), 120.78 (C_q , 4a), 121.94 (CH, 6), 127.01 (CH, 5), 136.29 (CH, 7), 160.59 (C_q , 8a), 163.20 (C_q , 4Dik), 167.96 (C_q , 1Dik), 190.96 ppm (C_q , ketone 4)

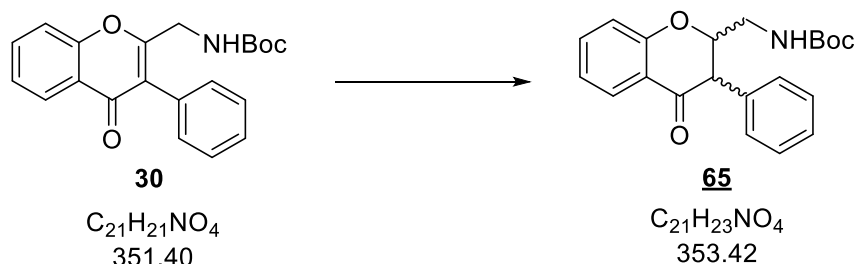
Isomer 2:

^1H NMR (500 MHz, CDCl_3): δ =1.86-1.99 (m, 1H, 7Dik-1), 1.99-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.35-2.46 (m, 1H, 8Dik-2), 2.63-2.80 (m, 2H, 3), 3.49 (dd, J=14.0, 7.7 Hz, 1H, $\text{CH}_2\text{N}-1$), 3.53-3.68 (m, 2H, 6Dik), 4.04 (d, J=15.6 Hz, 1H, $\text{CH}_2\text{N}-2$), 4.07 (d, J=17.8 Hz, 1H, 3Dik-1), 4.14 (t, J=7.8 Hz, 1H, 8aDik), 4.48 (d, J=16.6 Hz, 1H, 3Dik-2), 4.74 (t, J=7.9 Hz, 1H, 2), 6.95 (d, J=8.3 Hz, 1H, 8), 7.05 (t, J=7.6 Hz, 1H, 6), 7.49 (t, J=6.6 Hz, 1H, 7), 7.88 ppm (d, J=7.5 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.65 (CH_2 , 7Dik), 28.81 (CH_2 , 8Dik), 40.28 (CH_2 , 3), 45.23 (CH_2 , 6Dik), 50.08 (CH_2 , CH_2N), 54.31 (CH_2 , 3Dik), 59.02 (CH, 8aDik), 76.86 (CH, 2), 117.80 (CH, 8), 120.84 (C_q , 4a), 121.95 (CH, 6), 127.10 (CH, 5), 136.26 (CH, 7), 160.48 (C_q , 8a), 163.20 (C_q , 4Dik), 167.97 (C_q , 1Dik), 190.81 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{17}H_{18}N_2NaO_4$ m/z 337.1159 $[M+Na]^+$, found m/z 337.1162 $[M+Na]^+$.

A.26.4.3. Synthesis of tert-butyl ((4-oxo-3-phenylchroman-2-yl)methyl)-carbamate (**65**)



Following the same procedure as for **63**, **65** was prepared from **30** (664 mg, 1 eq, 1.89 mmol) and kept for 2 h 30 min at $-78^\circ C$ after the addition of L-selectride. The crude product was purified by column chromatography (petrol ether:EtOAc=10:1 to 5:1) yielding 429 mg (64%) of **65** as a white solid (1:0.18 mixture of cis:trans isomers)

Major isomer (cis):

^1H NMR (500 MHz, $CDCl_3$): δ =1.44 (s, 9 H, Boc- CH_3), 3.04-3.19 (m, 1H, CH_2N -1), 3.39-3.53 (m, 1H, CH_2N -2), 3.77 (s, 1H, 3), 4.75-4.82 (m, 1H, 2), 4.89 (brs, 1H, NH), 7.08-7.13 (m, 2H, 8 & 6), 7.14-7.22 (m, 2H, 2',6' or 3',5'), 7.23-7.30 (m, 3H, 4' & 2',6' or 3',5'), 7.58 (t, J =7.6 Hz, 1H, 7), 7.95 ppm (d, J =7.0 Hz, 1H, 5)

^1H NMR (200 MHz, $CDCl_3$): δ =1.44 (s, 9 H, Boc- CH_3), 3.15 (dd, J =8.8, 4.3, 1H, CH_2N -1), 3.33-3.57 (m, 1H, CH_2N -2), 3.77 (d, J =3.4 Hz, 1H, 3), 4.78 (dt, J =8.4, 3.6 Hz, 1H, 2), 4.88 (brs, 1H, NH), 7.01-7.22 (m, 4H, 8, 6 & 2',6' or 3',5'), 7.22-7.31 (m, 3H, 4' & 2',6' or 3',5'), 7.57 (ddd, J =8.3, 7.3, 1.6 Hz, 1H, 7), 7.95 ppm (dd, J =8.1, 1.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =28.32 (CH_3 , 3C, Boc), 42.36 (CH_2 , CH_2N), 54.69 (CH, 3), 79.37 (CH, 2), 79.90 (C_q , $C(CH_3)_3$), 117.96 (CH, 8), 120.60 (C_q , 4a), 122.21 (CH, 6), 127.97 (CH, 4'), 128.11 (CH, 5), 128.99 (CH, 2C, 3', 5' or 2', 6'), 129.02 (CH, 2C, 3',5' or 2', 6'), 133.41 (C_q , 1'), 136.39 (CH, 7), 155.76 (C_q , O(CO)N), 160.90 (C_q , 8a), 191.24 ppm (C_q , ketone 4)

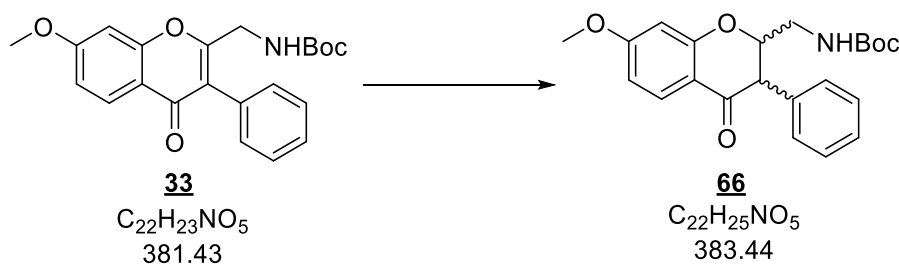
Minor isomer (trans):

^1H NMR (500 MHz, $CDCl_3$): δ =1.44 (s, 9 H, Boc- CH_3), 3.23-3.43 (m, 2H, CH_2N), 3.88 (d, J =11.8 Hz, 1H, 3), 4.57-4.77 (m, 1H, 2), 4.89 (brs, 1H, NH), 7.03-7.08 (m, 2H, 8 & 6), 7.13-7.22 (m, 2H, 2',6' or 3',5'), 7.23-7.42 (m, 3H, 4' & 2',6' or 3',5'), 7.54 (t, J =7.5 Hz, 1H, 7), 7.92-7.98 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =28.32 (CH_3 , 3C, Boc), 42.97 (CH_2 , CH_2N), 55.75 (CH, 3), 79.90 (C_q , $C(CH_3)_3$), 80.74 (CH, 2), 117.79 (CH, 8), 120.84 (C_q , 4a), 121.84 (CH, 6), 127.64 (CH, 4'), 128.03 (CH, 5), 129.06 (CH, 2C, 3', 5' or 2', 6'), 129.26 (CH, 2C, 3',5' or 2', 6'), 134.13 (C_q , 1'), 136.13 (CH, 7), 155.76 (C_q , O(CO)N), 160.44 (C_q , 8a), 192.24 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{21}H_{23}NNaO_4$ m/z 376.1519 $[M+Na]^+$, found m/z 376.1521 $[M+Na]^+$.

A.26.4.4. Synthesis of tert-butyl ((7-methoxy-4-oxo-3-phenylchroman-2-yl)methyl)carbamate (**66**)



Following the same procedure as for **63**, **66** was prepared from **33** (1.80 g, 1 eq, 4.7 mmol) and kept for 2 h 15 min at -78 °C after the addition of L-selectride. The crude product was purified by column chromatography (petrol ether:EtOAc=10:1 to 4:1) yielding 1.49 g (83%) of **66** as a white solid (1:0.21 mixture of cis:trans isomers?)

Major isomer (cis):

^1H NMR (500 MHz, $CDCl_3$): δ =1.44 (s, 9 H, Boc- CH_3), 3.04-3.14 (m, 1H, CH_2N -1), 3.40-3.49 (m, 1H, CH_2N -2), 3.71 (s, 1H, 3), 3.89 (s, 3H, OMe), 4.73-4.81 (m, 1H, 2), 4.87 (brs, 1H, NH), 6.55 (s, 1H, 8), 6.66 (d, J =8.9 Hz, 1H, 6), 7.12-7.21 (m, 2H 2', 6' or 3', 5'), 7.22-7.29 (m, 3H, 4' & 2', 6' or 3', 5'), 7.89 ppm (d, J =7.9 Hz, 1H, 5)

^1H NMR (200 MHz, $CDCl_3$): δ =1.44 (s, 9 H, Boc- CH_3), 3.12 (dd, J =8.8, 4.3 Hz, 1H, CH_2N -1), 3.29-3.54 (m, 1H, CH_2N -2), 3.70 (d, J =3.5 Hz, 1H, 3), 3.89 (s, 3H, OMe), 4.77 (dt, J =8.0, 4.0 Hz, 1H, 2), 4.86 (brs, 1H, NH), 6.54 (d, J =2.3 Hz, 1H, 8), 6.66 (dd, J =8.9, 2.3 Hz, 1H, 6), 7.10-7.21 (m, 2H 2', 6' or 3', 5'), 7.21-7.31 (m, 3H, 4' & 2', 6' or 3', 5'), 7.88 ppm (d, J =8.9 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =28.33 (CH_3 , 3C, Boc), 42.42 (CH_2 , CH_2N), 54.47 (CH, 3), 55.71 (CH_3 , OMe), 79.69 (CH, 2), 79.87 (C_q , C(CH_3)₃), 100.83 (CH, 8), 110.76 (CH, 6), 114.39 (C_q , 4a), 127.87 (CH, 4'), 128.96 (CH, 2C, 2', 6' or 3', 5'), 128.98 (CH, 2C, 2', 6' or 3', 5'), 129.82 (CH, 5), 133.76 (C_q , 1'), 155.74 (C_q , O(CO)N), 162.87 (C_q , 8a), 166.32 (C_q , 7), 189.97 ppm (C_q , ketone 4)

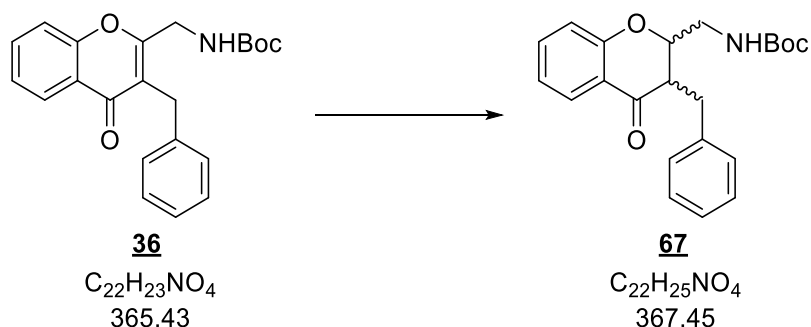
Minor isomer (trans):

^1H NMR (500 MHz, $CDCl_3$): δ =1.44 (s, 9 H, Boc- CH_3), 3.21-3.44 (m, 1H, CH_2N), 3.82 (d, J =11.3 Hz, 1H, 3), 3.87 (s, 3H, OMe), 4.56-4.78 (m, 1H, 2), 4.92 (brs, 1H, NH), 6.49 (s, 1H, 8), 6.63 (d, J =9.5 Hz, 1H, 6), 7.22-7.40 (m, 5H 2', 6', 3', 5' & 4'), 7.85-7.92 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =28.33 (CH_3 , 3C, Boc), 42.99 (CH_2 , CH_2N), 55.28 (CH, 3), 55.72 (CH_3 , OMe), 79.87 (C_q , C(CH_3)₃), 81.15 (CH, 2), 100.74 (CH, 8), 110.30 (CH, 6), 114.67 (C_q , 4a), 127.91 (CH, 4'), 129.00 (CH, 2C, 2', 6' or 3', 5'), 129.24 (CH, 2C, 2', 6' or 3', 5'), 129.39 (CH, 5), 134.59 (C_q , 1'), 155.74 (C_q , O(CO)N), 162.33 (C_q , 8a), 166.12 (C_q , 7), 190.80 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{22}H_{25}NNaO_5$ m/z 406.1625 [$M+Na$]⁺, found m/z 406.1630 [$M+Na$]⁺.

A.26.4.5. Synthesis of tert-butyl ((3-benzyl-4-oxochroman-2-yl)methyl)-carbamate (**67**)



Following the same procedure as for **63**, **67** was prepared from **36** (395 mg, 1 eq, 1.08 mmol) and kept for 2 h 30 min at -78 °C after the addition of L-selectride. The crude product was purified by column chromatography (petrol ether:EtOAc=10:1 to 2:1) yielding 339 mg (85%) of **67** as a clear honey (0.20:1 mixture of cis:trans isomers?)

Major isomer (trans):

¹H NMR (500 MHz, CDCl₃): δ=1.42 (s, 9 H, Boc-CH₃), 2.85-2.95 (m, 1H, 3), 3.04-3.14 (m, 1H, CH₂Ph-1), 3.14-3.23 (m, 1H, CH₂Ph-2), 3.44-3.60 (m, 2H, CH₂N), 4.27-4.35 (m, 1H, 2), 4.85 (brs, 1H, NH), 6.96 (d, J=7.9 Hz, 1H, 8), 7.03 (t, J=6.7 Hz, 1H, 6), 7.16-7.23 (m, 1H, 4'), 7.24-7.33 (m, 4H, 2', 6' & 3', 5'), 7.48 (t, J=7.4 Hz, 1H, 7), 7.90 ppm (d, J=7.8 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=28.31 (CH₃, 3C, Boc), 32.46 (CH₂, CH₂Ph), 41.84 (CH₂, CH₂N), 49.29 (CH, 3), 78.81 (CH, 2), 79.80 (C_q, C(CH₃)₃), 117.85 (CH, 8), 120.28 (C_q, 4a), 121.66 (CH, 6), 126.62 (CH, 4'), 127.31 (CH, 5), 128.60 (CH, 2C, 3', 5'), 129.39 (CH, 2C, 2', 6'), 136.18 (CH, 7), 137.99 (C_q, 1'), 155.62 (C_q, O(CO)N), 159.45 (C_q, 8a), 193.26 ppm (C_q, ketone 4)

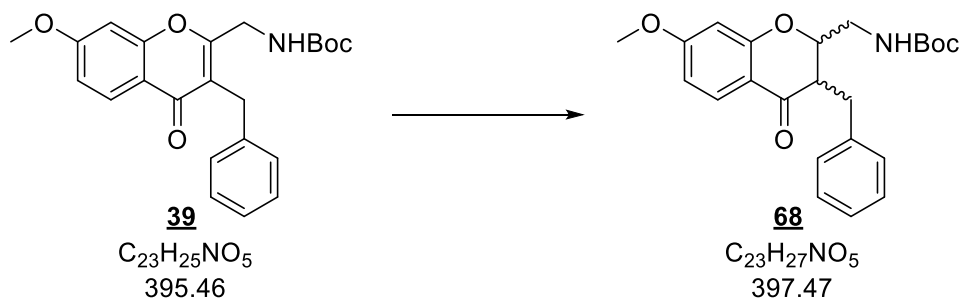
Minor isomer (cis):

¹H NMR (500 MHz, CDCl₃): δ=1.42 (s, 9 H, Boc-CH₃), 3.03-3.14 (m, 1H, 3), 3.28-3.40 (m, 2H, CH₂Ph), 3.44-3.61 (m, 2H, CH₂N), 4.47-4.57 (m, 1H, 2), 4.85 (brs, 1H, NH), 6.92-7.06 (m, 2H, 6 & 8), 7.14-7.33 (m, 5H, 4', 2', 6' & 3', 5'), 7.44-7.53 (m, 1H, 7), 7.84 ppm (d, J=7.8 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=28.31 (CH₃, 3C, Boc), 30.62 (CH₂, CH₂Ph), 40.30 (CH₂, CH₂N), 49.97 (CH, 3), 79.43 (CH, 2), 79.80 (C_q, C(CH₃)₃), 117.71 (CH, 8), 120.42 (C_q, 4a), 121.71 (CH, 6), 126.66 (CH, 4'), 127.38 (CH, 5), 128.63 (CH, 2C, 3', 5' or 2', 6'), 128.89 (CH, 2C, 2', 6' or 3', 5'), 135.99 (CH, 7), 138.24 (C_q, 1'), 155.62 (C_q, O(CO)N), 159.67 (C_q, 8a), 193.32 ppm (C_q, ketone 4)

HRMS (ESI): calcd for C₂₂H₂₅NNaO₄ m/z 390.1676 [M+Na]⁺, found m/z 390.1680 [M+Na]⁺.

A.26.4.6. Synthesis of tert-butyl ((3-benzyl-7-methoxy-4-oxochroman-2-yl)methyl)carbamate (**68**)



Following the same procedure as for **63**, **68** was prepared from **39** (288 mg, 1 eq, 0.73 mmol) and kept for 2 h 30 min at -78 °C after the addition of L-selectride. The crude product was purified by column

chromatography (petrol ether:EtOAc=15:1 to 5:1) yielding 239 mg (82%) of **68** as a white solid (0.22:1 mixture of cis:trans isomers?)

Major isomer (trans):

^1H NMR (500 MHz, CDCl_3): δ =1.42 (s, 9 H, Boc- CH_3), 2.78-2.90 (m, 1H, 3), 3.01-3.20 (m, 2H, CH_2Ph), 3.41-3.59 (m, 2H, CH_2N), 3.84 (s, 3H, OMe), 4.26-4.33 (m, 1H, 2), 4.82 (brs, 1H, NH), 6.40 (s, 1H, 8), 6.59 (d, J =8.7, 2.3 Hz, 1H, 6), 7.17 (t, J =7.0 Hz, 1H, 4'), 7.23-7.32 (m, 4H, 2', 6' & 3', 5'), 8.11 ppm (d, J =8.8 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =28.32 (CH_3 , 3C, Boc), 32.76 (CH_2 , CH_2Ph), 41.89 (CH_2 , CH_2N), 48.92 (CH, 3), 55.65 (CH_3 , OMe), 79.06 (CH, 2), 79.76 (C_q , $\text{C}(\text{CH}_3)_3$), 100.84 (CH, 8), 110.07 (CH, 6), 114.11 (C_q , 4a), 126.57 (CH, 4'), 128.59 (CH, 2C, 3', 5'), 129.07 (CH, 5), 129.38 (CH, 2C, 2', 6'), 138.11 (C_q , 1'), 155.59 (C_q , O(CO)N), 161.34 (C_q , 8a), 166.19 (C_q , 7), 191.79 ppm (C_q , ketone 4)

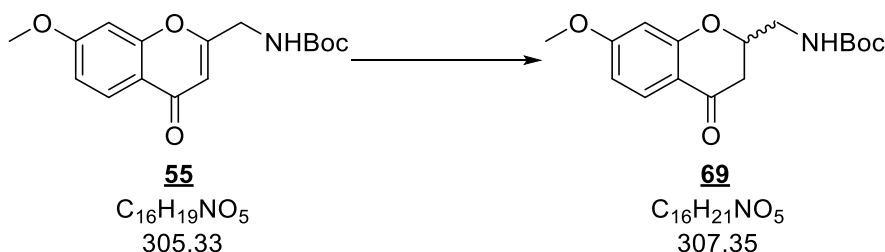
Minor isomer (cis):

^1H NMR (500 MHz, CDCl_3): δ =1.42 (s, 9 H, Boc- CH_3), 2.78-2.91 (m, 1H, CH_2Ph -1), 3.01-3.12 (m, 2H, CH_2Ph -2 & 3), 3.41-3.59 (m, 2H, CH_2N), 3.84 (s, 3H, OMe), 4.44-4.57 (m, 1H, 2), 4.82 (brs, 1H, NH), 6.43 (s, 1H, 8), 6.55-6.64 (m, 1H, 6), 7.15-7.35 (m, 5H, 4', 2', 6' & 3', 5'), 7.79 ppm (d, J =8.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =28.32 (CH_3 , 3C, Boc), 30.78 (CH_2 , CH_2Ph), 40.39 (CH_2 , CH_2N), 49.66 (CH, 3), 55.65 (CH_3 , OMe), 79.76 (C_q , $\text{C}(\text{CH}_3)_3$), 79.80 (CH, 2), 100.79 (CH, 8), 110.03 (CH, 6), 114.22 (C_q , 4a), 126.57 (CH, 4'), 128.60 (CH, 2C, 3', 5'), 128.88 (CH, 5), 129.38 (CH, 2C, 2', 6'), 138.45 (C_q , 1'), 155.59 (C_q , O(CO)N), 161.65 (C_q , 8a), 165.99 (C_q , 7), 192.02 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{27}\text{NNaO}_5$ m/z 420.1781 $[\text{M}+\text{Na}]^+$, found m/z 420.1788 $[\text{M}+\text{Na}]^+$.

A.26.4.7. Synthesis of tert-butyl ((7-methoxy-4-oxochroman-2-yl)methyl)-carbamate (**69**)



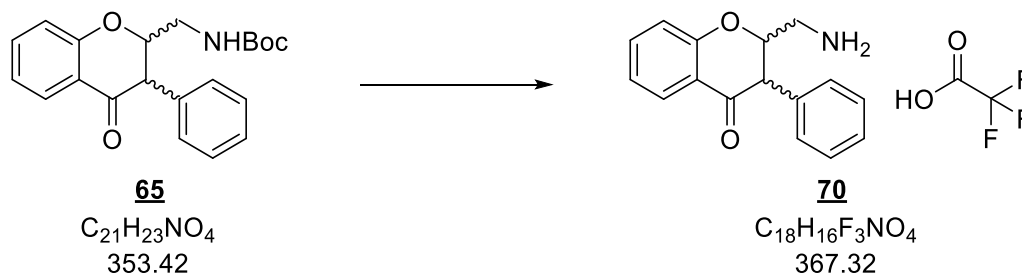
Following the same procedure as for **63**, **69** was prepared from **55** (1.25 g, 1 eq, 4.1 mmol) and kept for 2 h 20 min at -78°C after the addition of L-selectride. The crude product was purified by column chromatography (petrol ether:EtOAc=10:1 to 2:1) yielding 643 mg (51%) of **69** as a cream solid.

^1H NMR (500 MHz, CDCl_3): δ =1.46 (s, 9 H, Boc- CH_3), 2.61 (d, J =16.6 Hz, 1H, 3-1), 2.71 (dd, J =16.5, 13.9 Hz, 1H, 3-2), 3.39-3.51 (m, 1H, CH_2N -1), 3.53-3.63 (m, 1H, CH_2N -2), 3.84 (s, 3H, OMe), 4.47-4.58 (m, 1H, 2), 4.99 (brs, 1H, NH), 6.43 (s, 1H, 8), 6.59 (d, J =8.5 Hz, 1H, 6), 7.82 ppm (d, J =8.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =28.34 (CH_3 , 3C, Boc), 39.77 (CH_2 , 3), 44.00 (CH_2 , CH_2N), 55.65 (CH_3 , OMe), 77.35 (CH, 2), 79.91 (C_q , $\text{C}(\text{CH}_3)_3$), 100.78 (CH, 8), 110.02 (CH, 6), 114.78 (C_q , 4a), 128.78 (CH, 5), 155.80 (C_q , O(CO)N), 162.93 (C_q , 8a), 166.04 (C_q , 7), 191.65 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{16}\text{H}_{21}\text{NNaO}_5$ m/z 330.1312 $[\text{M}+\text{Na}]^+$, found m/z 330.1315 $[\text{M}+\text{Na}]^+$.

A.26.4.8. Synthesis of 2-(aminomethyl)-3-phenylchroman-4-one 2,2,2-trifluoroacetate (**70**)



Following the same procedure as for **56**, starting from **65** (409 mg, 1 eq, 1.16 mmol), **70** was obtained as a brown honey which was used without further purification (410 mg, quant., 1:1.20 mixture of cis:trans isomers).

HRMS (ESI): calcd for C₁₆H₁₆NO₂ m/z 254.1176 [M+H]⁺, found m/z 254.1178 [M+H]⁺.

Isomer 1 (cis):

¹H NMR (500 MHz, CDCl₃): δ=2.91-3.05 (m, 1H, CH₂N-1), 3.17 (d, J=14.0 Hz, 1H, CH₂N-2), 3.71-3.75 (m, 1H, 3), 4.91 (d, J=10.1, 1H, 2), 6.74 (brs, 1H, NH), 7.03-7.11 (m, 3H, 8 & 2', 6'), 7.11-7.18 (m, 1H, 6), 7.22-7.38 (m, 3H, 4' & 3',5'), 7.58 (t, J=7.6 Hz, 1H, 7), 7.53 (brs, 2H, NH), 7.89-7.95 ppm (m, 1H, 5)

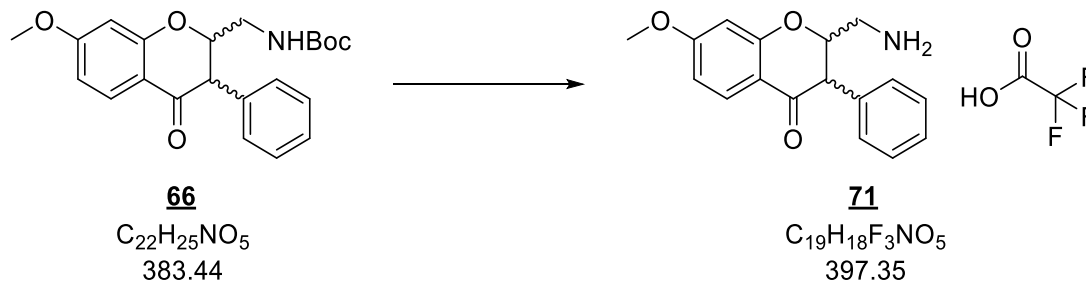
¹³C NMR (125 MHz, CDCl₃): δ=41.77 (CH₂, CH₂N), 53.93 (CH, 3), 75.99 (CH, 2), 115.21 (q, J=287.9 Hz, C_q, CF₃), 117.79 (CH, 8), 120.19 (C_q, 4a), 123.30 (CH, 6), 128.19 (CH, 5), 128.75 (CH, 4'), 128.69 (CH, 2C, 2', 6'), 129.47 (CH, 2C, 3',5'), 131.90 (C_q, 1'), 137.22 (CH, 7), 159.63 (C_q, 8a), 190.35 ppm (C_q, ketone 4)

Isomer 2 (trans):

¹H NMR (500 MHz, CDCl₃): δ=2.91-3.05 (m, 1H, CH₂N-1), 3.21 (t, J=12.1 Hz, 1H, CH₂N-2), 3.80 (d, J=12.4 Hz, 1H, 3), 4.83 (t, J=11.1, 1H, 2), 6.74 (brs, 1H, NH), 7.00 (d, J=8.1 Hz, 1H, 8) 7.05-7.11 (m, 2H, 2', 6'), 7.11-7.18 (m, 1H, 6), 7.22-7.38 (m, 3H, 4' & 3',5'), 7.53 (t, J=7.5 Hz, 1H, 7), 7.53 (brs, 2H, NH), 7.89-7.95 ppm (m, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=42.58 (CH₂, CH₂N), 55.77 (CH, 3), 77.20 (CH, 2), 115.21 (q, J=287.9 Hz, C_q, CF₃), 117.58 (CH, 8), 120.58 (C_q, 4a), 122.95 (CH, 6), 127.81 (CH, 5), 128.75 (CH, 4'), 129.01 (CH, 2C, 2', 6'), 129.49 (CH, 2C, 3',5'), 132.55 (C_q, 1'), 136.91 (CH, 7), 159.36 (C_q, 8a), 161.18 (q, J=39.4 Hz, C_q, CF₃(CO)), 191.09 ppm (C_q, ketone 4)

A.26.4.9. Synthesis of 2-(aminomethyl)-7-methoxy-3-phenylchroman-4-one 2,2,2-trifluoroacetate (**71**)



Following the same procedure as for **56**, starting from **66** (1000 mg, 1 eq, 2.6 mmol), **71** was obtained as a brown honey which was used without further purification (1.0 g, quant., 1:1.10 mixture of cis:trans isomers).

Isomer 1 (cis):

¹H NMR (500 MHz, CDCl₃): δ=2.88-2.99 (m, 1H, CH₂N-1), 3.10 (d, J=13.1 Hz, 1H, CH₂N-2), 3.61-3.70 (m, 1H, 3), 3.79 (s, 3H, OMe), 4.89 (d, J=8.5 Hz, 1H, 2), 5.45 (brs, 1H, NH), 6.51 (s, 1H, 8), 6.67 (d, J=9.2 Hz,

^1H NMR (500 MHz, CDCl_3): δ =7.07 (d, J =5.3 Hz, 2H, 2', 6'), 7.21-7.36 (m, 3H, 4' & 3', 5'), 7.82 (d, J =8.7 Hz, 1H, 5), 7.91 ppm (brs, 2H, NH)

^{13}C NMR (125 MHz, CDCl_3): δ =41.72 (CH_2 , CH_2N), 53.64 (CH, 3), 55.68 (CH_3 , OMe), 76.36 (CH, 2), 100.68 (CH, 8), 111.93 (CH, 6), 113.74 (C_q , 4a), 115.48 (q, J =288.2 Hz, C_q , CF_3), 128.57 (CH, 4'), 128.69 (CH, 2C, 2', 6'), 129.37 (CH, 2C, 3', 5'), 129.85 (CH, 5), 132.28 (C_q , 1'), 161.35 (q, J =38.5 Hz, C_q , $\text{CF}_3(\text{CO})$), 161.87 (C_q , 8a), 167.04 (C_q , 7), 189.27 ppm (C_q , ketone 4)

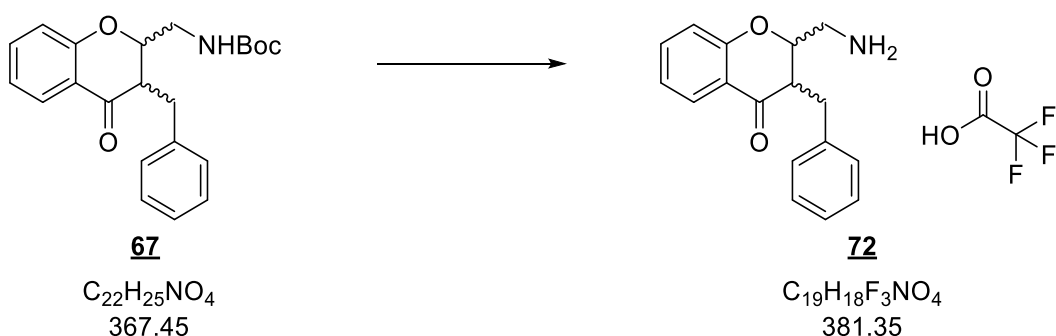
Isomer 2 (trans):

^1H NMR (500 MHz, CDCl_3): δ =2.88-2.99 (m, 1H, CH_2N -1), 3.16 (t, J =11.2 Hz, 1H, CH_2N -2), 3.74 (d, J =11.9 Hz, 1H, 3), 3.82 (s, 3H, OMe), 4.79 (t, J =10.2 Hz, 1H, 2), 5.45 (brs, 1H, NH), 6.45 (s, 1H, 8), 6.64 (d, J =8.9 Hz, 1H, 6), 7.07 (d, J =5.3 Hz, 2H, 2', 6'), 7.21-7.36 (m, 3H, 4' & 3', 5'), 7.82 (d, J =8.7 Hz, 1H, 5), 7.91 ppm (brs, 2H, NH)

^{13}C NMR (125 MHz, CDCl_3): δ =42.45 (CH_2 , CH_2N), 55.23 (CH, 3), 55.72 (CH_3 , OMe), 77.62 (CH, 2), 100.61 (CH, 8), 111.40 (CH, 6), 114.22 (C_q , 4a), 115.48 (q, J =288.2 Hz, C_q , CF_3), 128.63 (CH, 4'), 129.03 (CH, 2C, 2', 6'), 129.40 (CH, 2C, 3', 5'), 129.50 (CH, 5), 133.04 (C_q , 1'), 161.35 (q, J =38.5 Hz, C_q , $\text{CF}_3(\text{CO})$), 161.45 (C_q , 8a), 166.72 (C_q , 7), 189.75 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{17}\text{H}_{18}\text{NO}_3$ m/z 284.1281 $[\text{M}+\text{H}]^+$, found m/z 284.1283 $[\text{M}+\text{H}]^+$.

A.26.4.10. Synthesis of (2S,3R)-2-(aminomethyl)-3-benzylchroman-4-one 2,2,2-trifluoroacetate (**72**)



Following the same procedure as for **56**, starting from **67** (300 mg, 1 eq, 0.82 mmol), **72** was obtained as a brown honey which was used without further purification (313 mg, quant., 0.25:1 mixture of cis:trans isomers).

Major isomer (trans):

^1H NMR (500 MHz, CDCl_3): δ =2.85-2.92 (m, 1H, 3), 3.00 (dd, J =14.9, 8.4 Hz, 1H, CH_2Ph -1), 3.16-3.22 (m, 2H, CH_2N), 3.26 (d, J =14.7 Hz, 1H, CH_2Ph -2), 4.48-4.57 (m, 1H, 2), 6.35 (brs, 1H, NH), 6.97 (d, J =8.7 Hz, 1H, 8), 7.12 (t, J =7.2 Hz, 1H, 6), 7.17 (d, J =6.7 Hz, 2H, 2', 6'), 7.19-7.24 (m, 1H, 4'), 7.27 (t, 2H, 3', 5'), 7.52 (t, J =7.5 Hz, 1H, 7), 7.91 ppm (d, J =7.6 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =32.55 (CH_2 , CH_2Ph), 41.71 (CH_2 , CH_2N), 49.79 (CH, 3), 75.85 (CH, 2), 115.19 (C_q , q, J =287.8 Hz, CF_3), 117.87 (CH, 8), 119.81 (C_q , 4a), 122.90 (CH, 6), 127.22 (CH, 4'), 127.56 (CH, 5), 128.88 (CH, 2C, 2', 6'), 129.01 (CH, 2C, 3', 5'), 136.96 (CH, 7), 137.01 (C_q , 1'), 158.14 (C_q , 8a), 161.15 (C_q , q, J =39.5 Hz, $\text{TFA}(\text{CO})$), 191.40 ppm (C_q , ketone 4)

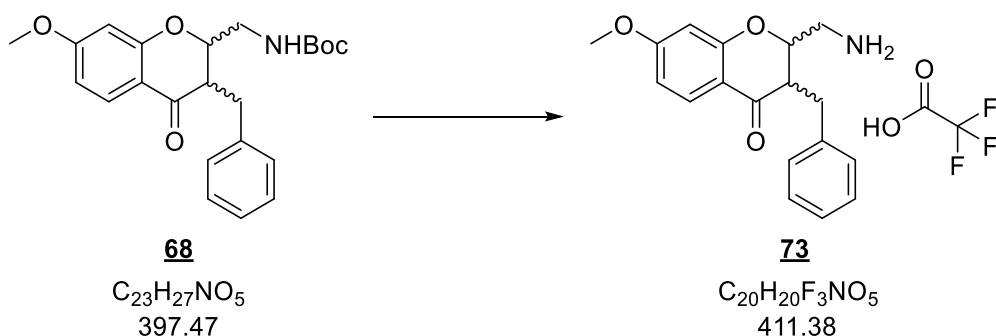
Minor isomer (cis):

^1H NMR (500 MHz, CDCl_3): δ =2.81 (dd, J =14.0, 8.2 Hz, 1H, CH_2Ph -1), 3.02-3.17 (m, 3H, CH_2N & 3), 3.33 (t, J =11.8 Hz, 1H, CH_2Ph -2), 4.73 (d, J =10.4 Hz, 1H, 2), 6.35 (brs, 1H, NH), 7.00 (d, J =8.7 Hz, 1H, 8), 7.08-7.14 (m, 3H, 2', 6' & 6), 7.19-7.31 (m, 3H, 3', 5' & 4'), 7.52 (t, J =7.5 Hz, 1H, 7), 7.70 (brs, NH), 7.84 ppm (d, J =7.6 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =30.67 (CH_2 , CH_2Ph), 40.26 (CH_2 , CH_2N), 49.31 (CH , 3), 76.13 (CH , 2), 115.19 (C_q , q, J =287.8 Hz, CF_3), 117.67 (CH , 8), 119.75 (C_q , 4a), 122.90 (CH , 6), 127.22 (CH , 4'), 127.64 (CH , 5), 128.68 (CH , 2C, 2', 6'), 128.92 (CH , 2C, 3', 5'), 136.86 (CH , 7), 137.29 (C_q , 1'), 158.70 (C_q , 8a), 161.15 (C_q , q, J =39.5 Hz, TFA-(CO)), 192.62 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{17}\text{H}_{18}\text{NO}_2$ m/z 268.1332 [$\text{M}+\text{H}$] $^+$, found m/z 268.1335 [$\text{M}+\text{H}$] $^+$.

A.26.4.11. Synthesis of (2S,3R)-2-(aminomethyl)-3-benzyl-7-methoxychroman-4-one 2,2,2-trifluoroacetate (**73**)



Following the same procedure as for **56**, starting from **68** (210 mg, 1 eq, 0.53 mmol), **73** was obtained as a brown honey which was used without further purification (218 mg, quant., 0.36:1 mixture of cis:trans isomers).

Major isomer (trans):

^1H NMR (500 MHz, CDCl_3): δ =2.75-2.84 (m, 1H, 3), 2.98 (dd, J =14.4, 8.1 Hz, 1H, CH_2Ph -1), 3.09-3.18 (m, 2H, CH_2N), 3.22 (d, J =14.6 Hz, 1H, CH_2Ph -2), 3.80 (s, 3H, OMe), 4.46-4.54 (m, 1H, 2), 4.96 (brs, 2H, NH), 6.43 (s, 1H, 8), 6.65 (d, J =8.7 Hz, 1H, 6), 7.16 (d, J =7.5 Hz, 2H, 2', 6'), 7.20 (t, J =7.3 Hz, 1H, 4'), 7.23-7.29 (m, 2H, 3', 5'), 7.82 ppm (d, J =8.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =33.00 (CH_2 , CH_2Ph), 41.62 (CH_2 , CH_2N), 49.34 (CH , 3), 55.66 (CH_3 , OMe), 79.15 (CH , 2), 100.86 (CH , 8), 111.46 (CH , 6), 113.35 (C_q , 4a), 115.31 (C_q , q, J =288.2 Hz, CF_3), 127.13 (CH , 4'), 128.92 (CH , 2C, 2', 6' or 3', 5'), 129.94 (CH , 2C, 2', 6' or 3', 5'), 129.26 (CH , 5), 137.20 (C_q , 1'), 160.20 (C_q , 8a), 161.16 (C_q , q, J =38.9 Hz, TFA-(CO)), 166.86 (C_q , 7), 190.17 ppm (C_q , ketone 4)

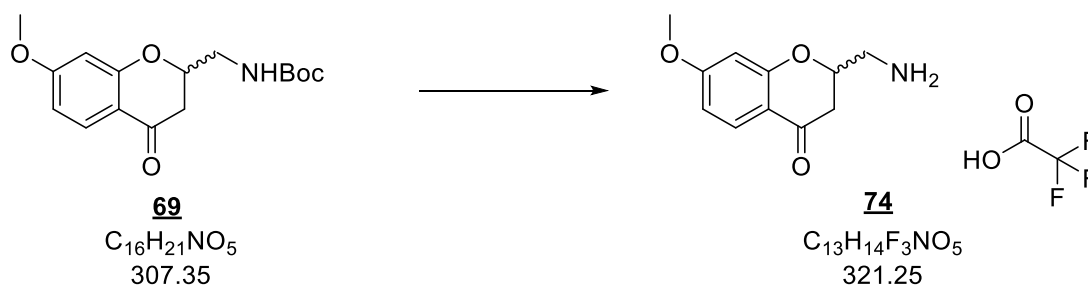
Minor isomer (cis):

^1H NMR (500 MHz, CDCl_3): δ =2.75-2.84 (m, 1H, CH_2Ph -1), 2.99-3.10 (m, 3H, CH_2Ph -2, CH_2N -1 & 3), 3.31 (t, J =11.8 Hz, 1H, CH_2N -2), 3.81 (s, 3H, OMe), 4.70 (d, J =10.3 Hz, 1H, 2), 4.96 (brs, 2H, NH), 6.46 (s, 1H, 8), 6.65 (d, J =8.7 Hz, 1H, 6), 7.09 (d, J =6.7 Hz, 2H, 2', 6'), 7.18-7.29 (m, 3H, 3', 5' & 4'), 7.74 (d, J =8.5 Hz, 1H, 5), 7.81 ppm (brs, 1H, NH)

^{13}C NMR (125 MHz, CDCl_3): δ =30.87 (CH_2 , CH_2Ph), 40.36 (CH_2 , CH_2N), 48.98 (CH , 3), 55.69 (CH_3 , OMe), 76.53 (CH , 2), 100.63 (CH , 8), 111.48 (CH , 6), 113.27 (C_q , 4a), 115.31 (C_q , q, J =288.2 Hz, CF_3), 127.13 (CH , 4'), 128.73 (CH , 2C, 2', 6'), 128.85 (CH , 2C, 3', 5'), 129.30 (CH , 5), 137.24 (C_q , 1'), 160.20 (C_q , 8a), 161.16 (C_q , q, J =38.9 Hz, TFA-(CO)), 166.78 (C_q , 7), 191.64 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{18}\text{H}_{20}\text{NO}_3$ m/z 298.1438 [$\text{M}+\text{H}$] $^+$, found m/z 298.1438 [$\text{M}+\text{H}$] $^+$.

A.26.4.12. Synthesis of (2S,3R)-2-(aminomethyl)-3-benzylchroman-4-one 2,2,2-trifluoroacetate (**74**)



Following the same procedure as for **56**, starting from **69** (500 mg, 1 eq, 1.6 mmol), **74** was obtained as a brown honey which was used without further purification (513 mg, quant).

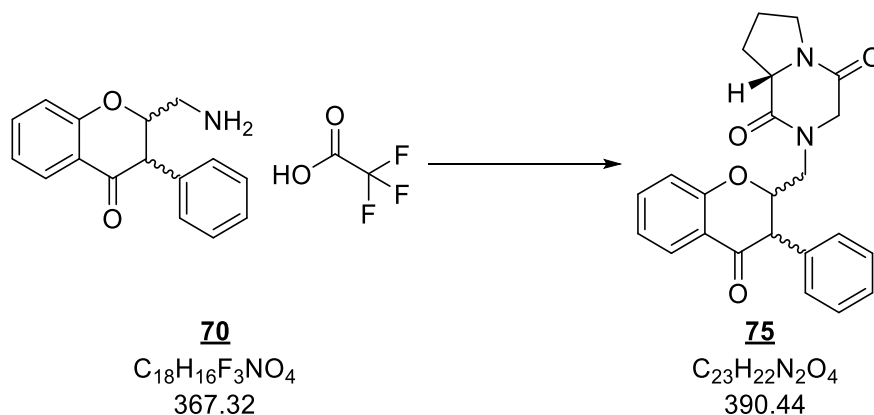
^1H NMR (500 MHz, $CDCl_3$): δ =2.64-2.79 (m, 2H, 3), 3.42 (s, 2H, CH_2N), 3.78 (s, 3H, OMe), 4.66-4.84 (m, 1H, 2), 6.41 (s, 1H, 8), 6.59 (d, J =8.7 Hz, 1H, 6), 7.75 (d, J =8.4 Hz, 1H, 5), 8.04 ppm (brs, 3H, NH_3^+)

^{13}C NMR (125 MHz, $CDCl_3$): δ =39.13 (CH_2 , 3), 43.28 (CH_2 , CH_2N), 55.65 (CH_3 , OMe), 73.59 (CH , 2), 100.64 (CH , 8), 111.39 (CH , 6), 114.01 (C_q , 4a), 115.24 (C_q , q, J =286.9 Hz, CF_3), 128.91 (CH , 5), 160.91 (C_q , q, J =39.1 Hz, TFA-(CO)), 162.18 (C_q , 8a), 166.98 (C_q , 7), 189.55 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{11}H_{14}NO_3$ m/z 208.0968 $[M+H]^+$, found m/z 208.0969 $[M+H]^+$.

A.26.5. Synthesis of diketopiperazine derivatives of chromanone compounds

A.26.5.1. Synthesis of 2-((4-oxo-3-phenylchroman-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**75**)



The trifluoroacetate of the amine **70** (600 mg, 1 eq, 1.6 mmol), methyl (2-chloroacetyl)prolinate (**7**, 331 mg, 1 eq, 1.6 mmol) and triethyl amine (0.58 mL, 2.6 eq, 4.2 mmol) were dissolved in 2-ethoxyethanol (15 mL) and heated at reflux for 3 days. Then the volatiles were evaporated and the residue was redissolved in deionized water/ sat. $NaHCO_3$ mixture and DCM. After separation of the phases the aqueous phase was extracted four more times with DCM, the combined organic phases were dried over Na_2SO_4 , filtered and evaporated. The crude product was purified by column chromatography (twice, first PE:EtOAc=5:1 to pure EtOAc to EtOAc:MeOH=95:5, then DCM to DCM:MeOH=50:1) and then by chiral preparative HPLC yielding 22.7 mg of Peak 1 (90% isomer 1), 9.4 mg of Peak 2 (1:0.82 mixture of isomers 1&2) and 3.6 mg of Peak 3 (92% isomer 3) (combined 35.6 mg, 6%) of **75** as a white foam.

Experimental Part

Isomer 1 (Peak 1, trans)

^1H NMR (500 MHz, CDCl_3): δ =1.83-1.96 (m, 1H, 7Dik-1), 1.97-2.14 (m, 2H, 7Dik-2 & 8Dik-1), 2.28-2.39 (m, 1H, 8Dik-2), 3.46 (dd, J =11.3, 11.0 Hz, 1H, $\text{CH}_2\text{N-1}$), 3.50-3.66 (m, 2H, 6Dik), 3.70 (d, J =14.2 Hz, 1H, $\text{CH}_2\text{N-2}$), 3.80 (d, J =11.1 Hz, 1H, 3), 4.05 (t, J =7.7 Hz, 1H, 8aDik), 4.10 (d, J =16.5 Hz, 1H, 3Dik-1), 4.15 (d, J =16.2 Hz, 1H, 3Dik-2), 4.94 (t, J =10.1 Hz, 1H, 2), 7.03-7.10 (m, 2H, 8 & 6), 7.21 (d, J =6.1 Hz, 2H, 2', 6'), 7.30-7.44 (m, 3H, 3', 5' & 4'), 7.53 (t, J =7.3 Hz, 1H, 7), 7.92 ppm (d, J =6.4 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.73 (CH_2 , 7Dik), 28.48 (CH_2 , 8Dik), 45.19 (CH_2 , 6Dik), 49.35 (CH_2 , CH_2N), 53.59 (CH_2 , 3Dik), 56.14 (CH, 3), 58.82 (CH, 8aDik), 79.54 (CH, 2), 118.10 (CH, 8), 120.68 (C_q , 4a), 122.05 (CH, 6), 127.59 (CH, 4'), 128.12 (CH, 5), 128.99 (CH, 2C, 2', 6'), 129.10 (CH, 2C, 3', 5'), 134.02 (C_q , 1'), 136.35 (CH, 7), 160.07 (C_q , 8a), 163.19 (C_q , 4Dik), 167.70 (C_q , 1Dik), 191.44 ppm (C_q , ketone 4)

Isomer 2 (Peak 2, cis)

^1H NMR (500 MHz, CDCl_3): δ =1.83-1.96 (m, 1H, 7Dik-1), 1.96-2.14 (m, 2H, 7Dik-2 & 8Dik-1), 2.27-2.39 (m, 1H, 8Dik-2), 3.16 (dd, J =14.2, 9.0 Hz, 1H, $\text{CH}_2\text{N-1}$), 3.52-3.64 (m, 2H, 6Dik), 3.78 (s, 1H, 3), 3.88-3.97 (m, 2H, 8aDik & $\text{CH}_2\text{N-2}$), 4.07-4.14 (m, 1H, 3Dik-1), 4.17 (d, J =16.2 Hz, 1H, 3Dik-2), 4.90 (d, J =9.5 Hz, 1H, 2), 7.07-7.16 (m, 2H, 8 & 6), 7.17-7.24 (m, 2H, 2', 6'), 7.24-7.44 (m, 3H, 3', 5' & 4'), 7.56 (t, J =8.5 Hz, 1H, 7), 7.89-7.95 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.71 (CH_2 , 7Dik), 28.49 (CH_2 , 8Dik), 45.18 (CH_2 , 6Dik), 48.33 (CH_2 , CH_2N), 53.83 (CH_2 , 3Dik), 54.74 (CH, 3), 58.76 (CH, 8aDik), 79.14 (CH, 2), 118.28 (CH, 8), 120.32 (C_q , 4a), 122.48 (CH, 6), 128.12 (CH, 5), 128.95 (CH, 4'), 129.00 (CH, 2C, 2', 6'), 129.09 (CH, 2C, 3', 5'), 133.99 (C_q , 1'), 136.53 (CH, 7), 160.63 (C_q , 8a), 163.27 (C_q , 4Dik), 167.87 (C_q , 1Dik), 190.70 ppm (C_q , ketone 4)

Isomer 3 (Peak 3, cis)

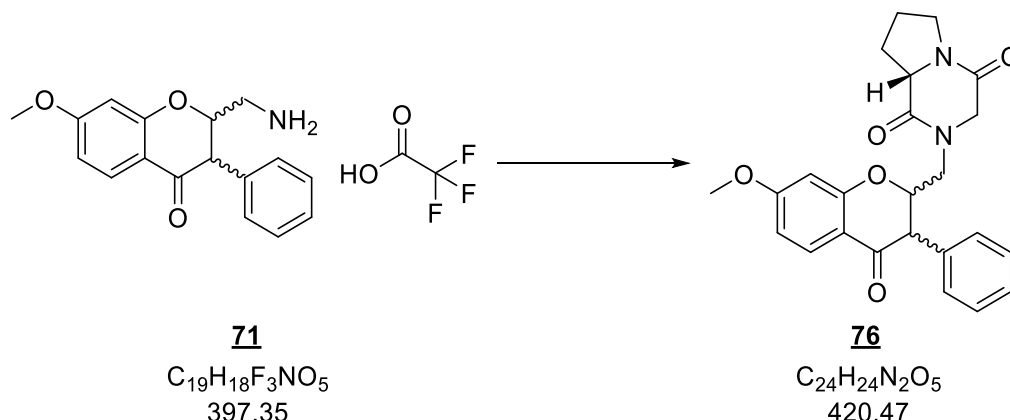
^1H NMR (500 MHz, CDCl_3): δ =1.83-1.96 (m, 1H, 7Dik-1), 1.96-2.07 (m, 2H, 7Dik-2 & 8Dik-1), 2.29-2.40 (m, 1H, 8Dik-2), 3.01 (t, J =11.6 Hz, 1H, $\text{CH}_2\text{N-1}$), 3.50-3.62 (m, 2H, 6Dik), 3.81 (s, 1H, 3), 3.88 (d, J =16.8 Hz, 1H, 3Dik-1), 4.05-4.13 (m, 2H, 8aDik & $\text{CH}_2\text{N-2}$), 4.48 (d, J =16.8 Hz, 1H, 3Dik-2), 4.97 (d, J =9.2 Hz, 1H, 2), 7.05 (d, J =8.1 Hz, 1H, 8), 7.12 (t, J =7.5 Hz, 1H, 6), 7.24-7.31 (m, 2H, 2', 6'), 7.31-7.46 (m, 3H, 3', 5' & 4'), 7.58 (t, J =7.3 Hz, 1H, 7), 7.95 ppm (d, J =7.9 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.67 (CH_2 , 7Dik), 28.61 (CH_2 , 8Dik), 45.12 (CH_2 , 6Dik), 49.18 (CH_2 , CH_2N), 54.67 (CH_2 , 3Dik), 54.69 (CH, 3), 59.05 (CH, 8aDik), 79.25 (CH, 2), 117.89 (CH, 8), 120.38 (C_q , 4a), 122.52 (CH, 6), 128.15 (CH, 5), 128.71 (CH, 4'), 128.94 (CH, 2C, 2', 6'), 129.15 (CH, 2C, 3', 5'), 133.06 (C_q , 1'), 136.50 (CH, 7), 160.48 (C_q , 8a), 163.31 (C_q , 4Dik), 167.85 (C_q , 1Dik), 190.66 ppm (C_q , ketone 4)

One isomer (trans) could not be isolated from impurities and could therefore not be annotated without doubt.

HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{NaO}_4$ m/z 413.1472 $[\text{M}+\text{Na}]^+$, found m/z 413.1474 $[\text{M}+\text{Na}]^+$.

A.26.5.2. Synthesis of 2-((4-oxo-3-phenylchroman-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**76**)



Following the same procedure as for **75**, **76** was prepared from **71** (314 mg, 1 eq, 0.78 mmol). The crude product was purified by column chromatography (DCM to DCM:MeOH=30:1) and preparative HPLC yielding 20.4 mg (5%) of **76** as a white solid (mixture of 4 isomers, trans:trans:cis:cis=1:0.73:0.10:0.10, 18 mol% impurity).

Isomer 1 (major isomer, trans):

^1H NMR (500 MHz, $CDCl_3$): δ =1.83-1.97 (m, 1H, 7Dik-1), 1.97-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.30-2.40 (m, 1H, 8Dik-2), 3.49 (t, J =14.1 Hz, 1H, CH_2N -1), 3.50-3.70 (m, 3H, 6Dik & CH_2N -2), 3.74 (d, J =10.3 Hz, 1H, 3), 3.87 (s, 3H, OMe), 4.02-4.10 (m, 2H, 8aDik & 3Dik-1), 4.09-4.17 (m, 1H, 3Dik-2), 4.93 (t, J =9.8 Hz, 1H, 2), 6.50 (s, 1H, 8), 6.60-6.65 (m, 1H, 6), 7.21 (d, J =6.1 Hz, 2H, 2', 6'), 7.30-7.34 (m, 1H, 4'), 7.34-7.41 (m, 2H, 3', 5'), 7.82-7.88 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =22.76 (CH_2 , 7Dik), 28.51 (CH_2 , 8Dik), 45.21 (CH_2 , 6Dik), 49.37 (CH_2 , CH_2N), 53.58 (CH_2 , 3Dik), 55.53 (CH, 3), 55.84 (CH_3 , OMe), 58.86 (CH, 8aDik), 79.95 (CH, 2), 100.75 (CH, 8), 110.90 (CH, 6), 114.38 (C_q , 4a), 128.00 (CH, 4'), 128.93 (CH, 2C, 2', 6'), 129.05 (CH, 2C, 3', 5'), 129.30 (CH, 5), 134.59 (C_q , 1'), 161.97 (C_q , 8a), 163.32 (C_q , 4Dik), 166.36 (C_q , 7), 167.71 (C_q , 1Dik), 189.95 ppm (C_q , ketone 4)

Isomer 2 (2nd biggest isomer, trans):

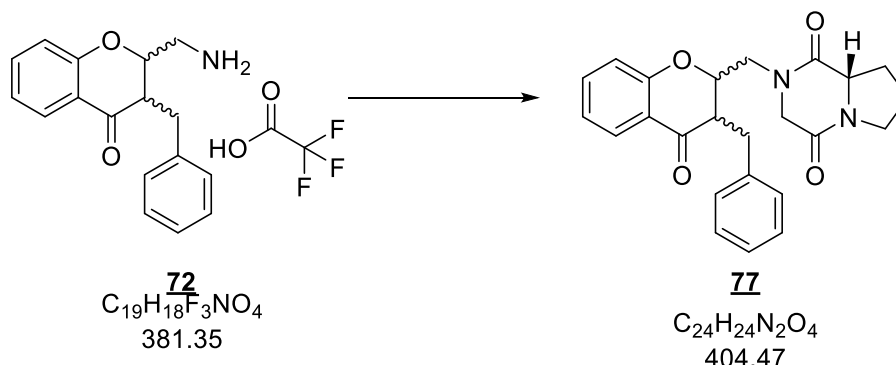
^1H NMR (500 MHz, $CDCl_3$): δ =1.83-1.97 (m, 1H, 7Dik-1), 1.97-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.30-2.40 (m, 1H, 8Dik-2), 3.23 (dd, J =15.0, 8.6 Hz, 1H, CH_2N -1), 3.50-3.66 (m, 2H, 6Dik), 3.74 (d, J =10.3 Hz, 1H, 3), 3.87 (s, 3H, OMe), 3.89-3.94 (m, 1H, 3Dik-1), 3.98 (d, J =13.9 Hz, 1H, CH_2N -2), 4.02-4.10 (m, 1H, 8aDik), 4.49 (d, J =16.8 Hz, 1H, 3Dik-2), 4.98 (t, J =10.0 Hz, 1H, 2), 6.40 (s, 1H, 8), 6.60-6.65 (m, 1H, 6), 7.21 (d, J =6.1 Hz, 2H, 2', 6'), 7.30-7.34 (m, 1H, 4'), 7.34-7.41 (m, 2H, 3', 5'), 7.82-7.88 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =22.68 (CH_2 , 7Dik), 28.63 (CH_2 , 8Dik), 45.18 (CH_2 , 6Dik), 50.09 (CH_2 , CH_2N), 54.64 (CH_2 , 3Dik), 55.53 (CH, 3), 55.77 (CH_3 , OMe), 59.02 (CH, 8aDik), 80.46 (CH, 2), 100.71 (CH, 8), 110.51 (CH, 6), 114.49 (C_q , 4a), 128.05 (CH, 4'), 128.91 (CH, 2C, 2', 6'), 129.05 (CH, 2C, 3', 5'), 129.53 (CH, 5), 134.44 (C_q , 1'), 161.75 (C_q , 8a), 163.21 (C_q , 4Dik), 166.27 (C_q , 7), 167.62 (C_q , 1Dik), 189.91 ppm (C_q , ketone 4)

Two minor isomers (both cis, different stereochemistry at C-2) could not be annotated without doubt.

HRMS (ESI): calcd for $C_{24}H_{24}N_2NaO_5$ m/z 443.1577 $[M+Na]^+$, found m/z 443.1575 $[M+Na]^+$.

A.26.5.3. Synthesis of 2-((3-benzyl-4-oxochroman-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**77**)



Following the same procedure as for **75**, **77** was prepared from **72** (373 mg, 1 eq, 0.97 mmol). The crude product was purified by column chromatography (DCM to DCM:MeOH=50:1) and preparative HPLC yielding 27.4 mg (7%) of **77** as a yellow semi-solid (mixture of 4 isomers, trans:trans:cis:cis=1:0.79:0.46:0.23).

Isomer 1 (biggest isomer, trans):

^1H NMR (500 MHz, $CDCl_3$): δ =1.83-1.97 (m, 1H, 7Dik-1), 1.97-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.31-2.43 (m, 1H, 8Dik-2), 2.80-2.88 (m, 1H, 3), 3.10 (dd, J =14.4, 8.2 Hz, 1H, CH_2Ph -1), 3.21-3.29 (m, 1H, CH_2Ph -2), 3.49-3.61 (m, 2H, 6Dik), 3.63-3.74 (m, 2H, CH_2N), 3.91 (d, J =16.2 Hz, 1H, 3Dik-1), 4.02-4.12 (m, 2H, 8aDik & 3Dik-2), 4.49 (t, J =8.1 Hz, 1H, 2), 7.00 (d, J =8.2 Hz, 1H, 8), 7.04-7.10 (m, 1H, 6), 7.19-7.34 (m, 5H, 2', 6', 3', 5' & 4'), 7.49-7.56 (m, 1H, 7), 7.90 ppm (d, J =7.8 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =22.69 (CH_2 , 7Dik), 28.63 (CH_2 , 8Dik), 33.11 (CH_2 , CH_2Ph), 45.21 (CH_2 , 6Dik), 48.01 (CH_2 , CH_2N), 49.88 (CH, 3), 53.52 (CH_2 , 3Dik), 58.75 (CH, 8aDik), 78.58 (CH, 2), 118.10 (CH, 8), 120.06 (C_q , 4a), 122.00 (CH, 6), 126.78 (CH, 4'), 127.28 (CH, 5), 128.71 (CH, 2C, 3',5'), 129.23 (CH, 2C, 2', 6'), 136.59 (CH, 7), 137.85 (C_q , 1'), 159.08 (C_q , 8a), 163.14 (C_q , 4Dik), 167.81 (C_q , 1Dik), 192.60 ppm (C_q , ketone 4)

Isomer 2 (2nd biggest isomer, trans):

^1H NMR (500 MHz, $CDCl_3$): δ =1.83-1.97 (m, 1H, 7Dik-1), 1.97-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.31-2.43 (m, 1H, 8Dik-2), 2.80-2.88 (m, 1H, 3), 3.00 (dd, J =13.4, 8.9 Hz, 1H, CH_2Ph -1), 3.14-3.25 (m, 1H, CH_2Ph -2), 3.27-3.38 (m, 1H, CH_2N -1), 3.49-3.61 (m, 2H, 6Dik), 3.63-3.74 (m, 1H, 3Dik-1), 3.98 (d, J =14.2 Hz, 1H, CH_2N -2), 4.02-4.12 (m, 1H, 8aDik), 4.31 (d, J =16.3 Hz, 1H, 3Dik-2), 4.54-4.60 (m, 1H, 2), 6.94 (d, J =8.2 Hz, 1H, 8), 7.04-7.10 (m, 1H, 6), 7.19-7.34 (m, 5H, 2', 6', 3', 5' & 4'), 7.49-7.56 (m, 1H, 7), 7.90 ppm (d, J =7.8 Hz, 1H, 5)

^{13}C NMR (125 MHz, $CDCl_3$): δ =22.74 (CH_2 , 7Dik), 28.52 (CH_2 , 8Dik), 33.80 (CH_2 , CH_2Ph), 45.18 (CH_2 , 6Dik), 48.42 (CH_2 , CH_2N), 50.05 (CH, 3), 54.16 (CH_2 , 3Dik), 58.96 (CH, 8aDik), 78.45 (CH, 2), 117.76 (CH, 8), 119.94 (C_q , 4a), 122.07 (CH, 6), 126.84 (CH, 4'), 127.39 (CH, 5), 128.73 (CH, 2C, 3',5'), 129.17 (CH, 2C, 2', 6'), 136.69 (CH, 7), 137.69 (C_q , 1'), 159.58 (C_q , 8a), 163.10 (C_q , 4Dik), 167.79 (C_q , 1Dik), 192.53 ppm (C_q , ketone 4)

Isomer 3 (3rd biggest isomer, cis):

^1H NMR (500 MHz, $CDCl_3$): δ =1.83-1.97 (m, 1H, 7Dik-1), 1.97-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.31-2.43 (m, 1H, 8Dik-2), 2.86-2.94 (m, 1H, CH_2Ph -1), 3.14-3.38 (m, 3H, 3, CH_2Ph -2 & CH_2N -1), 3.49-3.61 (m, 2H, 6Dik), 3.88-3.94 (m, 1H, CH_2N -2), 4.02-4.12 (m, 3H, 3Dik & 8aDik), 4.67 (d, J =9.5 Hz, 1H, 2), 6.99-7.06 (m, 1H, 8), 7.04-7.10 (m, 1H, 6), 7.19-7.34 (m, 5H, 2', 6', 3', 5' & 4'), 7.49-7.56 (m, 1H, 7), 7.86 ppm (d, J =7.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.65 (CH_2 , 7Dik), 28.67 (CH_2 , 8Dik), 30.63 (CH_2 , CH_2Ph), 45.21 (CH_2 , 6Dik), 45.82 (CH_2 , CH_2N), 50.24 (CH , 3), 53.16 (CH_2 , 3Dik), 58.82 (CH , 8aDik), 78.73 (CH , 2), 118.00 (CH , 8), 120.28 (C_q , 4a), 121.97 (CH , 6), 126.85 (CH , 4'), 127.39 (CH , 5), 128.71 (CH , 2C, 3',5'), 128.89 (CH , 2C, 2', 6'), 136.33 (CH , 7), 138.13 (C_q , 1'), 159.43 (C_q , 8a), 162.91 (C_q , 4Dik), 167.69 (C_q , 1Dik), 192.86 ppm (C_q , ketone 4)

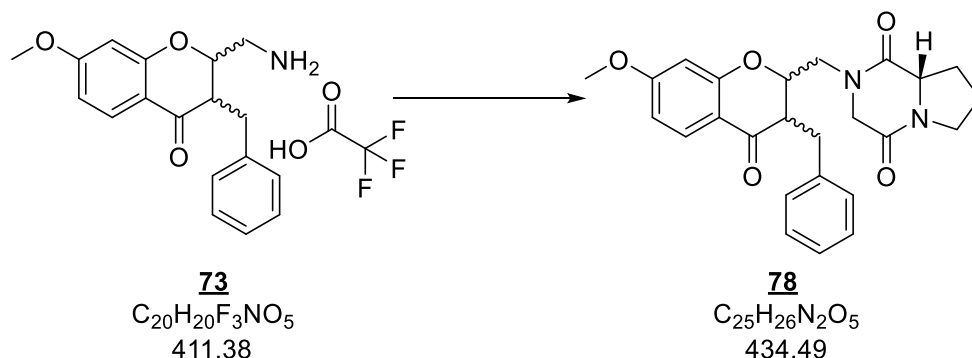
Isomer 4 (smallest isomer, cis)

^1H NMR (500 MHz, CDCl_3): δ =1.83-1.97 (m, 1H, 7Dik-1), 1.97-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.31-2.43 (m, 1H, 8Dik-2), 2.86-2.94 (m, 1H, CH_2Ph -1), 3.10-3.29 (m, 3H, 3, CH_2Ph -2 & CH_2N -1), 3.49-3.61 (m, 2H, 6Dik), 3.67-3.75 (m, 1H, 3Dik-1), 4.02-4.12 (m, 1H, 8aDik), 4.15 (d, J =14.2 Hz, 1H, CH_2N -2), 4.41 (d, J =16.6 Hz, 1H, 3Dik-2), 4.74 (d, J =9.6 Hz, 1H, 2), 6.92-6.97 (m, 1H, 8), 7.04-7.10 (m, 1H, 6), 7.19-7.34 (m, 5H, 2', 6', 3', 5' & 4'), 7.49-7.56 (m, 1H, 7), 7.86 ppm (d, J =7.7 Hz, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.65 (CH_2 , 7Dik), 28.67 (CH_2 , 8Dik), 30.60 (CH_2 , CH_2Ph), 45.21 (CH_2 , 6Dik), 46.40 (CH_2 , CH_2N), 50.45 (CH , 3), 54.37 (CH_2 , 3Dik), 59.03 (CH , 8aDik), 79.15 (CH , 2), 117.64 (CH , 8), 120.39 (C_q , 4a), 121.97 (CH , 6), 126.85 (CH , 4'), 127.39 (CH , 5), 128.71 (CH , 2C, 3',5'), 128.89 (CH , 2C, 2', 6'), 136.39 (CH , 7), 138.20 (C_q , 1'), 159.43 (C_q , 8a), 163.20 (C_q , 4Dik), 167.61 (C_q , 1Dik), 192.74 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{NaO}_4$ m/z 427.1628 $[\text{M}+\text{Na}]^+$, found m/z 427.1634 $[\text{M}+\text{Na}]^+$.

A.26.5.4. Synthesis of 2-((3-benzyl-7-methoxy-4-oxochroman-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**78**)



Following the same procedure as for **75**, **78** was prepared from **73** (240 mg, 1 eq, 0.58 mmol). The crude product was purified by column chromatography (DCM to DCM:MeOH=50:1) and preparative HPLC yielding 35.6 mg (13%) of **78** as a white solid (mixture of 4 isomers, trans:trans:cis:cis=1:1:0.45:0.38, 31 mol% impurity).

Isomer 1 (trans):

^1H NMR (500 MHz, CDCl_3): δ =1.84-1.99 (m, 1H, 7Dik-1), 1.99-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.32-2.43 (m, 1H, 8Dik-2), 2.72-2.81 (m, 1H, 3), 3.04-3.15 (m, 1H, CH_2Ph -1), 3.16-3.25 (m, 1H, CH_2Ph -2), 3.46-3.59 (m, 2H, 6Dik), 3.59-3.77 (m, 2H, CH_2N), 3.86 (s, 3H, OMe), 3.86-3.92 (m, 1H, 3Dik-1), 4.02-4.14 (m, 2H, 8aDik & 3Dik-2), 4.48 (t, J =7.3 Hz, 1H, 2), 6.43 (s, 1H, 8), 6.59-6.66 (m, 1H, 6), 7.19-7.27 (m, 3H, 2', 6' & 4'), 7.27-7.34 (m, 2H, 3', 5'), 7.82-7.87 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.69 (CH_2 , 7Dik), 28.50 (CH_2 , 8Dik), 33.45 (CH_2 , CH_2Ph), 45.21 (CH_2 , 6Dik), 48.03 (CH_2 , CH_2N), 49.40 (CH , 3), 53.58 (CH_2 , 3Dik), 55.79 (CH_3 , OMe), 58.79 (CH , 8aDik), 78.82 (CH , 2), 100.75 (CH , 8), 110.82 (CH , 6), 113.77 (C_q , 4a), 126.79 (CH , 4'), 128.69 (CH , 2C, 3', 5'), 129.00 (CH , 5), 129.24 (CH , 2C, 2', 6'), 137.97 (C_q , 1'), 161.03 (C_q , 8a), 163.23 (C_q , 4Dik), 166.52 (C_q , 7), 167.81 (C_q , 1Dik), 191.12 ppm (C_q , ketone 4)

Isomer 2 (trans):

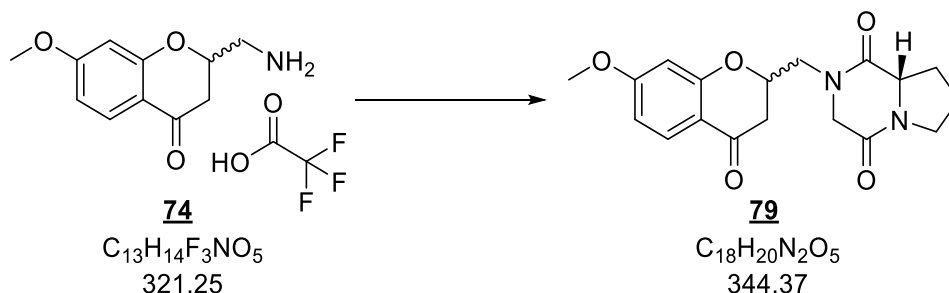
^1H NMR (500 MHz, CDCl_3): δ =1.84-1.99 (m, 1H, 7Dik-1), 1.99-2.15 (m, 2H, 7Dik-2 & 8Dik-1), 2.32-2.43 (m, 1H, 8Dik-2), 2.72-2.81 (m, 1H, 3), 2.97 (dd, J =13.4, 9.5 Hz, 1H, CH_2Ph -1), 3.15-3.25 (m, 1H, CH_2Ph -2), 3.25-3.42 (m, 1H, CH_2N -1), 3.46-3.59 (m, 2H, 6Dik), 3.70 (d, J =17.7 Hz, 1H, 3Dik-1), 3.86 (s, 3H, OMe), 3.99 (d, J =13.3 Hz, 1H, CH_2N -2), 4.02-4.13 (m, 1H, 8aDik), 4.26-4.37 (m, 1H, 3Dik-2), 4.55 (t, J =6.0 Hz, 1H, 2), 6.36 (s, 1H, 8), 6.59-6.66 (m, 1H, 6), 7.19-7.27 (m, 3H, 2', 6' & 4'), 7.27-7.34 (m, 2H, 3', 5'), 7.82-7.87 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.69 (CH_2 , 7Dik), 28.66 (CH_2 , 8Dik), 33.97 (CH_2 , CH_2Ph), 45.21 (CH_2 , 6Dik), 48.53 (CH_2 , CH_2N), 49.69 (CH, 3), 54.25 (CH_2 , 3Dik), 55.73 (CH_3 , OMe), 58.99 (CH, 8aDik), 78.75 (CH, 2), 100.91 (CH, 8), 110.25 (CH, 6), 113.77 (C_q , 4a), 126.74 (CH, 4'), 128.71 (CH, 2C, 3', 5'), 128.92 (CH, 5), 129.19 (CH, 2C, 2', 6'), 137.89 (C_q , 1'), 160.49 (C_q , 8a), 163.17 (C_q , 4Dik), 166.52 (C_q , 7), 167.72 (C_q , 1Dik), 191.17 ppm (C_q , ketone 4)

Two minor isomers (both cis, different stereochemistry at C-2) could not be annotated without doubt.

HRMS (ESI): calcd for $\text{C}_{25}\text{H}_{26}\text{N}_2\text{NaO}_5$ m/z 457.1734 $[\text{M}+\text{Na}]^+$, found m/z 457.1735 $[\text{M}+\text{Na}]^+$.

A.26.5.5. Synthesis of 2-((7-methoxy-4-oxochroman-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**79**)

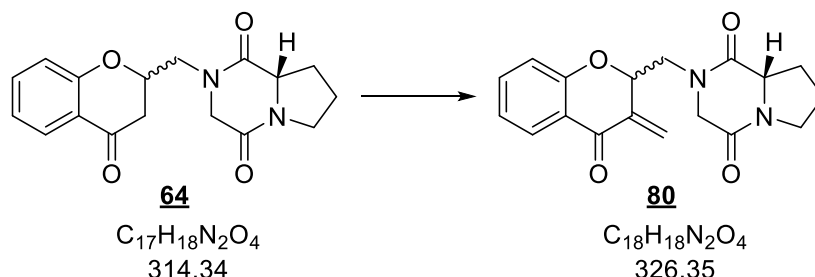


Following the same procedure as for **75**, **79** was prepared from **74** (180 mg, 1 eq, 0.56 mmol). The crude product was purified by column chromatography (DCM to DCM:MeOH=20:1) and preparative HPLC yielding 30.2 mg (16%) of **79** as an orange semi-solid (1:1 mixture of 2 isomers).

^1H NMR (500 MHz, CDCl_3): δ =1.89-2.02 (m, 1H, 7Dik-1), 2.02-2.18 (m, 2H, 7Dik-2 & 8Dik-1), 2.37-2.49 (m, 1H, 8Dik-2), 2.60-2.74 (m, 2H, 3), 3.50 (dd, J =14.4, 8.0 Hz, 0.5 H, CH_2N -1), 3.54-3.71 (m, 2H, 6Dik), 3.77 (dd, J =14.4, 6.7 Hz, 0.5 H, CH_2N -1*), 3.83 (d, J =14.9 Hz, 0.5 H, CH_2N -2*), 3.87 (s, 3H, OMe), 4.05 (d, J =14.6 Hz, 0.5 Hz, CH_2N -2), 4.10 (d, J =16.9 Hz, 0.5 H, 3Dik-1), 4.13-4.23 (m, 1.5 H, 8aDik & 3Dik-1*), 4.33 (d, J =16.5 Hz, 0.5 H, 3Dik-2*), 4.50 (d, J =16.7 Hz, 0.5H, 3Dik-2), 4.67-4.78 (m, 1H, 2), 6.40/6.46 (s, 1H, 8), 6.59-6.65 (m, 1H, 6), 7.81-7.87 ppm (m, 1H, 5)

^{13}C NMR (125 MHz, CDCl_3): δ =22.65/22.72 (CH_2 , 7Dik), 28.64/28.85 (CH_2 , 8Dik), 39.92/39.93 (CH_2 , 3), 45.25 (CH_2 , 6Dik), 49.62/50.06 (CH_2 , CH_2N), 53.98/54.42 (CH_2 , 3Dik), 55.72/55.77 (CH_3 , OMe), 58.85/59.04 (CH, 8aDik), 77.16/77.29 (CH, 2), 100.74/100.75 (CH, 8), 110.40/110.63 (CH, 6), 114.62/114.70 (C_q , 4a), 128.71/128.84 (CH, 5), 162.45/162.59 (C_q , 8a), 163.21 (C_q , 4Dik), 166.17/166.21 (C_q , 7), 167.89/167.91 (C_q , 1Dik), 189.41/189.48 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $\text{C}_{18}\text{H}_{20}\text{N}_2\text{NaO}_5$ m/z 367.1264 $[\text{M}+\text{Na}]^+$, found m/z 367.1265 $[\text{M}+\text{Na}]^+$.

A.26.6. Attempts at the synthesis of final target molecule G**A.26.6.1. Synthesis of (8aS)-2-((3-methylene-4-oxochroman-2-yl)methyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**80**)**

Prepared according to ¹⁴³. The chromanone **64** (100 mg, 1 eq, 0.32 mmol) was dissolved in DMF (5 mL) and $FeCl_3 \cdot 6H_2O$ (8.4 mg, 0.1 eq, 0.03 mmol) and $K_2S_2O_8$ (165 mg, 2 eq, 0.64 mmol) were added. The flask was closed with a septum (no Argon!) and the reaction was heated at 115 °C for 2 h. Subsequently water and EtOAc were added, the phases were separated and the aqueous phase was extracted four more times with EtOAc. The combined organic phases were washed twice with brine, dried over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography (DCM to DCM:MeOH=20:1) yielding 36 mg (35%) of **80** as a brown solid (1:0.39 mixture of isomers

Isomer 1 (minor isomer):

¹H NMR (500 MHz, $CDCl_3$): δ =1.90-1.96 (m, 1H, 7Dik-1), 1.96-2.16 (m, 2H, 7Dik-2 & 8Dik-1), 2.34-2.46 (m, 1H, 8Dik-2), 3.34 (dd, J =15.6, 8.7 Hz, 1H, CH_2N -1), 3.50-3.62 (m, 2H, 6Dik), 3.73 (d, J =16.2 Hz, 1H, 3Dik-1), 4.06-4.14 (m, 2H, 8aDik & CH_2N -2), 4.46 (d, J =16.8 Hz, 1H, 3Dik-2), 5.40 (d, J =9.9 Hz, 1H, 2), 5.69 (s, 1H, = CH_2 -1), 6.46 (s, 1H, = CH_2 -2), 6.93 (d, J =7.8 Hz, 1H, 8), 7.09 (t, J =7.1 Hz, 1H, 6), 7.54 (t, J =6.9 Hz, 1H, 7), 7.98 ppm (d, J =6.6 Hz, 1H, 5)

¹³C NMR (125 MHz, $CDCl_3$): δ =22.72 (CH_2 , 7Dik), 28.61 (CH_2 , 8Dik), 45.23 (CH_2 , 6Dik), 50.54 (CH_2 , CH_2N), 54.56 (CH_2 , 3Dik), 59.07 (CH, 8aDik), 78.94 (CH, 2), 118.17 (CH, 8), 121.03 (C_q , 4a), 122.46 (CH, 6), 124.63 (CH_2 , = CH_2), 128.03 (CH, 5), 136.75 (CH, 7), 138.86 (C_q , 3), 159.08 (C_q , 8a), 163.15 (C_q , 4Dik), 167.98 (C_q , 1Dik), 180.42 ppm (C_q , ketone 4)

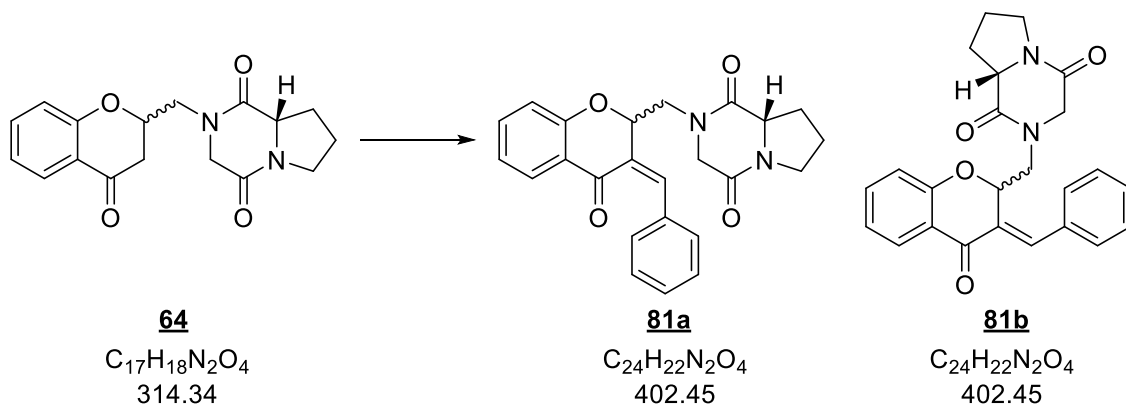
Isomer 2 (major isomer):

¹H NMR (500 MHz, $CDCl_3$): δ =1.90-1.96 (m, 1H, 7Dik-1), 1.96-2.16 (m, 2H, 7Dik-2 & 8Dik-1), 2.34-2.46 (m, 1H, 8Dik-2), 3.50-3.69 (m, 3H, CH_2N -1 & 6Dik), 3.88 (d, J =13.7 Hz, 1H, CH_2N -2), 3.94 (d, J =16.2 Hz, 1H, 3Dik-1), 4.09 (t, J =8.0 Hz, 8aDik), 4.15 (d, J =16.2 Hz, 1H, 3Dik-2), 5.34 (d, J =9.2 Hz, 1H, 2), 5.69 (s, 1H, = CH_2 -1), 6.46 (s, 1H, = CH_2 -2), 7.03 (d, J =8.4 Hz, 1H, 8), 7.09 (t, J =7.1 Hz, 1H, 6), 7.54 (t, J =6.9 Hz, 1H, 7), 7.98 ppm (d, J =6.6 Hz, 1H, 5)

¹³C NMR (125 MHz, $CDCl_3$): δ =22.76 (CH_2 , 7Dik), 28.61 (CH_2 , 8Dik), 45.26 (CH_2 , 6Dik), 49.31 (CH_2 , CH_2N), 53.51 (CH_2 , 3Dik), 58.87 (CH, 8aDik), 78.41 (CH, 2), 118.56 (CH, 8), 121.00 (C_q , 4a), 122.43 (CH, 6), 124.50 (CH_2 , = CH_2), 127.87 (CH, 5), 136.79 (CH, 7), 138.95 (C_q , 3), 159.45 (C_q , 8a), 162.99 (C_q , 4Dik), 167.94 (C_q , 1Dik), 180.54 ppm (C_q , ketone 4)

HRMS (ESI): calcd for $C_{18}H_{18}N_2NaO_4$ m/z 349.1159 [$M+Na$]⁺, found m/z 349.1159 [$M+Na$]⁺.

A.26.6.2. Synthesis of (8aS)-2-((3-((Z)-benzylidene)-4-oxochroman-2-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (81a**) and (8aS)-2-((3-((E)-benzylidene)-4-oxochroman-2-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**81b**)**



Preparation following ¹⁵⁸. The chromanone **64** (30 mg, 1 eq, 0.09 mmol) and benzaldehyde dimethyl acetal (22.7 mg, 1.6 eq, 0.14 mmol) were dissolved in DCM (5 mL), cooled to -78 °C and TMSOTf (0.025 mL, 1.6 eq, 0.14 mmol) was added during 5 min. The reaction was kept at -78 °C for another hour, then put in an ice bath for 30 min and subsequently warmed to room temperature overnight. On the following day, sat. NaHCO₃ solution was added and the reaction was extracted three times with EtOAc. The combined organic phases were dried over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography (DCM to DCM:MeOH=20:1) yielding 19 mg (52%) of **81** as a yellow semi-solid (mixture of isomers, E1:E2:Z1:Z2=1:0.30:0.92:0.27).

Isomer 1 (**81a**, Z1):

¹H NMR (500 MHz, CDCl₃): δ=1.85-1.98 (m, 1H, 7Dik-1), 1.98-2.24 (m, 2H, 7Dik-2 & 8Dik-1), 2.34-2.49 (m, 1H, 8Dik-2), 3.50-3.70 (m, 3H, 6Dik & CH₂N-1), 3.80 (d, J=16.2 Hz, 1H, 3Dik-1), 3.86 (d, J=14.0 Hz, 1H, CH₂N-2), 4.02-4.16 (m, 2H, 8aDik & 3Dik-2), 5.29-5.35 (m, 1H, 2), 6.95 (s, 1H, =CH), 7.00-7.10 (m, 2H, 6 & 8), 7.33-7.40 (m, 3H, 3',5' & 4'), 7.48-7.57 (m, 1H, 7), 7.72-7.79 (m, 2H, 2', 6'), 7.97 ppm (d, J=7.5 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=22.71 (CH₂, 7Dik), 28.65 (CH₂, 8Dik), 45.24 (CH₂, 6Dik), 48.91 (CH₂, CH₂N), 53.45 (CH₂, 3Dik), 58.86 (CH, 8aDik), 81.87 (CH, 2), 118.12 (CH, 8), 122.07 (CH, 6), 122.11 (C_q, 4a), 127.74 (CH, 5), 128.06 (CH, 2C, 3', 5'), 129.56 (C_q, 3), 130.00 (CH, 4'), 130.64 (CH, 2C, 2', 6'), 133.91 (C_q, 1'), 136.65 (CH, 7), 142.17 (CH, =CH), 158.08 (C_q, 8a), 162.88 (C_q, 4Dik), 167.91 (C_q, 1Dik), 181.70 ppm (C_q, ketone 4)

Isomer 2 (**81b**, E1, major isomer):

¹H NMR (500 MHz, CDCl₃): δ=1.85-1.98 (m, 1H, 7Dik-1), 1.98-2.24 (m, 2H, 7Dik-2 & 8Dik-1), 2.34-2.49 (m, 1H, 8Dik-2), 3.50-3.70 (m, 3H, 6Dik & CH₂N-1), 3.77 (d, J=16.2 Hz, 1H, 3Dik-1), 4.00 (d, J=14.5 Hz, 1H, CH₂N-2), 4.02-4.16 (m, 2H, 8aDik & 3Dik-2), 5.96 (d, J=10.2 Hz, 1H, 2), 7.00-7.10 (m, 1H, 8), 7.12 (t, J=7.4 Hz, 1H, 6), 7.44 (t, J=7.1 Hz, 1H, 4'), 7.48-7.56 (m, 2H, 3',5'), 7.58 (t, J=7.5 Hz, 1H, 7), 7.69 (d, J=7.9 Hz, 2H, 2', 6'), 7.91 (s, 1H, =CH), 8.04 ppm (d, J=6.7 Hz, 1H, 5)

¹³C NMR (125 MHz, CDCl₃): δ=22.78 (CH₂, 7Dik), 28.60 (CH₂, 8Dik), 45.21 (CH₂, 6Dik), 48.43 (CH₂, CH₂N), 53.36 (CH₂, 3Dik), 58.88 (CH, 8aDik), 75.11 (CH, 2), 118.57 (CH, 8), 121.26 (C_q, 4a), 122.33 (CH, 6), 127.81 (CH, 5), 129.06 (CH, 2C, 3', 5'), 130.13 (C_q, 3), 130.14 (CH, 4'), 130.70 (CH, 2C, 2', 6'), 133.46 (C_q, 1'), 137.04 (CH, 7), 140.54 (CH, =CH), 157.92 (C_q, 8a), 162.91 (C_q, 4Dik), 168.17 (C_q, 1Dik), 181.51 ppm (C_q, ketone 4)

Two minor isomers (different stereochemistry at C-2) could not be annotated without doubt.

Experimental Part

HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{22}\text{N}_2\text{NaO}_4$ m/z 425.1472 $[\text{M}+\text{Na}]^+$, found m/z 425.1470 $[\text{M}+\text{Na}]^+$.

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Appendix

Publication: “Subtle Structural Differences Trigger Inhibitory Activity of Propafenone Analogues at the Two Polyspecific ABC Transporters: P-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)”, proline and diketopiperazine derivatives of propafenones and 2-ethoxyacetophenones



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Subtle Structural Differences Trigger Inhibitory Activity of Propafenone Analogues at the Two Polyspecific ABC Transporters: P-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)

Theresa Schwarz,^[a] Floriane Montanari,^[a] Anna Cseke,^[a] Katrin Wlcek,^[a] Lene Visvader,^[a] Sarah Palme,^[a] Peter Chiba,^[b] Karl Kuchler,^[c] Ernst Urban,^[a] and Gerhard F. Ecker^{*,[a]}

The transmembrane ABC transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are widely recognized for their role in cancer multidrug resistance and absorption and distribution of compounds. Furthermore, they are linked to drug–drug interactions and toxicity. Nevertheless, due to their polyspecificity, a molecular understanding of the ligand-transporter interaction, which allows designing of both selective and dual inhibitors, is still in its infancy. This study comprises a combined approach of synthesis, *in silico* prediction, and *in vitro* testing to identify molecular features trigger-

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Thesis author contributions:

The thesis author synthesized and characterized the compounds presented in this article (except compounds 16a-c, which were synthesized by Lene Visvader). *In silico* predictions were conducted by Floriane Montanari, and *in vitro* assays were conducted by Anna Cseke, Katrin Wlcek and Sarah Palme. The thesis author was supervised by Ernst Urban and Gerhard F. Ecker, Peter Chiba and Karl Kuchler supervised the biological experiments. The manuscript was written and revised by Theresa Schwarz, Floriane Montanari, Anna Cseke, Katrin Wlcek, Ernst Urban and Gerhard F. Ecker in collaboration.



Subtle Structural Differences Trigger Inhibitory Activity of Propafenone Analogues at the Two Polyspecific ABC Transporters: P-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)

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Introduction

P-glycoprotein (P-gp, ABCB1) and the breast cancer resistance protein (BCRP, ABCG2) belong to the superfamily of ABC proteins. In humans, this superfamily is composed of 49 members, subdivided into 7 subfamilies named ABCA to ABCG.^[1] Based on their structural organization, ABC transporters form a passageway across cellular membranes enabling the transport of substrates. They consist of two transmembrane (TMDs) and two nucleotide binding domains (NBDs), whereby ATP hydroly-

sis at the NBDs provides the energy necessary for the transport process.^[1]

P-gp and BCRP are efflux transporters, mediating transport of their substrates out of cells in which they are expressed. Thus, they are physiologically expressed in organs with barrier and/or excretion functions including the gastrointestinal (GI) tract, the blood-brain barrier, the liver, and the kidney.^[2]

In the GI tract, both ABC family members are located in the apical membrane of enterocytes, facing towards the intestinal lumen, affecting the absorption of compounds from the intestinal lumen into the blood. Similarly, P-gp and BCRP are localized at the apical side of proximal tubule cells and hepatocytes in the kidney and the liver, respectively. There, they are involved in the excretion of endogenous and exogenous compounds, as well as of metabolites, into the urine or the bile.^[3] At the blood–brain barrier, P-gp and BCRP are located at the apical/luminal side in the endothelial cells of brain capillaries, where they protect the brain from xenobiotics.^[3]

Based on their physiological expression, P-gp and BCRP are important determinants in absorption, distribution, and excretion of drugs and metabolites that are P-gp and/or BCRP substrates. Inhibition of the proper transport function of these ABC proteins has been reported to be responsible for drug–drug interactions.^[4] In addition, P-gp and BCRP are also known for their involvement in multidrug resistance (MDR).^[5] As efflux transporters for different anticancer agents, including anthracyclines, kinase inhibitors (imatinib), and alkaloids (e.g. topotecan),^[6] P-gp and/or BCRP overexpression increases the transport of these agents out of the tumor cells resulting in decreased drug response.^[5]

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With the idea to tackle MDR, several medicinal chemistry efforts have already been taken to create compounds that would specifically inhibit either P-gp or BCRP individually, or in combination. Brooks and colleagues^[7] designed and tested 18 taxane derivatives for their inhibition of P-gp, MRP1, and BCRP. They found that taxane derivatives are substrates for P-gp, and to a lesser extent for MRP1, explaining their MDR reversal effect by competitive binding. With respect to BCRP, however, the inhibition was found to be noncompetitive, and the authors suggested that taxane derivatives only bind to one binding site on BCRP and are not transported. Colabufo and colleagues^[8] were looking for selective P-gp inhibitors since they expected that the *in vivo* toxicity of existing MDR modulating agents is related to the inhibition of other ABC-transporters that are instrumental in protecting noncancerous cells. They synthesized a series of arylmethoxy and arylmethylaminoderivatives that showed good P-gp inhibition. Specificity for P-gp as compared with BCRP seemed to be conferred by the linker, that is, only arylmethylamine derivatives showed activity for BCRP. Sim and colleagues^[9] focused on a type of flavonoids, aurones, which are known inhibitors of BCRP and tried to probe into the selectivity profile towards P-gp by generating more than one hundred derivatives. While most compounds preferentially inhibited BCRP, this publication found methoxyaurones, methoxyindanones, and methoxyflavones to be active on both P-gp and BCRP. Kühnle et al.^[10] found that by modifying the P-gp inhibitor tariquidar, they could greatly increase the selectivity for BCRP. Valdameri et al. synthesized a series of chalcone derivatives^[11] and chromone derivatives^[12] aiming at selective BCRP inhibitors. In the case of the chalcones, they showed the influence of the position of methoxy substitutions on both activity and cytotoxicity. While investigating the chromone derivatives, they found a very selective and active derivative for BCRP with a high therapeutic index. Also in case of propafenones, both compounds showing P-gp and BCRP specificity could be identified.^[13] Here, we extend these studies and aim to understand the molecular features triggering transporter selectivity. For this, we combined synthesis of novel propafenone derivatives, *in silico* prediction models to prioritize our compounds, and *in vitro* activity measurements.

Results and Discussion

Both P-glycoprotein and BCRP show polyspecific ligand recognition patterns with considerable overlap in their substrate and inhibitor profile. Previous results obtained with propafenone-type inhibitors pointed towards the vicinity of the nitrogen atom as potential structural moiety for selectivity profiling. In particular, compounds that contain a nonionizable nitrogen atom showed high selectivity indices. Furthermore, also the number of rotatable bonds as well as the number of H-bond acceptors seemed to influence both activity and selectivity.^[13] Thus, the compound design focused on two main features, the basicity of the nitrogen atom as well as the rigidity of the substituents in this region. This was also inspired by the polycyclic

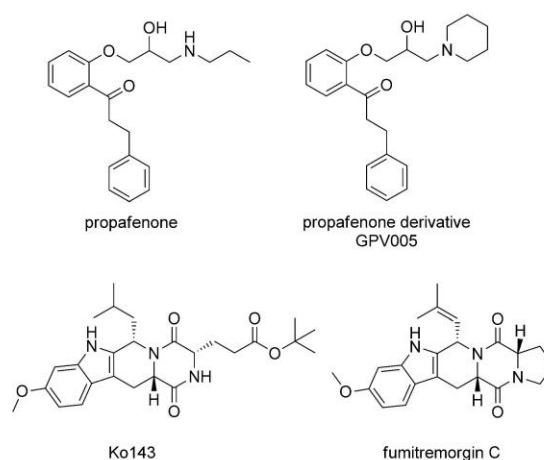


Figure 1. P-gp and BCRP inhibitors.

structure of fumitremorgin C and its derivative Ko143, which are selective BCRP inhibitors (Figure 1).

In silico classification models for P-gp and BCRP

In order to assess the likelihood of success, *in silico* classification models for P-gp and BCRP were established, and the inhibitor profiles of the compounds of interest were predicted. Two binary classification models for BCRP and P-gp inhibition were built on previously published large datasets.^[14] Both models are based on ECFP-like (extended connectivity fingerprint) 1024-bit fingerprints as descriptors and use logistic regression (for BCRP) and support vector machine (for P-gp) as base classifiers. The models are classification models, thus predicting whether a compound is likely to be an inhibitor of any of the two transporters. As threshold for active/inactive an IC_{50} value of 10 μ M was used. The predictive capability of the two models was evaluated by classical 10-fold cross-validation, results of which are shown in Table 1.

The two models were used to subsequently predict the inhibition against BCRP and P-gp for a series of compounds (Figure 2). As outlined above, transporter selectivity for propafenones might be triggered by the substituents in the vicinity

Table 1. Evaluation of the predictive power of the *in silico* models by 10-fold cross-validation.^[a]

	Accuracy	Sensitivity	Specificity	MCC	AUC
BCRP inhibition	0.83	0.77	0.87	0.65	0.90
P-gp inhibition	0.87	0.86	0.89	0.75	0.94

[a] Accuracy: the amount of correct predictions among all predictions; sensitivity: the proportion of inhibitors correctly identified; specificity: the proportion of noninhibitors correctly identified; Matthews correlation coefficient (MCC): correlation coefficient between the real and predicted classifications (between -1 and 1); area under the receiver operating characteristic (ROC) curve (AUC): ranking capability of the model.

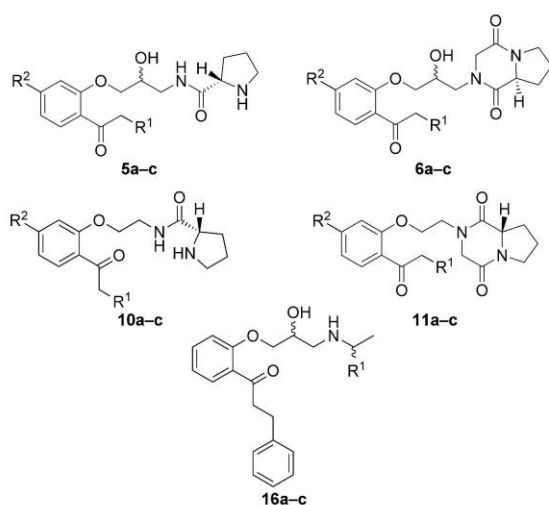


Figure 2. Synthesized target structures.

of the nitrogen atom. Flexibility of the substituents was triggered by the introduction of a proline (compounds **5** and **10**) and a diketopiperazine moiety (compounds **6** and **11**), whereby the latter contained only nonbasic nitrogen atoms. Furthermore, also lipophilicity of the compounds was varied by different substituents at the phenone moiety (R^1). Finally, the linker region between the nitrogen atom and the central aromatic ring (compound **5** vs. **10** and **6** vs. **11**), as well as the substitution on the central aromatic ring, was modified. In order to specifically analyze the influence of the basicity of the nitrogen atom on the transporter interaction profile of propafenones, the respective isopropyl, fluoroisopropyl, and difluoroisopropyl analogues **16a-c** were synthesized. The predictions for the

whole set of compounds indicate that both dual inhibitors as well as selective P-gp inhibitors should be present in this compound series.

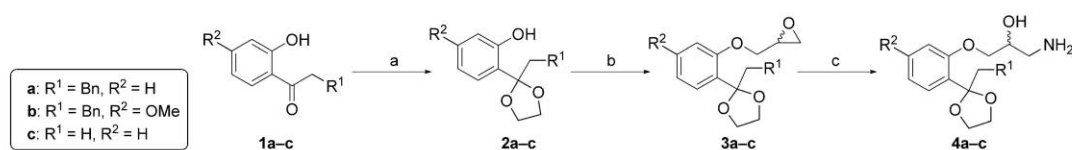
Synthesis

The target compounds **5**, **6**, **10**, and **11** (Figure 2) were synthesized following two general strategies.

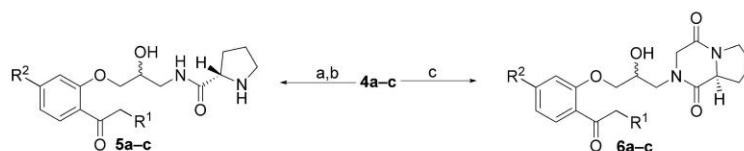
Respective hydroxyphenones **1a-c** were prepared from 1-(2-hydroxy-4-methoxyphenyl)ethanone by condensation with benzaldehyde and reduction of the double bond by hydrogenation.^[15] For the synthesis of the aryloxypropanolamines **4** and **6**, the ketone functionality in differently substituted 2'-hydroxyacetophenones **1a-c** was protected as a ketal using ethylene glycol and trimethylorthoformate similar to Ref. [16] as shown in Scheme 1. Subsequently, the phenols **2a-c** were O-alkylated with epichlorohydrine, and the epoxides **3a-c** were reacted with ammonia in methanol to yield the aryloxypropanolamines **4a-c**. Attempts to avoid the step of the protection of the ketone group failed and led to complex mixtures after the epoxide ring opening with ammonia.

Amines **4a-c** were converted to the respective amides **5a-c** with *N*-(tert-butoxycarbonyl)-L-proline (boc-proline) using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) (see Scheme 2). Unfortunately, the NMR analysis of the proline derivatives **5a-c** proved to be difficult due to the occurrence of rotamers that lead to broad signals. In addition, a mixture of diastereomers was formed that could not be separated by column chromatography.

For synthesis of the diketopiperazines **6a-c**, amines **4a-c** were reacted with methyl(2-chloroacetyl)prolinate, which was prepared from proline methyl ester and 2-chloroacetyl chloride.^[17] The diketopiperazine analogues showed NMR spectra that were easier to interpret due to the fixed conformation. The last step in both synthetic routes was the deprotection of



Scheme 1. Synthesis of intermediate products **4a-c**. *Reagents and conditions:* a) ethylene glycol, trifluoromethanesulfonic acid (TfOH), trimethylorthoformate, hexane, 40 °C, 3 d, 63–99%; b) epichlorohydrine, NaH, DMF, 70 °C, o/n, 74–99%; c) NH_3 in MeOH, RT, 2 d 14–54%.



Scheme 2. Synthetic route for final compounds **5a-c** and **6a-c**. *Reagents and conditions:* a) boc-proline, DCC, DMAP, CH_2Cl_2 , 2 h to o/n, 41–71%; b) 4 N HCl, dioxane, RT, 4 h, 98%–quant.; c) methyl (2-chloroacetyl)prolinate, Et_3N , RT, CH_3CN , 2.5 d or reflux, 2-ethoxyethanol, 2–3 d, then 2 N HCl, CH_3CN , RT, 4 h, 6–61% (2 steps).

the carbonyl group under acidic conditions, and, for the proline derivatives, formation of a hydrochloride. In some cases, the deprotection of the ketone occurred already partially during the column chromatography step.

For the synthesis of the aryloxyethanolamines **10** and **11**, the substituted 2'-hydroxyphenones **1a–c** were first reacted with bromoacetonitrile, followed by a protection of the carbonyl group as outlined above (see Scheme 3). Subsequently, the nitriles **8a–c** were reduced using lithium aluminium hydride^[18] to yield the amines **9a–c**. Attempts to improve the yield by using other reducing agents, such as Red-Al (sodium bis(2-methoxyethoxy)aluminumhydride, used for the synthesis of **9a**) or borane-tetrahydrofuran (THF)-complex failed.

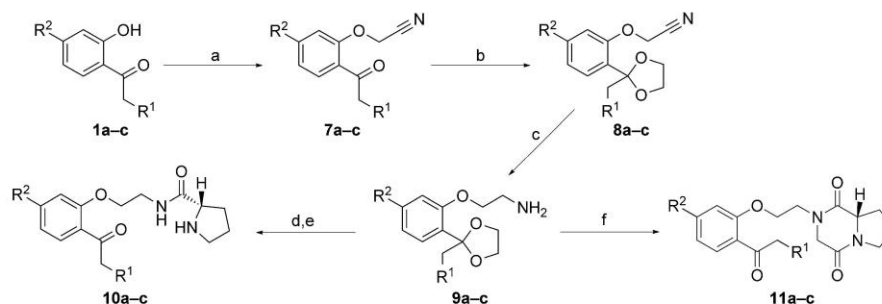
An alternative route for the synthesis of intermediate products **9** comprised the O-alkylation of the phenol **1a** with 1,2-dibromoethane^[19] as shown in Scheme 4. Then the carbonyl group of **12a** is protected with ethylene glycol, and the bromide is substituted with trifluoroacetamide^[20] to give **14a**. This derivative can easily be cleaved with base, furnishing the amine **9a** in higher yields and with less tedious steps. The proline amide and diketopiperazine derivatives were synthesized in analogy to the procedures as described above, and after deprotection and, if possible, hydrochloride formation, the target compounds **10a–c** and **11a–c** were obtained.

Following the standard synthetic route for propafenone derivatives, compound **16a** was prepared by reacting the epoxide **15** with isopropylamine. For the fluorinated derivatives **16b** and **16c**, this was not possible due to the decreased ba-

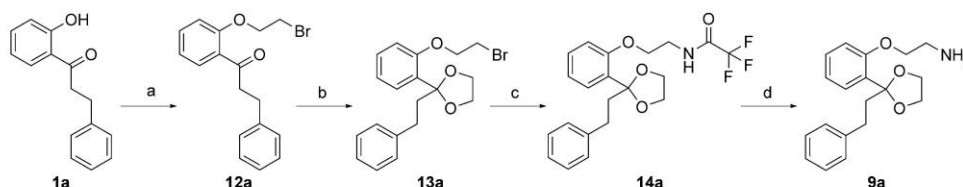
sicity and thus reactivity of the fluorinated amines. Therefore the amine **4a** was reacted with the respective fluorosubstituted acetone derivatives in a reductive amination reaction followed by deprotection of the ketone (Scheme 5).

In vitro studies

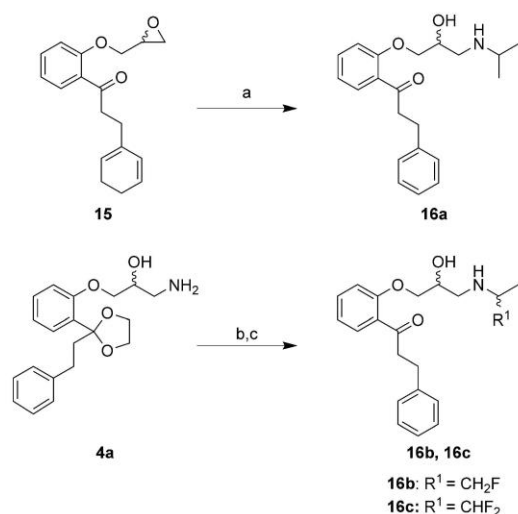
P-gp and BCRP inhibitory activity was assessed by implementing an intracellular accumulation assay of daunorubicin or mitoxantrone in cells overexpressing P-gp or BCRP, respectively. In a first run, the effect of compounds **5**, **6**, **10**, **11**, and **16** (Figure 2) at 100 μM final concentration was tested (Figure 3). All compounds showing an inhibitory effect of more than 50% compared with full inhibition by the known inhibitors of P-gp (verapamil) and BCRP (Ko143) were further processed, and IC_{50} values were determined (IC_{50} curves and Hill coefficients are available in Figure S1 and Table S3 in the Supporting Information). In order to allow comparison with the *in silico* classification models, compounds with IC_{50} values below 10 μM were considered as active. As shown in Table 2, eight compounds were identified as inhibitors for BCRP (**5b**, **6a**, **6b**, **10a**, **10b**, **11b**, **16b**, **16c**), while one compound (**5a**) showed borderline activity on BCRP with an IC_{50} value of $16.13 \pm 3 \mu\text{M}$. For P-gp, nine compounds were classified as inhibitors (**5a**, **5b**, **6a**, **10a**, **10b**, **11b**, **16a**, **16b**, **16c**). In addition, compounds **6b** and **11a** showed borderline inhibitory activity on P-gp with IC_{50} values of 11 ± 1 and $13.9 \pm 1.5 \mu\text{M}$, respectively.



Scheme 3. Synthesis of compounds **10a–c** and **11a–c**. *Reagents and conditions:* a) bromoacetonitrile, K_2CO_3 , acetone, 50°C , 4 d (88–100%); b) ethylene glycol, *p*-toluenesulfonic acid (pTosOH), toluene, reflux, o/n, 72–97%; c) LiAlH_4 or Red-Al, Et_2O , reflux or RT, 3–4 h, 15–42%; d) boc-proline, DCC, DMAP, CH_2Cl_2 , 2 h to o/n, 57–78%; e) 4 N HCl, dioxane, RT, 4 h, 97%–quant.; f) methyl (2-chloroacetyl)prolinate, Et_3N , reflux, 2-ethoxyethanol, 2 d, then 2 N HCl, CH_3CN , RT, 4 h, 19–39% (2 steps).



Scheme 4. Alternative route to compound **9a**. *Reagents and conditions:* a) 1,2-dibromoethane, K_2CO_3 , Bu_4NBr , toluene, water, reflux, 6 d, 79%; b) ethylene glycol, TfOH, trimethylorthoformate, hexane, 40°C , 3 d, 99%; c) trifluoroacetamide, K_2CO_3 , Bu_4NBr , DMF, 80°C , 4.5 h, 65%; d) KOH, MeOH, water, RT, 2 h, 99%.



Scheme 5. Synthesis of compounds **16a–c**. *Reagents and conditions:* a) *i*PrNH₂, reflux, 21 h, 57%; b) NaBH₃CN, mono or difluoroacetone, AcOH, MeOH, reflux, 4 h, quant.; c) 2 N HCl, EtOAc, 2 h, 84–86%.

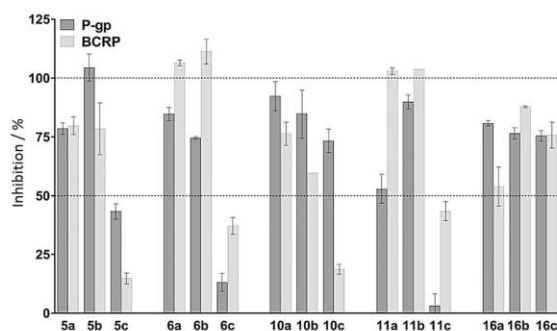


Figure 3. Evaluation of compounds used for IC₅₀ measurements. In a first set of experiments, the effect of all compounds at 100 μM final concentration was tested on P-gp (dark grey bars) and BCRP (light grey bars) using intracellular accumulation assay as described in the Experimental Section. Data are shown as % inhibition compared with the positive control verapamil (P-gp) or Ko143 (BCRP) and are given as mean ± SD of a single experiment with at least duplicate measurements.

Comparing the inhibitory activity on P-gp and BCRP, seven compounds (**5a**, **6a**, **10a**, **10b**, **16a**, **16b**, **16c**) were more effective on P-gp than on BCRP (Table 2). The most pronounced difference in the inhibitory activity on P-gp and BCRP was observed for the fluorinated compounds **16a**, **16b**, and **16c** and the aryloxyethanolamine **5a** giving more than fivefold higher IC₅₀ values for BCRP than for P-gp. Another three compounds, namely **6a**, **10a**, and **10b** were more than 2.6-fold better inhibitors for P-gp than for BCRP. Compounds **5b** and **11a** showed comparable inhibitory activity on both ABC proteins (1.4- and 1.5-fold higher IC₅₀ values for BCRP). The methoxy-substituted compounds **6b** and **11b** bearing a piperazinedione

Table 2. Results of in silico prediction and in vitro IC₅₀ evaluation of BCRP and P-gp inhibition for the synthesized compounds (Figure 2).

Compd	log P ^[a]	Substituents		BCRP		P-gp	
		R ¹	R ²	in silico ^[b]	IC ₅₀ [μM] ^[c]	in silico ^[b]	IC ₅₀ [μM] ^[c]
5a	2.37	Bn	H	0.25	16.1 ± 3.0	0.97	2.1 ± 0.4
5b	2.36	Bn	OMe	0.37	3.1 ± 0.5	0.92	2.2 ± 0.6
5c	0.53	H	H	0.08	n.d.	0.46	58.7 ± 9.2
6a	1.93	Bn	H	0.39	5.8 ± 1.0	0.98	1.6 ± 0.6
6b	1.92	Bn	OMe	0.64	3.8 ± 0.6	0.96	11.0 ± 1.0
6c	0.09	H	H	0.15	n.d.	0.78	n.d.
10a	2.94	Bn	H	0.17	3.3 ± 0.6	0.88	1.3 ± 0.1
10b	2.93	Bn	OMe	0.39	2.9 ± 0.5	0.83	1.0 ± 0.2
10c	1.10	H	H	0.07	n.d.	0.19	34.8 ± 1.8
11a	2.50	Bn	H	0.40	21.5 ± 2.9	0.94	13.9 ± 1.5
11b	2.49	Bn	OMe	0.75	2.3 ± 0.0	0.91	6.2 ± 0.4
11c	0.66	H	H	0.19	n.d.	0.42	n.d.
16a	2.86	CH ₃	–	0.22	n.d.	0.97	0.3 ± 0.0
16b	3.08	CH ₂ F	–	0.19	5.5 ± 0.9	0.97	0.5 ± 0.1
16c	3.47	CH ₂ F	–	0.24	4.9 ± 1.1	0.97	0.9 ± 0.2

[a] Calculated partition coefficient log P_{octanol/water} determined using Molecular Operating Environment (MOE) version 2014.09. [b] Score between 0 and 1, given by in silico models, roughly corresponds to the probability of being an inhibitor; in silico predictions over 0.5 are marked in bold and represent compounds that are predicted as inhibitors by the models. [c] IC₅₀ values represent the mean ± SD of at least *n* = 3 independent experiments performed at least in duplicate; for inhibition of BCRP, **11a** was measured in *n* = 2 independent experiments; the exact number of experiments is given in the Supporting Information (Figure S1 and Table S3); IC₅₀ values were evaluated as described in the Experimental Section; n.d.: not determined.

moiety were the only ones showing a higher inhibitory activity on BCRP than on P-gp, with 2.9- and 2.7-fold lower IC₅₀ values for BCRP, respectively. Finally, **5c**, **6c**, **10c**, and **11c** were identified as noninhibitors for both ABC proteins, as their IC₅₀ values were either far above 10 μM (**5c** and **10c** on P-gp) or did not show more than 50% inhibition at 100 μM.

In addition, compound **11a** was shown to be a weak inhibitor for BCRP (IC₅₀: 21.5 ± 2.9 μM) and a borderline inhibitor for P-gp (IC₅₀: 13.9 ± 1.5 μM).

Structure–activity relationships (SAR)

In order to gain insights into molecular features that might trigger P-gp vs. BCRP selectivity of propafenone-type inhibitors, we modified the substituent at the nitrogen atom towards more rigid and less basic properties. These modifications were complemented by a set of distinct variations, which should allow assessing if the compounds still show the basic SAR pattern reported for propafenones. Specifically, these include the linker region (propanol vs. ethylene), the phenone moiety (phenylpropylphenone vs. propylphenone), as well as the central aromatic ring (4-methoxy vs. 4-H). In addition, we calculated the logP values of all compounds and put them in relation to previously published logP/pIC₅₀ correlations for this scaffold.

For P-glycoprotein, the SAR pattern retrieved is mainly in line with those reported for other propafenone analogues: i) there is a significant correlation of pIC₅₀ values with the logP

of the compounds ($r^2=0.71$),^[21] ii) elimination of the phenyl ring of the phenylpropionophenone moiety strongly decreases biological activity (which is most probably related to the logP decrease), iii) shortening the distance between the central aromatic ring and the nitrogen atom slightly decreases biological activity,^[22] and iv) increasing electron density of the central aromatic ring slightly increases activity.^[23] However, the ethylene compound **6a** with a diketopiperazine moiety at the nitrogen atom does not follow this pattern. It is tenfold more active than its propanol analogue **11a**, and is also more active than its methoxy analogue **6b** by a factor of 7.

For BCRP, the picture is more complex, and there are no obvious trends. Also with respect to lipophilicity of the compounds, there is no correlation with pIC_{50} values ($r^2=0.02$). Interestingly, the most active compound (**11b**) belongs to the diketopiperazine series, whereas in case of P-gp it is a proline analogue (**10b**).

Prospective performance of the in silico models

The BCRP inhibition model tends to give low scores in our chemical series, that is, rarely predicts a compound as an inhibitor. In our specific case, 6 compounds out of 15 are mispredicted as inactive (**5b**, **6a**, **10a**, **b**, **16b**, **c**), which results in an accuracy of 60%. The P-gp inhibition model, on the contrary, tends to give high scores, that is, it often predicts a compound as an inhibitor. In our specific case, the model makes one clear mistake (predicting the des-phenyl analogue **6c** as active) and 2 misclassifications of borderline compounds (predicting **6b** ($\text{IC}_{50}=11.0 \pm 1 \mu\text{M}$) and **11a** ($\text{IC}_{50}=13.9 \pm 1.5 \mu\text{M}$) as inhibitors when they show activity slightly above $10 \mu\text{M}$, the IC_{50} threshold applied for categorizing the compounds).

To understand this behavior of both our models, for each compound of our series we retrieved the five nearest structural neighbors of the training sets (see Supporting Information for Tables S1 and S2 showing the average similarity values). In the BCRP training set, all neighbors retrieved were propafenone derivatives from the study of Cramer et al.,^[13] and all these compounds were inactive for BCRP. This explains the tendency of the model to predict most of the propafenone derivatives as noninhibitors of BCRP. In contrast, in the P-gp training set, all retrieved neighbors were propafenone derivatives annotated as inhibitors of P-gp. This explains the tendency of the P-gp model to predict most propafenone derivatives as active.

The difference between the prospective results and the predicted cross-validation results seems striking, but is inherently linked to the composition of the training set. Indeed, the in silico models are applied on structurally very similar compounds. As a result, the fingerprint features encoding these structures are very similar to each other, and the results given by the models cannot show a high variability. The cross-validation rather evaluates the generalizability of the model than its specific capability to properly predict SAR subtleties within the propafenone family. However, if one directly uses the score to rank the compounds according to their probability to inhibit P-gp or BCRP, instead of applying a fixed threshold, the calculated prospective AUCs would be 0.78 for BCRP and 0.85 for P-

gp. Thus, these models are still valuable tools for prioritizing propafenone-type compounds in medicinal chemistry projects.

Selectivity vs. multitarget inhibition

Although the activity differences for compounds **5–11** between P-gp and BCRP are less than a factor of 10 [selectivity indices (SI) P-gp/BCRP=0.13–2.82], some trends can be deduced from the data. Highest selectivity for P-gp was obtained for the two ethylene analogues **5a** (SI=0.13) and **6a** (SI=0.27), both being unsubstituted at the central aromatic ring and having either a proline or a diketopiperazine in the vicinity of the nitrogen atom. In case of BCRP selectivity, the two diketopiperazines **6b** and **11b** showed the highest SI values (2.82 and 2.70, respectively). Both of them show a methoxy moiety at position 4 of the central aromatic ring and belong either to the ethylene (**6b**) or the propanolamine (**11b**) class of compounds. The remaining compounds may be regarded as dual transporter inhibitors, showing SI values in the range of 0.34–0.71 and pIC_{50} values in the low micromolar range. Selectivity towards BCRP thus seems to be triggered mainly by the substituent on the central aromatic ring, as well as the diketopiperazine moiety. This might at least in part be due to the basic feature of the nitrogen atom, as exemplified by the **16a–c** series of compounds. By introduction of fluorine atoms, the basicity of the nitrogen atom is systematically varied. Although in all cases the compounds are more active with respect to P-gp than to BCRP, there is a clear tendency in P-gp that lower basicity/H-bond donor properties lead to lower activity. This is not seen in BCRP, which is in line with previous observations for propafenones.^[13,24] Furthermore, the switch from proline to diketopiperazine also decreases flexibility in the vicinity of the nitrogen atom. It is tempting to speculate that a further morphing towards a more fumitremorgin C like scaffold would increase selectivity.

Conclusions

The ABC-transporters P-glycoprotein (P-gp) and the breast cancer resistance protein (BCRP) are characterized by a broad and partly overlapping ligand profile. Due to their importance for absorption, distribution, metabolism, excretion (ADME), and toxicity, design of multitransporter inhibitors as well as transporter-specific compounds would be a valuable effort for obtaining a set of tool compounds. Building on previous work, we extended our studies on the propafenone scaffold as a versatile chemotype for triggering transporter selectivity and for increasing our understanding of the molecular features driving ligand–transporter interaction. Results indicate that both the flexibility of the substituent at the nitrogen atom, as well as the basicity of the nitrogen atom, triggers transporter selectivity. Furthermore, inhibitory activity of compounds for P-gp seems to be much more influenced by logP than that for BCRP. Exploiting these differences should thus further allow to dissect the molecular features triggering ligand selectivity for the two polyspecific ABC-transporters: P-gp and BCRP.

Experimental Section

Chemistry

General. All moisture-sensitive reactions were conducted in anhydrous solvents (Sigma–Aldrich) under dry argon atmosphere. All solvents and reagents were obtained from Sigma–Aldrich, ABCR, TCI, or Acros and used without further purification. Reactions were monitored by analytical thin-layer chromatography (TLC) using pre-coated TLC sheets ALUGRAM Xtra SIL G/UV254 (Macherey–Nagel, 0.20 mm silica gel 60 layer with fluorescent indicator UV254). Visualization was carried out by UV light (254 nm), ninhydrin, and/or Seebach stain. Flash chromatography was performed using Merck silica gel 60 μm (0.040–0.063 mm). Yields are not optimized.

NMR spectroscopy. NMR spectra were registered either on a Bruker Avance 200 or Ultrashield 500 spectrometer (Billerica, MA, USA) at 200 or 500 MHz, using CDCl₃ or D₆DMSO as solvents. Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS), and coupling constants (*J*) are given in Hertz (Hz). Multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), septet, or m (multiplet). ¹H and ¹³C spectra of tested compounds are given in the Supporting Information. Purity of target compounds was defined by ¹³C NMR (absence of additional signals).

High-resolution electrospray ionization mass spectrometry (HR-ESI-MS). HR-ESI-MS spectra were obtained on a maXis HD ESI-Qq-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) using direct infusion. The ESI ion source was operated as follows: capillary voltage: 1.0 to 4.0 kV (individually optimized), nebulizer: 0.4 bar (N₂), dry gas flow: 4 L min^{−1} (N₂), and dry temperature: 200 °C. Mass spectra were recorded in the range of *m/z* 50–1550 in the positive-ion mode. The sum formulas were determined using Bruker Compass Data Analysis 4.2 based on the mass accuracy ($\Delta m/z \leq 2$ ppm) and isotopic pattern matching (SmartFormula algorithm).

2-(2-Phenethyl-1,3-dioxolan-2-yl)phenol (1a): 2'-Hydroxy-3-phenylpropionophenone (1 g, 1 eq, 4.4 mmol), ethylene glycol (0.37 mL, 1.5 eq, 6.6 mmol), trimethylorthoformate (0.73 mL, 1.5 eq, 6.6 mmol), and trifluoromethanesulfonic acid (3.9 μL, 0.01 eq, 0.04 mmol) in hexane (7 mL) were heated at 42 °C o/n, then ethylene glycol (0.2 mL) and trimethylorthoformate (0.4 mL) were added, and the reaction was heated for two more days. Then the mixture was cooled to RT, and Et₃N (3 mL) was added followed by saturated aq NaHCO₃ (25 mL). This was extracted with Et₂O (4 × 50 mL, a few drops Et₃N added), and the combined organic extracts were washed once with brine (30 mL), dried over Na₂SO₄, and concentrated to afford **1a** as a white solid (99%, 1.17 g), which was used without further purification. NMR data were in good accordance with the literature.^[25]

5-Methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenol (1b): Preparation like **1a**; used without further purification. Brown oil (4.54 g, 97%); ¹H NMR (500 MHz, CDCl₃): δ = 2.20–2.28 (m, 2H), 2.70–2.79 (m, 2H), 3.79 (s, 3H), 3.94 (brs, 2H), 4.13 (brs, 2H), 6.41–6.51 (m, 2H), 7.13–7.23 (m, 4H), 7.23–7.36 (m, 2H), 8.37 ppm (s, 1H); ¹³C NMR (125 MHz, CDCl₃): δ = 29.5, 41.4, 53.7, 64.5 (2C), 101.9, 106.3, 111.3, 117.5, 125.8, 127.8, 128.3 (2C), 128.3 (2C), 141.5, 155.9, 161.6 ppm.

2-(2-Methyl-1,3-dioxolan-2-yl)phenol (1c): Preparation like **1a**. Purification by column chromatography (petrol ether:EtOAc, 20:1 → 15:1) yielded **1c** as a yellow oil (9.99 g, 63%). NMR data were in good accordance with the literature.^[16]

2-(2-(Oxiran-2-ylmethoxy)phenyl)-2-phenethyl-1,3-dioxolane

(2a): The ketal **1a** (5 g, 1 eq, 18.5 mmol) was dissolved in anhydrous dimethylformamide (40 mL) and heated to 70 °C, then NaH (60% dispersion, 1.11 g, 1.5 eq, 27.7 mmol) was added in portions during 30 min. After stirring for 1 h at this temperature, epichlorohydrine (4.4 mL, 3 eq, 55.5 mmol) was added and heated overnight. TLC showed no starting material; therefore, the reaction mixture was concentrated, diluted with Et₂O (100 mL) and extracted once with sat. NaHCO₃ solution (100 mL) and water (100 mL). The combined aqueous extracts were extracted once with Et₂O (100 mL), and the combined organic phases were dried over Na₂SO₄, and the solvent was evaporated. The crude product was triturated with petrol ether (20 mL) under cooling, and the petrol ether phase was removed, which yielded the product as a yellowish oil (6.0 g, 99%) after drying. NMR data were in good accordance with the literature.^[25]

2-(4-Methoxy-2-(oxiran-2-ylmethoxy)phenyl)-2-phenethyl-1,3-dioxolane (2b):

Preparation like **2a**; purification by column chromatography (petrol ether:EtOAc, 4:1 to 2:1) afforded a colorless oil (3.52 g, 74%); ¹H NMR (500 MHz, CDCl₃): δ = 2.39–2.49 (m, 2H), 2.59–2.67 (m, 2H), 2.87 (d, *J* = 3.5 Hz, 2H), 3.35–3.39 (m, 1H), 3.80 (s, 3H), 3.85–3.92 (m, 2H), 4.02–4.08 (m, 3H), 4.24 (dd, *J* = 11.2, 3.0 Hz, 1H), 6.47 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.49 (d, *J* = 2.2 Hz, 1H), 7.13 (t, *J* = 7.1 Hz, 1H), 7.17 (d, *J* = 7.3 Hz, 2H), 7.23 (d, *J* = 7.6 Hz, 2H), 7.24–7.29 (m, 1H), 7.42 ppm (d, *J* = 8.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃): δ = 30.2, 39.7, 44.7, 50.2, 55.3, 64.70, 64.72, 68.8, 101.1, 104.2, 109.9, 122.5, 125.4, 128.1 (2C), 128.30, 128.34 (2C), 128.36, 142.4, 157.0, 160.7 ppm; HRMS (ESI): calcd for C₂₁H₂₄NaO₅ *m/z* 357.1697 [*M*+Na]⁺, found *m/z* 357.1697 [*M*+Na]⁺.

2-Methyl-2-(2-(oxiran-2-ylmethoxy)phenyl)-1,3-dioxolane (2c):

Preparation like **2a**. The crude product was purified by column chromatography (petrol ether: EtOAc 6:1 to 2:1) to yield **2c** as a colorless oil (3.26 g, 84%); ¹H NMR (500 MHz, CDCl₃): δ = 1.79 (s, 3H), 2.87–2.92 (m, 2H), 3.38–3.43 (m, 1H), 3.79–3.88 (m, 2H), 4.01–4.06 (m, 2H), 4.08 (dd, *J* = 11.2, 4.9 Hz, 1H), 4.31 (dd, *J* = 11.2, 3.0 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 6.94 (td, *J* = 7.4, 1.1 Hz, 1H), 7.26 (td, *J* = 7.4, 1.1 Hz, 1H), 7.50 ppm (dd, *J* = 7.7, 1.7 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃): δ = 25.4, 44.7, 50.3, 64.5 (2C), 68.9, 108.5, 113.6, 120.8, 126.8, 129.4, 131.1, 156.0 ppm; HRMS (ESI): calcd for C₁₃H₁₆NaO₄ *m/z* 259.0941 [*M*+Na]⁺, found *m/z* 259.0945 [*M*+Na]⁺.

1-Amino-3-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propan-2-ol (3a):

The epoxide **2a** (2 g, 1 eq, 6.12 mmol), was dissolved in MeOH (23 mL) and NH₃ in MeOH (7 mL, 23 mL) was added. It was stirred for 2 d at RT and then evaporated to yield a crude yellow oil (2.28 g, 109%), used without further purification; ¹H NMR (500 MHz, CDCl₃): δ = 2.44 (t, *J* = 8.5 Hz, 2H), 2.55–2.73 (m, 2H), 2.80–2.99 (m, 2H), 3.80–3.93 (m, 2H), 3.93–4.14 (m, 4H), 4.17–4.25 (m, 1H), 6.91 (d, *J* = 6.8 Hz, 1H), 6.97 (t, *J* = 6.8 Hz, 1H), 7.09–7.18 (m, 1H), 7.15 (d, *J* = 7.0 Hz, 2H), 7.23 (d, *J* = 6.3 Hz, 2H), 7.26–7.33 (m, 1H), 7.48 ppm (d, *J* = 7.3 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃): δ = 30.0, 40.0, 44.2, 64.7, 64.8, 70.8, 72.4, 110.3, 114.4, 120.0, 125.6, 127.1, 128.25 (2C), 128.30 (2C), 129.6, 130.0, 142.1, 156.6 ppm; HRMS (ESI): calcd for C₂₀H₂₆NO₄ *m/z* 344.1856 [*M*+H]⁺, found *m/z* 344.1857 [*M*+H]⁺.

1-Amino-3-(5-methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propan-2-ol (3b):

Prepared like **3a**; yellow oil (1.70 g, 54%); ¹H NMR (500 MHz, CDCl₃): δ = 2.40 (t, *J* = 8.7 Hz, 2H), 2.54–2.70 (m, 2H), 2.83–2.97 (m, 2H), 3.79 (s, 3H), 3.82–3.90 (m, 2H), 3.92 (d, *J* = 6.3 Hz, 1H), 3.91–3.99 (m, 1H), 4.00–4.10 (m, 2H), 4.17 (d, *J* = 6.9 Hz, 1H), 6.44–6.52 (m, 2H), 7.09–7.18 (m, 3H), 7.20–7.26 (m, 2H),

7.37 ppm (d, $J = 8.5$ Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 30.1$, 40.2, 44.2, 55.4, 64.6, 64.7, 70.7, 72.4, 101.7, 105.1, 110.3, 122.5, 125.6, 127.9, 128.2 (2C), 128.3 (2C), 142.0, 157.5, 160.9 ppm; HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{28}\text{NO}_5$ m/z 374.1962 $[\text{M}+\text{H}]^+$, found m/z 374.1964 $[\text{M}+\text{H}]^+$.

1-Amino-3-(2-(2-methyl-1,3-dioxolan-2-yl)phenoxy)propan-2-ol (3c): Preparation like **3a**; the crude product (mixture with dimer) was purified by column chromatography ($\text{CH}_2\text{Cl}_2 + 1\%$ Et_3N to $\text{CH}_2\text{Cl}_2:\text{MeOH} = 20:1 + 1\%$ Et_3N) to yield the pure product as a yellowish solid (378 mg, 14%) and the rest (1.61 g) as a mixture with the dimer; ^1H NMR (500 MHz, CDCl_3): $\delta = 1.77$ (s, 3 H), 2.82–2.92 (m, 2 H), 3.77–3.88 (m, 2 H), 3.92–3.99 (m, 2 H), 4.01–4.08 (m, 2 H), 4–17–4.26 (m, 1 H), 6.91 (d, $J = 8.2$ Hz, 1 H), 6.94 (td, $J = 7.6$, 0.9 Hz, 1 H), 7.23–7.29 (m, 1 H), 7.46 ppm (dd, $J = 7.7$, 1.7 Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 25.8$, 44.3, 64.4, 64.5, 71.1, 72.3, 108.8, 114.4, 120.9, 126.3, 129.5, 130.9, 156.5 ppm; HRMS (ESI): calcd for $\text{C}_{13}\text{H}_{20}\text{NO}_4$ m/z 254.1387 $[\text{M}+\text{H}]^+$, found m/z 254.1390 $[\text{M}+\text{H}]^+$.

2-(2-(3-Phenylpropanoyl)phenoxy)acetonitrile (7a): 2'-Hydroxy-3-phenylpropiophenone (4 g, 1 eq, 17.7 mmol) was dissolved in acetone (200 mL), and K_2CO_3 (9.77 g, 4 eq, 70.7 mmol) was added. Bromoacetonitrile (2.54 g, 1.2 eq, 21.2 mmol) dissolved in acetone (100 mL) was added dropwise throughout 30 min. The reaction was stirred at 50°C for 4 d, then it was filtered, washed once with acetone, and concentrated. The residue was dissolved in CH_2Cl_2 (100 mL) and water (100 mL), and the aqueous phase was extracted a $\text{CH}_2\text{Cl}_2/\text{CHCl}_3$ mixture (10% CHCl_3 , 3×100 mL). The combined organic extracts were washed once with brine (70 mL), dried over Na_2SO_4 , and the solvent was evaporated to yield a brown solid (4.15 g, 88%). NMR data were in good accordance with the literature.^[18]

2-(5-Methoxy-2-(3-phenylpropanoyl)phenoxy)acetonitrile (7b): Preparation like **7a**; brown solid (4.86 g, 93%); ^1H NMR (500 MHz, CDCl_3): $\delta = 3.02$ (t, $J = 7.6$ Hz, 2 H), 3.24 (t, $J = 7.6$ Hz, 2 H), 3.87 (s, 3 H), 4.80 (s, 2 H), 6.52 (d, $J = 2.2$ Hz, 1 H), 6.66 (dd, $J = 8.7$, 2.0 Hz, 1 H), 7.17–7.22 (m, 1 H), 7.22–7.25 (m, 2 H), 7.27–7.32 (m, 2 H), 7.81 ppm (d, $J = 8.8$ Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 30.4$, 44.9, 54.0, 55.7, 100.4, 107.5, 114.5, 121.8, 126.0, 128.4 (4C), 133.1, 141.4, 157.0, 164.1, 198.5 ppm; HRMS (ESI): calcd for $\text{C}_{18}\text{H}_{17}\text{NNaO}_3$ m/z 318.1101 $[\text{M}+\text{Na}]^+$, found m/z 318.1100 $[\text{M}+\text{Na}]^+$.

2-(2-Acetylphenoxy)acetonitrile (7c): Preparation like **7a**. The product was a white solid (3.54 g, 100%), which was used without further purification. NMR data were in good accordance with the literature.^[26]

2-(2-(2-Phenethyl-1,3-dioxolan-2-yl)phenoxy)acetonitrile (8a): The nitrile **7a** (4 g, 1 eq, 15.1 mmol), ethylene glycol (1.7 mL, 2 eq, 30.9 mmol), and *p*-toluenesulfonic acid monohydrate (0.29 g, 0.1 eq, 1.5 mmol) were dissolved/suspended in toluene (50 mL) and heated o/n in a Dean–Stark apparatus. Then the reaction mixture was poured into ice-cold sat. NaHCO_3 solution (50 mL) and extracted with EtOAc (4×70 mL). The combined organic extracts were dried over Na_2SO_4 , filtered, and evaporated to yield a brown oil (4.52 g, 97%); ^1H NMR (500 MHz, CDCl_3): $\delta = 2.35$ –2.44 (m, 2 H), 2.60–2.69 (m, 2 H), 3.84–3.93 (m, 2 H), 4.04–4.13 (m, 2 H), 4.81 (s, 2 H), 7.07 (d, $J = 8.2$ Hz, 1 H), 7.09–7.18 (m, 4 H), 7.20–7.26 (m, 2 H), 7.35 (td, $J = 7.7$, 1.7 Hz, 1 H), 7.58 ppm (dd, $J = 7.7$, 1.7 Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 29.9$, 40.0, 55.2, 64.9 (2C), 109.5, 115.5, 115.7, 123.3, 125.6, 128.1, 128.2 (2C), 128.3 (2C), 129.8, 132.0, 142.0, 154.4 ppm; HRMS (ESI): calcd for $\text{C}_{19}\text{H}_{19}\text{NNaO}_3$ m/z 332.1257 $[\text{M}+\text{Na}]^+$, found m/z 332.1258 $[\text{M}+\text{Na}]^+$.

2-(5-Methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)acetonitrile (8b): Preparation like **8a**. The crude product was purified by column chromatography (petrol ether:EtOAc 4:1 to 2:1) to yield **8b** as a yellow oil (3.83 g, 72%); ^1H NMR (500 MHz, CDCl_3): $\delta = 2.34$ –2.41 (m, 2 H), 2.59–2.66 (m, 2 H), 3.82 (s, 3 H), 3.85–3.91 (m, 2 H), 4.05–4.09 (m, 2 H), 4.79 (s, 2 H), 6.61 (s, 1 H), 6.62 (dd, $J = 6.5$, 2.4 Hz, 1 H), 7.12–7.17 (m, 3 H), 7.21–7.26 (m, 2 H), 7.48 ppm (dd, $J = 6.3$, 3.2 Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 30.1$, 40.2, 55.0, 55.5, 64.7 (2C), 102.9, 107.1, 125.6, 128.2 (2C), 128.3 (2C), 128.9, 109.5, 115.3, 124.0, 142.1, 155.1, 160.7 ppm; HRMS (ESI): calcd for $\text{C}_{20}\text{H}_{21}\text{NNaO}_4$ m/z 362.1363 $[\text{M}+\text{Na}]^+$, found m/z 362.1366 $[\text{M}+\text{Na}]^+$.

2-(2-(2-Methyl-1,3-dioxolan-2-yl)phenoxy)acetonitrile (8c): Preparation like **8a**. The crude product was purified by column chromatography (petrol ether:EtOAc 4:1 to 1:1) to yield **8c** as a white solid (3.01 g, 81%); ^1H NMR (500 MHz, CDCl_3): $\delta = 1.75$ (s, 3 H), 3.82–3.86 (m, 2 H), 4.04–4.08 (m, 2 H), 4.85 (s, 2 H), 7.08 (d, $J = 8.2$ Hz, 1 H), 7.10 (t, $J = 7.4$ Hz, 1 H), 7.34 (td, $J = 7.8$, 1.7 Hz, 1 H), 7.56 ppm (dd, $J = 7.6$, 1.6 Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 25.8$, 64.6 (2C), 55.1, 108.0, 114.5, 114.6, 123.4, 127.3, 129.7, 132.8, 154.3 ppm; HRMS (ESI): calcd for $\text{C}_{12}\text{H}_{13}\text{NNaO}_3$ m/z 242.0788 $[\text{M}+\text{Na}]^+$, found m/z 242.0789 $[\text{M}+\text{Na}]^+$.

2-(2-(2-Phenethyl-1,3-dioxolan-2-yl)phenoxy)ethan-1-amine (9a): Red-Al (~ 3.5 M in toluene, 4.5 mL, 9.7 eq, 15.8 mmol) was placed in a flask, and the nitrile **8a** (0.5 g, 1 eq, 1.62 mmol) dissolved in toluene (20 mL) was added dropwise during 30 min. The reaction mixture was stirred at RT for 3 h, and then water (20 mL) and Na-K-tartrate (13 g) was added and stirred for 30 min at RT. The mixture was filtered over celite and washed with EtOAc. Then the phases were separated, and the aqueous phase was extracted with EtOAc (3×80 mL). The combined organic extracts were washed once with brine (100 mL), dried over Na_2SO_4 , and evaporated. The crude product was purified by column chromatography ($\text{CH}_2\text{Cl}_2 + 1\%$ Et_3N to $\text{CH}_2\text{Cl}_2:\text{MeOH} = 20:1 + 1\%$ Et_3N) to yield **9a** as a colorless oil (76 mg, 15%); ^1H NMR (500 MHz, CDCl_3): $\delta = 2.43$ –2.50 (m, 2 H), 2.61–2.69 (m, 2 H), 3.08 (brs, 2 H), 3.82–3.92 (m, 2 H), 3.99–4.10 (m, 4 H), 6.89 (d, $J = 8.2$ Hz, 1 H), 6.94 (t, $J = 7.4$ Hz, 1 H), 7.11–7.18 (m, 3 H), 7.20–7.33 (m, 3 H), 7.51 ppm (dd, $J = 7.6$, 1.6 Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 30.1$, 39.7, 41.7, 64.7 (2C), 70.7, 110.0, 113.0, 120.2, 125.5, 127.4, 128.2 (2C), 128.3 (2C), 129.4, 129.7, 142.2, 156.5 ppm; HRMS (ESI): calcd for $\text{C}_{19}\text{H}_{24}\text{NO}_3$ m/z 314.1751 $[\text{M}+\text{H}]^+$, found m/z 314.1752 $[\text{M}+\text{H}]^+$.

2-(5-Methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)ethan-1-amine (9b): LiAlH_4 (671 mg, 2 eq, 17.7 mmol) was suspended in absolute Et_2O (200 mL), and the nitrile **8b** (3 g, 1 eq, 8.8 mmol, dissolved in 50 mL Et_2O) was added carefully throughout 30 min. After completion of the addition, the reaction mixture was heated at reflux for 4 h and afterwards stirred at RT o/n. First EtOAc, and then saturated Na-K-tartrate solution, was added carefully with cooling, and the mixture was stirred for 3 h. Then the aqueous phase was extracted with EtOAc (3×150 mL). The organic phases were dried over Na_2SO_4 , filtered, and evaporated. The crude product was purified by column chromatography (CH_2Cl_2 to $\text{CH}_2\text{Cl}_2:\text{MeOH}$ 20:1, always 1% conc. aq. NH_4OH added) to yield a yellow oil (1.27 g, 42%); ^1H NMR (500 MHz, CDCl_3): $\delta = 2.40$ –2.46 (m, 2 H), 2.59–2.66 (m, 2 H), 3.07 (t, $J = 4.9$ Hz, 2 H), 3.80 (s, 3 H), 3.83–3.88 (m, 2 H), 4.01 (t, $J = 4.9$ Hz, 2 H), 4.02–4.06 (m, 2 H), 6.43–6.47 (m, 2 H), 7.11–7.17 (m, 3 H), 7.20–7.26 (m, 2 H), 7.40 ppm (d, $J = 8.8$ Hz, 1 H); ^{13}C NMR (125 MHz, CDCl_3): $\delta = 30.2$, 39.9, 41.6, 55.3, 64.6 (2C), 70.5, 100.7, 103.6, 110.0, 122.2, 125.5, 128.2 (3C), 128.3 (2C), 142.5, 157.4, 160.7 ppm; HRMS (ESI): calcd for $\text{C}_{20}\text{H}_{26}\text{NO}_4$ m/z 344.1856 $[\text{M}+\text{H}]^+$, found m/z 344.1860 $[\text{M}+\text{H}]^+$.

2-(2-(2-Methyl-1,3-dioxolan-2-yl)phenoxy)ethan-1-amine (9c): Preparation like **9b**. The crude product was purified by column chromatography (CH_2Cl_2 with 1% Et_3N to CH_2Cl_2 : MeOH =12:1 + 1% Et_3N) to yield a brown oil (744 mg, 39%); ^1H NMR (500 MHz, CDCl_3): δ =1.78 (s, 3H), 3.10 (t, J =5.0 Hz, 2H), 3.77–3.85 (m, 2H), 3.99–4.04 (m, 2H), 4.05 (t, J =5.0 Hz, 2H), 6.90 (d, J =8.2 Hz, 1H), 6.91 (td, J =7.5, 1.2 Hz, 1H), 7.25 (ddd, J =8.0, 7.4, 1.9 Hz, 1H), 7.49 ppm (dd, J =7.6, 1.6 Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =25.6, 41.7, 64.4 (2C), 70.7, 108.5, 113.0, 120.2, 126.7, 129.3, 130.6, 156.4 ppm; HRMS (ESI): calcd for $\text{C}_{12}\text{H}_{18}\text{NO}_3$ m/z 224.1281 $[\text{M}+\text{H}]^+$, found m/z 224.1284 $[\text{M}+\text{H}]^+$.

N-(2-hydroxy-3-(2-(3-phenylpropanoyl)phenoxy)propyl)pyrrolidine-2-carboxamide (5a): *Coupling reaction:* The amine **4a** (1000 mg, 1 eq, 2.91 mmol) and boc-proline (627 mg, 1 eq, 2.91 mmol) were dissolved in anhydrous CH_2Cl_2 (15 mL), and dimethylaminopyridine (18 mg, 0.05 eq, 0.15 mmol) and dicyclohexylcarbodiimide (601 mg, 1 eq, 2.91 mmol) were added and stirred until TLC indicated completion (2 h to o/n). The reaction mixture was filtered, washed with sat. NaHCO_3 solution (20 mL) and water (20 mL), and the aqueous extracts were extracted with CH_2Cl_2 (2 $\times$ 50 mL). The combined organic extracts were washed once with brine (50 mL), dried over Na_2SO_4 , and the solvent was evaporated. The product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2 : MeOH =30:1 to 15:1) to yield tert-butyl 2-((2-hydroxy-3-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propyl)carbamoyl)pyrrolidine-1-carboxylate as a white solid (mixture of diastereomers, 986 mg, 63%); ^1H NMR (500 MHz, CDCl_3): δ =1.41 (s, 9H), 1.86 (brs, 2H), 2.07–2.31 (m, 2H), 2.42 (t, J =8.4 Hz, 2H), 2.55–2.70 (m, 2H), 3.34 (brs, 1H), 3.39–3.51 (m, 2H), 3.55 (brs, 1H), 3.80–3.94 (m, 2H), 4.00–4.12 (m, 2H), 4.16–4.31 (m, 2H), 6.90 (d, J =7.9 Hz, 1H), 6.96 (t, J =7.9 Hz, 1H), 7.11–7.17 (m, 3H), 7.20–7.29 (m, 3H), 7.47 ppm (d, J =7.9 Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =22.1/23.7, 28.2(3C), 30.0, 31.1, 40.0, 41.5, 47.0/47.1, 61.3, 64.7, 64.8, 69.0/69.3, 71.6/71.9, 79.9, 110.4, 114.6, 121.0/121.2, 125.6, 127.0, 128.3 (4C), 129.7, 130.0, 142.0, 156.5, 172.1 ppm; HRMS (ESI): calcd for $\text{C}_{30}\text{H}_{40}\text{N}_2\text{NaO}_7$ m/z 563.2728 $[\text{M}+\text{Na}]^+$, found m/z 563.2733 $[\text{M}+\text{Na}]^+$.

Deprotection: The protected derivative (500 mg, 1 eq, 0.92 mmol) was dissolved in dioxane (3 mL) and aq. HCl (4 mL, 5 mL) was added and stirred at RT for 4 h. Then the reaction mixture was basified with aq. NaOH solution, diluted with water, and extracted with EtOAc (4 $\times$ 50 mL), dried over Na_2SO_4 , and the solvent was evaporated to yield a brown oil (380 mg, 98%); ^1H NMR (500 MHz, CDCl_3): δ =1.63–1.73 (m, 2H), 1.83–1.93 (m, 2H), 2.04–2.19 (m, 1H), 2.74–2.94 (m, 2H), 3.03 (t, J =7.6 Hz, 2H), 3.28 (t, J =7.7 Hz, 2H), 3.30–3.39 (m, 1H), 3.46–3.55 (m, 1H), 3.70–3.78 (m, 1H), 3.94–4.08 (m, 3H), 6.95 (d, J =8.2 Hz, 1H), 7.02 (t, J =7.6 Hz, 1H), 7.18 (t, J =7.3 Hz, 1H), 7.22 (d, J =7.0 Hz, 2H), 7.28 (d, J =8.2 Hz, 2H), 7.43 (t, J =7.9 Hz, 1H), 7.60 (d, J =7.6 Hz, 1H), 8.02 ppm (brs, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =26.1, 30.1, 30.7/30.8, 42.7/42.8, 44.7, 47.2, 60.3, 69.8, 70.5/70.6, 113.3/113.4, 121.1, 126.0, 128.3 (2C), 128.4 (2C), 129.9/130.0, 133.4, 141.3, 157.3, 177.3, 201.6 ppm; HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{NaO}_4$ m/z 419.1941 $[\text{M}+\text{Na}]^+$, found m/z 419.1939 $[\text{M}+\text{Na}]^+$.

Hydrochloride formation: The amine (320 mg, 0.8 mmol) was dissolved in 3 mL methyl *tert*-butyl ether (MTBE) and CH_3CN (1 mL) and treated with 6N HCl (1 mL), stirred for 1 h, and then the solvent was evaporated to yield the hydrochloride as a yellow semi-solid (347 mg, 87%); HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{NaO}_4$ m/z 419.1941 $[\text{M}+\text{Na}]^+$, found m/z 419.1939 $[\text{M}+\text{Na}]^+$.

N-(2-hydroxy-3-(5-methoxy-2-(3-phenylpropanoyl)phenoxy)propyl)pyrrolidine-2-carboxamide (5b): Preparation of the protected derivative like **5a**; purification by column chromatography (petrol ether:EtOAc=1:1 to pure EtOAc to yield tert-butyl 2-((2-hydroxy-3-(5-methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propyl)carbamoyl)pyrrolidine-1-carboxylate as a colorless honey (220 mg, 71%); ^1H NMR (500 MHz, CDCl_3): δ =1.80–1.91 (m, 2H), 1.32–1.47 (m, 9H), 2.04–2.24 (m, 2H), 2.38 (t, J =8.5 Hz, 2H), 2.54–2.66 (m, 2H), 3.21–3.58 (m, 4H), 3.79 (s, 3H), 3.81–3.91 (m, 3H), 3.99–4.10 (m, 3H), 4.11–4.19 (m, 1H), 4.20–4.32 (m, 1H), 6.40–6.52 (m, 2H), 7.08–7.17 (m, 3H), 7.19–7.25 (m, 2H), 7.36 ppm (d, J =8.5 Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =24.6, 28.2/28.3 (3C), 30.1/30.2, 31.2, 40.2, 41.4/41.5, 47.1, 55.4, 61.3, 64.6, 64.7, 68.9, 71.9, 80.1/80.7, 101.7/101.8, 105.3, 110.6, 122.4, 125.6, 127.9, 128.2 (2C), 128.3 (2C), 142.1, 157.4, 160.9, 173.3 ppm; HRMS (ESI): calcd for $\text{C}_{31}\text{H}_{42}\text{N}_2\text{NaO}_8$ m/z 593.2833 $[\text{M}+\text{Na}]^+$, found m/z 593.2838 $[\text{M}+\text{Na}]^+$.

Deprotection like for **5a**; orange honey (134 mg, 99%); ^1H NMR (500 MHz, CDCl_3): δ =1.73 (quin, J =6.9 Hz, 2H), 1.86–1.96 (m, 1H), 2.12–2.22 (m, 1H), 2.87–2.98 (m, 1H), 2.98–3.06 (m, 1H), 3.02 (t, J =7.6 Hz, 2H), 3.25 (t, J =7.7 Hz, 2H), 3.30–3.39 (m, 1H), 3.49–3.61 (m, 1H), 3.81–3.87 (m, 1H), 3.84 (s, 3H), 3.97–4.04 (m, 2H), 4.04–4.11 (m, 1H), 6.46 (d, J =1.9 Hz, 1H), 6.53 (d, J =8.8 Hz, 1H), 7.19 (t, J =7.3 Hz, 1H), 7.23 (d, J =7.3 Hz, 2H), 7.26–7.34 (m, 2H), 7.74 (d, J =8.8 Hz, 1H), 8.05 ppm (vrs, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =26.1/26.2, 30.4, 30.8, 42.9, 44.5, 47.2/47.3, 55.6, 60.3, 69.8, 70.6/70.7, 99.9/100.0, 105.9/106.0, 121.0, 126.0, 128.3/128.4 (2C), 128.4/128.5 (2C), 132.5, 141.6, 159.8, 164.3, 177.4, 199.2 ppm; HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{NaO}_5$ m/z 449.2047 $[\text{M}+\text{Na}]^+$, found m/z 449.2050 $[\text{M}+\text{Na}]^+$.

Hydrochloride formation like for **5a** yielded a brown honey (108 mg, 98%); HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{NaO}_5$ m/z 449.2047 $[\text{M}+\text{Na}]^+$, found m/z 449.2047 $[\text{M}+\text{Na}]^+$.

N-(3-(2-acetylphenoxy)-2-hydroxypropyl)pyrrolidine-2-carboxamide (5c): Preparation of the protected derivative like for **5a**. The product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2 : MeOH =30:1) to yield tert-butyl 2-((2-hydroxy-3-(2-(2-methyl-1,3-dioxolan-2-yl)phenoxy)propyl)carbamoyl)pyrrolidine-1-carboxylate as a colorless semisolid (109 mg, 41%); ^1H NMR (500 MHz, CDCl_3): δ =1.38 (brs, 6H), 1.42 (brs, 3H), 1.76 (s, 3H), 1.79–1.90 (m, 2H), 1.95–2.27 (m, 2H), 3.22–3.41 (m, 1H), 3.41–3.51 (m, 2H), 3.52–3.73 (m, 1H), 3.76–3.95 (m, 3H), 4.00–4.12 (m, 3H), 4.15–4.32 (m, 2H), 6.84 (brs, 0.5H), 6.90 (d, J =7.9 Hz, 1H), 6.94 (t, J =7.7 Hz, 1H), 7.14 (brs, 0.5H), 7.26 (t, J =7.7 Hz, 1H), 7.45 ppm (d, J =7.6 Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =23.7/24.5, 25.8, 28.2/28.3 (3C), 31.1, 41.5/41.6, 47.0/47.2, 60.3/61.2, 64.4/64.5, 69.0/69.4, 71.5/71.8, 108.9, 114.5, 121.1, 126.2, 126.3, 129.6, 130.9, 156.4, 175.7 ppm; HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{34}\text{N}_2\text{NaO}_7$ m/z 473.2258 $[\text{M}+\text{Na}]^+$, found m/z 473.2255 $[\text{M}+\text{Na}]^+$.

Deprotection like for **5a** yielded a yellowish semi-solid (50 mg, 96%); ^1H NMR (500 MHz, CDCl_3): δ =1.62–1.78 (m, 2H), 1.81–1.94 (m, 1H), 2.08–2.21 (m, 1H), 2.61 (s, 3H), 2.81–2.95 (m, 1H), 2.95–3.06 (m, 1H), 3.38–3.49 (m, 1H), 3.56–3.66 (m, 1H), 3.72–3.87 (m, 1H), 3.97–4.09 (m, 2H), 4.09–4.19 (m, 1H), 6.96 (d, J =8.5 Hz, 1H), 7.01 (t, J =7.6 Hz, 1H), 7.44 (t, J =7.7 Hz, 1H), 7.68 (d, J =7.6 Hz, 1H), 8.13 ppm (brs, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ =26.1, 30.8, 31.2, 42.8, 47.2, 60.3, 69.8, 70.6/70.7, 113.3, 121.0, 128.3, 130.4, 133.7, 157.7, 177.3, 199.9 ppm; HRMS (ESI): calcd for $\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_4$ m/z 307.1652 $[\text{M}+\text{H}]^+$, found m/z 307.1657 $[\text{M}+\text{H}]^+$.

Hydrochloride formation like for **5a** yielded a brown honey (60 mg, 94%); HRMS (ESI): calcd for $C_{16}H_{23}N_2O_4$ m/z 307.1652 $[M+H]^+$, found m/z 307.1657 $[M+H]^+$.

N-(2-(2-(3-phenylpropanoyl)phenoxy)ethyl)pyrrolidine-2-carboxamide (10a): Preparation like **5a**; purification by column chromatography (petrol ether:EtOAc=2:1 to 1:2) led to tert-butyl 2-((2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)ethyl)carbamoylpyrrolidine-1-carboxylate as a yellowish honey (85 mg, 62%); ^1H NMR (500 MHz, $CDCl_3$): δ =1.33 (brs, 6H), 1.42 (brs, 3H), 1.73–1.92 (m, 2H), 1.95–2.02 (m, 1H), 2.11–2.22 (m, 1H), 2.38–2.52 (m, 2H), 2.55–2.67 (m, 2H), 3.42–3.55 (m, 2H), 3.61–3.72 (m, 2H), 3.81–3.95 (m, 2H), 4.02–4.26 (m, 5H), 6.90 (d, J =8.2 Hz, 1H), 6.97 (t, J =7.4 Hz, 1H), 7.08–7.17 (m, 3H), 7.19–7.25 (m, 2H), 7.28 (t, J =7.6 Hz, 1H), 7.50 ppm (d, J =7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =23.8, 28.3 (3C), 30.2, 31.6, 39.0, 39.7, 46.8, 61.2, 64.7, 64.8, 67.9, 80.0, 110.3, 113.7, 120.9, 125.6, 127.6, 128.2 (4C), 129.6, 129.8, 142.1, 156.0, 173.3 ppm; HRMS (ESI): calcd for $C_{29}H_{39}N_2O_6$ m/z 511.2803 $[M+H]^+$, found m/z 511.2807 $[M+H]^+$.

Deprotection like for **5a**; yellow honey (48 mg, quant.); ^1H NMR (500 MHz, $CDCl_3$): δ =1.62 (quin, J =6.9 Hz, 2H), 1.78–1.86 (m, 1H), 2.05–2.14 (m, 1H), 2.69–2.78 (m, 1H), 2.84–2.91 (m, 1H), 3.05 (t, J =7.3 Hz, 2H), 3.27–3.40 (m, 2H), 3.56–3.74 (m, 3H), 4.08–4.19 (m, 2H), 6.93 (d, J =8.5 Hz, 1H), 7.02 (t, J =7.6 Hz, 1H), 7.19 (t, J =7.3 Hz, 1H), 7.23 (d, J =7.0 Hz, 1H), 7.26–7.32 (m, 2H), 7.44 (ddd, J =8.7, 7.1, 1.6 Hz, 1H), 7.66 (dd, J =7.9, 1.6 Hz, 1H), 7.98 ppm (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =26.2, 30.3, 30.6, 38.4, 45.3, 47.2, 60.4, 67.3, 112.4, 121.1, 126.0, 128.4 (4C), 130.3, 132.3, 133.4, 141.7, 157.5, 174.2, 201.8 ppm; HRMS (ESI): calcd for $C_{22}H_{27}N_2O_3$ m/z 367.2016 $[M+H]^+$, found m/z 367.2019 $[M+H]^+$.

Hydrochloride formation like for **5a** yielded a brown semisolid (47 mg, 98%); HRMS (ESI): calcd for $C_{22}H_{27}N_2O_3$ m/z 367.2016 $[M+H]^+$, found m/z 37.2020 $[M+H]^+$.

N-(2-(4-methoxy-2-(3-phenylpropanoyl)phenoxy)ethyl)pyrrolidine-2-carboxamide (10b): Preparation like **5a**; purification by column chromatography (petrol ether:EtOAc=1:3 to 1:5) led to tert-butyl 2-((2-(5-methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)ethyl)carbamoyl)pyrrolidine-1-carboxylate as a colorless oil (989 mg, 78%); ^1H NMR (500 MHz, $CDCl_3$): δ =1.33 (brs, 6H), 1.42 (brs, 3H), 1.75–1.84 (m, 1H), 1.84–1.92 (m, 1H), 1.92–2.03 (m, 1H), 2.11–2.22 (m, 1H), 2.34–2.47 (m, 2H), 2.52–2.66 (m, 2H), 3.41–3.55 (m, 2H), 3.62–3.70 (m, 2H), 3.80 (s, 3H), 3.82–3.94 (m, 2H), 3.99–4.23 (m, 5H), 6.46 (s, 1H), 6.48 (d, J =8.8 Hz, 1H), 7.09–7.17 (m, 3H), 7.20–7.25 (m, 2H), 7.40 ppm (d, J =8.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =23.8, 28.3 (3C), 30.3, 31.6, 38.8, 39.9, 46.8, 55.4, 61.2, 64.6, 64.7, 67.9, 80.1, 101.2, 104.6, 110.2, 122.3, 125.6, 128.2 (4C), 128.4, 142.3, 156.7, 160.8, 171.3 ppm; HRMS (ESI): calcd for $C_{30}H_{41}N_2O_7$ m/z 541.2908 $[M+H]^+$, found m/z 541.2911 $[M+H]^+$.

Deprotection like for **5a** yielded a yellow semisolid (356 mg, 98%); ^1H NMR (500 MHz, $CDCl_3$): δ =1.56–1.64 (m, 2H), 1.76–1.86 (m, 1H), 2.01–2.12 (m, 1H), 2.67–2.75 (m, 1H), 2.81–2.89 (m, 1H), 3.03 (t, J =7.7 Hz, 2H), 3.21–3.38 (m, 2H), 3.53–3.59 (m, 1H), 3.59–3.68 (m, 2H), 3.84 (s, 3H), 4.05–4.18 (m, 2H), 6.42 (s, 1H), 6.54 (d, J =8.8 Hz, 1H), 7.18 (t, J =7.1 Hz, 1H), 7.23 (d, J =7.3 Hz, 2H), 7.28 (d, J =7.6 Hz, 2H), 7.81 (d, J =8.5 Hz, 1H), 7.99 ppm (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =26.1, 30.4, 30.6, 38.3, 45.2, 47.2, 55.5, 60.4, 67.3, 98.9, 105.7, 121.0, 125.9, 128.3 (2C), 128.4 (2C), 132.7, 141.9, 159.6, 164.3, 175.6, 199.1 ppm; HRMS (ESI): calcd for $C_{23}H_{29}N_2O_4$ m/z 397.2122 $[M+H]^+$, found m/z 397.2126 $[M+H]^+$.

Hydrochloride formation like for **5a** yielded a brown honey (286 mg, 99%); HRMS (ESI): calcd for $C_{23}H_{29}N_2O_4$ m/z 397.2122 $[M+H]^+$, found m/z 397.2124 $[M+H]^+$.

N-(2-(2-acetylphenoxy)ethyl)pyrrolidine-2-carboxamide (10c): Preparation like **5a**, purification by column chromatography (CH_2Cl_2 :MeOH=40:1 to 25:1) yielded tert-butyl 2-((2-(2-methyl-1,3-dioxolan-2-yl)phenoxy)ethyl)carbamoylpyrrolidine-1-carboxylate as a colorless oil (215 mg, 57%); ^1H NMR (500 MHz, $CDCl_3$): δ =1.36 (brs, 6H), 1.43 (brs, 3H), 1.77 (s, 3H), 1.81–1.96 (m, 2H), 1.97–2.07 (m, 1H), 2.16–2.25 (m, 1H), 3.44–3.57 (m, 2H), 3.64–3.74 (m, 2H), 3.77–3.89 (m, 2H), 4.03–4.18 (m, 4.5H), 4.22–4.29 (m, 0.5H), 6.91 (d, J =8.2 Hz, 1H), 6.95 (t, J =7.1 Hz, 1H), 7.27 (t, J =7.1 Hz, 1H), 7.48 ppm (d, J =7.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =23.8, 25.6, 28.3(3C), 31.5, 39.0, 46.9, 61.3, 64.4 (2C), 68.1, 80.2, 108.7, 114.1, 121.1, 126.8, 129.5, 131.1, 155.9, 165.7 ppm; HRMS (ESI): calcd for $C_{22}H_{33}N_2O_6$ m/z 421.2333 $[M+H]^+$, found m/z 421.2338 $[M+H]^+$.

Deprotection like **5a** yielded a yellowish solid (75 mg, 97%); ^1H NMR (500 MHz, $CDCl_3$): δ =1.66–1.77 (m, 2H), 1.85–1.97 (m, 1H), 2.11–2.23 (m, 1H), 2.64 (s, 3H), 2.84–2.94 (m, 1H), 2.98–3.07 (m, 1H), 3.62–3.77 (m, 2H), 3.78–3.85 (m, 1H), 4.16 (t, J =4.7 Hz, 2H), 6.94 (d, J =8.2 Hz, 1H), 7.02 (t, J =7.6 Hz, 1H), 7.45 (t, J =7.9 Hz, 1H), 7.73 (d, J =7.9 Hz, 1H), 8.13 ppm (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =26.1, 30.6, 31.8, 38.5, 47.1, 60.4, 67.3, 112.6, 121.0, 128.5, 130.5, 133.7, 157.8, 170.7, 199.7 ppm; HRMS (ESI): calcd for $C_{15}H_{21}N_2NaO_3$ m/z 299.1366 $[M+Na]^+$, found m/z 299.1368 $[M+Na]^+$.

Hydrochloride formation like for **5a** yielded a brown semisolid (86 mg, 98%); HRMS (ESI): calcd for $C_{15}H_{21}N_2NaO_3$ m/z 299.1366 $[M+Na]^+$, found m/z 299.1368 $[M+Na]^+$.

2-(2-Hydroxy-3-(2-(3-phenylpropanoyl)phenoxy)propyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (6a): The amine **4a** (371 mg, 1 eq, 1.08 mmol), methyl (2-chloroacetyl)prolinate (223 mg, 1 eq, 1.08 mmol), and Et_3N (0.16 mL, 1.2 eq, 1.13 mmol) were dissolved in CH_3CN (40 mL) and stirred at RT for 2.5 d. The solvent was evaporated, and the residue was redissolved in EtOAc and extracted with water. The aqueous phase was extracted with EtOAc (3 $\times$ 50 mL), the combined organic phases were dried over Na_2SO_4 , and the solvent was evaporated. The product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2 :MeOH=20:1) twice to yield a yellow semisolid (30 mg, 6%).

2-(2-Hydroxy-3-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione: **Diastereomer 1:** ^1H NMR (500 MHz, $CDCl_3$): δ =1.85–1.96 (m, 1H), 2.00–2.14 (m, 2H), 2.35–2.43 (m, 1H), 2.41 (t, J =8.7 Hz, 2H), 2.60–2.68 (m, 2H), 3.26 (dd, J =14.1, 7.4 Hz, 1H), 3.51–3.59 (m, 1H), 3.59–3.67 (m, 1H), 3.82–3.92 (m, 4H), 4.05–4.13 (m, 3H), 4.04 (d, J =17.0 Hz, 1H), 4.16–4.19 (m, 1H), 4.23 (dd, J =9.5, 3.2 Hz, 1H), 4.42 (d, J =16.7 Hz, 1H), 6.90 (dd, J =8.2, 2.8 Hz, 1H), 6.98 (t, J =7.6 Hz, 1H), 7.10–7.17 (m, 3H), 7.20–7.26 (m, 2H), 7.28 (t, J =7.7 Hz, 1H), 7.48 ppm (d, J =7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =22.7, 28.9, 30.0, 40.1, 45.2, 49.7, 54.5, 59.1, 64.8 (2C), 69.7, 72.1, 110.4, 114.7, 121.3, 125.6, 127.1, 128.3 (4C), 129.8, 130.0, 142.0, 156.5, 163.6, 168.0 ppm; **Diastereomer 2:** ^1H NMR (500 MHz, $CDCl_3$): δ =1.85–1.96 (m, 1H), 2.00–2.14 (m, 2H), 2.35–2.43 (m, 1H), 2.41 (t, J =8.7 Hz, 2H), 2.60–2.68 (m, 2H), 3.51–3.59 (m, 1H), 3.59–3.67 (m, 3H), 3.82–3.92 (m, 3H), 4.05–4.13 (m, 3H), 4.14 (d, J =17.0 Hz, 1H), 4.18–4.22 (m, 2H), 4.29 (d, J =16.4 Hz, 1H), 6.90 (dd, J =8.2, 2.8 Hz, 1H), 6.98 (t, J =7.6 Hz, 1H), 7.10–7.17 (m, 3H), 7.20–7.26 (m, 2H), 7.28 (t, J =7.7 Hz, 1H), 7.48 ppm (d, J =7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =22.6, 28.9, 30.0, 40.0, 45.2, 48.9, 54.0, 58.9, 64.8 (2C), 69.5, 72.0, 110.3,

114.7, 121.2, 125.6, 127.1, 128.3 (4C), 129.7, 130.0, 142.0, 156.5, 163.4, 168.0 ppm; HRMS (ESI): calcd for $C_{27}H_{32}N_2NaO_6$ m/z 503.2153 $[M+Na]^+$, found m/z 503.2151 $[M+Na]^+$.

The ketal (24 mg, 1 eq, 0.05 mmol) was dissolved in CH_3CN (2 mL), and aq. HCl solution (2 N, 2 mL) was added, and it was stirred for 4 h at RT. Then it was diluted with water and extracted with EtOAc (4 × 50 mL), dried over Na_2SO_4 , and evaporated to yield a colorless solid (21 mg, 96%). **Diastereomer 1**: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.86–1.97 (m, 1H), 2.00–2.12 (m, 2H), 2.35–2.43 (m, 1H), 3.04 (t, J = 7.9 Hz, 2H), 3.23–3.29 (m, 2H), 3.31 (dd, J = 14.5, 7.9 Hz, 1H), 3.54–3.65 (m, 2H), 3.79 (dd, J = 14.1, 3.0 Hz, 1H), 3.93–4.01 (m, 1H), 3.96 (d, J = 16.7 Hz, 1H), 4.07–4.15 (m, 2H), 4.17–4.22 (m, 1H), 4.33 (d, J = 17.0 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 7.16–7.22 (m, 1H), 7.23 (d, J = 7.3 Hz, 2H), 7.28 (td, J = 7.5, 1.6 Hz, 2H), 7.44 (t, J = 7.9 Hz, 1H), 7.60 ppm (ddd, J = 7.7, 4.6, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 28.8, 30.1, 44.4, 45.2, 50.2, 54.4, 59.0, 69.5, 71.5, 114.3, 121.4, 126.1, 128.4 (2C), 128.5, 128.5 (2C), 130.0, 133.5, 141.3, 157.3, 163.4, 168.7, 201.5 ppm; **Diastereomer 2**: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.86–1.97 (m, 1H), 2.00–2.12 (m, 2H), 2.35–2.43 (m, 1H), 3.04 (t, J = 7.9 Hz, 2H), 3.23–3.29 (m, 2H), 3.31 (dd, J = 14.4, 3.3 Hz, 1H), 3.54–3.65 (m, 2H), 3.68 (dd, J = 14.2, 6.6 Hz, 1H), 3.93–4.01 (m, 1H), 4.01 (d, J = 16.7 Hz, 1H), 4.07–4.15 (m, 2H), 4.16–4.20 (m, 1H), 4.23 (d, J = 16.7 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 7.16–7.22 (m, 1H), 7.23 (d, J = 7.3 Hz, 2H), 7.28 (td, J = 7.5, 1.6 Hz, 2H), 7.44 (t, J = 7.9 Hz, 1H), 7.60 ppm (ddd, J = 7.7, 4.6, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 28.8, 30.1, 44.3, 45.2, 49.6, 53.9, 58.9, 69.3, 71.4, 114.2, 121.4, 126.1, 127.0, 128.4 (2C), 128.5, 128.5 (2C), 133.5, 141.2, 157.3, 163.3, 168.5, 201.4 ppm; HRMS (ESI): calcd for $C_{25}H_{28}N_2NaO_5$ m/z 459.1890 $[M+Na]^+$, found m/z 459.1893 $[M+Na]^+$.

2-(2-Hydroxy-3-(5-methoxy-2-(3-phenylpropanoyl)phenoxy)propyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (6b): The amine **4b** (300 mg, 1 eq, 0.8 mmol), methyl (2-chloroacetyl)prolinate (165 mg, 1 eq, 0.8 mmol), and Et_3N (0.14 mL, 1.3 eq, 1.0 mmol) were dissolved in 2-ethoxyethanol (6 mL) and heated at reflux for 3 d. The solvent was evaporated and the residue was redissolved in a mixture of CH_2Cl_2 and toluene and extracted with water. The aqueous phase was extracted with CH_2Cl_2 (3 × 60 mL), the combined organic phases were washed once with brine (70 mL), dried over Na_2SO_4 and the solvent was evaporated. The product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2 :MeOH = 30:1) to yield 251 mg of a yellow solid that was already partially deprotected.

2-(2-Hydroxy-3-(5-methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (ESI): calcd for $C_{28}H_{34}N_2NaO_7$ m/z 533.2258 $[M+Na]^+$, found m/z 533.2252 $[M+Na]^+$.

The mixture from the previous step (120 mg, 1 eq, 0.07 mmol) was dissolved in CH_3CN (9 mL); aq. HCl solution (2 N, 9 mL) was added and it was stirred for 4 h at RT. Then it was diluted with water (5 mL) and aq. $NaHCO_3$ (10 mL), extracted with EtOAc (4 × 30 mL), dried over Na_2SO_4 , and evaporated to yield a yellowish solid (109 mg, 61%, 2 steps). **Diastereomer 1**: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.86–1.97 (m, 1H), 2.00–2.12 (m, 2H), 2.34–2.43 (m, 1H), 3.02 (t, J = 7.6 Hz, 1H), 3.19–3.25 (m, 2H), 3.30 (dd, J = 14.2, 7.6 Hz, 1H), 3.54–3.65 (m, 2H), 3.81 (dd, J = 14.3, 3.0 Hz, 1H), 3.84 (s, 3H), 3.91–4.03 (m, 1H), 3.93 (d, J = 17.1 Hz, 1H), 4.07–4.15 (m, 2H), 4.21–4.27 (m, 1H), 4.32 (d, J = 17.0 Hz, 1H), 6.45 (d, J = 2.2 Hz, 1H), 6.53 (dd, J = 8.7, 2.0 Hz, 1H), 7.18 (dd, J = 8.1, 5.9 Hz, 1H), 7.23 (d, J = 7.3 Hz, 2H), 7.28 (td, J = 7.6, 1.7 Hz, 2H), 7.57 ppm (t, J = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.7, 28.8, 30.4, 44.1, 45.2, 50.3, 54.4,

55.6, 59.0, 69.4, 71.6, 101.0, 106.1, 126.0, 128.4 (2C), 128.5 (2C), 132.5, 141.5, 159.8, 163.3, 164.2, 168.7, 199.2 ppm; **Diastereomer 2**: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.86–1.97 (m, 1H), 2.00–2.12 (m, 2H), 2.34–2.43 (m, 1H), 3.02 (t, J = 7.6 Hz, 1H), 3.19–3.25 (m, 2H), 3.52 (dd, J = 14.2, 7.6 Hz, 1H), 3.54–3.65 (m, 2H), 3.68 (dd, J = 14.3, 3.0 Hz, 1H), 3.84 (s, 3H), 3.91–4.03 (m, 1H), 3.98 (d, J = 17.1 Hz, 1H), 4.07–4.15 (m, 2H), 4.15–4.21 (m, 1H), 4.21 (d, J = 17.0 Hz, 1H), 6.45 (d, J = 2.2 Hz, 1H), 6.53 (dd, J = 8.7, 2.0 Hz, 1H), 7.18 (dd, J = 8.1, 5.9 Hz, 1H), 7.23 (d, J = 7.3 Hz, 2H), 7.28 (td, J = 7.6, 1.7 Hz, 2H), 7.57 ppm (t, J = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 28.8, 30.4, 44.0, 45.2, 49.7, 53.9, 55.6, 58.9, 69.2, 71.5, 100.9, 106.0, 126.0, 128.4 (2C), 128.5 (2C), 132.4, 141.5, 159.8, 163.2, 164.2, 168.5, 199.1 ppm; HRMS (ESI): calcd for $C_{26}H_{30}N_2NaO_6$ m/z 489.1996 $[M+Na]^+$, found m/z 489.2004 $[M+Na]^+$.

2-(3-(2-Acetylphenoxy)-2-hydroxypropyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (6c): Preparation like **6b**. The product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2 :MeOH = 30:1) to yield a colorless oil (81 mg, yield given over two steps below) that was already partially deprotected: 2-(2-hydroxy-3-(2-(2-methyl-1,3-dioxolan-2-yl)phenoxy)propyl) hexahydropyrrolo[1,2-a]pyrazine-1,4-dione; HRMS (ESI): calcd for $C_{20}H_{26}N_2NaO_6$ m/z 413.1683 $[M+Na]^+$, found m/z 413.1679 $[M+Na]^+$.

Deprotection like **6b** yielded a yellowish solid (23 mg, 35%, 2 steps). **Diastereomer 1**: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.87–1.97 (m, 1H), 2.00–2.14 (m, 2H), 2.36–2.44 (m, 1H), 2.62 (s, 3H), 3.57 (dd, J = 13.9, 3.5 Hz, 1H), 3.53–3.59 (m, 1H), 3.59–3.66 (m, 1H), 3.80 (dd, J = 14.3, 6.8 Hz, 1H), 3.97–4.04 (m, 1H), 4.07 (d, J = 16.7 Hz, 1H), 4.10–4.19 (m, 2H), 4.22–4.31 (m, 1H), 4.34 (d, J = 16.1 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.70 ppm (ddd, J = 7.7, 2.0, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.7, 28.8, 30.8, 45.2, 49.6, 54.0, 59.0, 69.5, 71.4, 114.2, 121.4, 130.6, 133.8, 128.5, 157.4, 163.2, 168.6, 198.2 ppm; **Diastereomer 2**: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.87–1.97 (m, 1H), 2.00–2.14 (m, 2H), 2.36–2.44 (m, 1H), 2.61 (s, 3H), 3.42 (dd, J = 14.2, 7.9 Hz, 1H), 3.53–3.59 (m, 1H), 3.59–3.66 (m, 1H), 3.85 (dd, J = 14.2, 3.2 Hz, 1H), 3.97–4.04 (m, 1H), 4.03 (d, J = 17.0 Hz, 1H), 4.10–4.19 (m, 2H), 4.22–4.31 (m, 1H), 4.42 (d, J = 16.4 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.70 ppm (ddd, J = 7.7, 2.0, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.7, 28.8, 30.7, 45.2, 50.4, 54.5, 58.9, 69.3, 71.3, 114.1, 121.4, 130.6, 133.8, 128.5, 157.4, 163.2, 168.6, 198.2 ppm; HRMS (ESI): calcd for $C_{18}H_{22}N_2NaO_5$ m/z 369.1421 $[M+Na]^+$, found m/z 369.1427 $[M+Na]^+$.

2-(2-(2-(3-Phenylpropanoyl)phenoxy)ethyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (11a): Preparation like **6b** yielded a mixture of partially deprotected 2-(2-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)ethyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (114 mg, partially deprotected, therefore yield given for two steps below); HRMS (ESI): calcd for $C_{26}H_{30}N_2NaO_5$ m/z 473.2047 $[M+Na]^+$, found m/z 473.2050 $[M+Na]^+$.

Deprotection like **6b** yielded a yellowish semisolid (80 mg, 39%, 2 steps); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.82–1.94 (m, 1H), 1.97–2.08 (m, 2H), 2.29–2.37 (m, 1H), 3.02 (t, J = 7.7 Hz, 2H), 3.23–3.29 (m, 2H), 3.49–3.61 (m, 3H), 3.86 (d, J = 16.4 Hz, 1H), 3.94 (t, J = 7.7 Hz, 1H), 4.02 (ddd, J = 14.3, 5.8, 4.3 Hz, 1H), 4.15 (ddd, J = 10.1, 5.7, 4.4 Hz, 1H), 4.23 (d, J = 16.4 Hz, 1H), 4.23–4.29 (m, 1H), 6.92 (d, J = 8.2 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 7.18 (t, J = 7.1 Hz, 1H), 7.23 (d, J = 7.0 Hz, 2H), 7.26–7.30 (m, 2H), 7.42 (ddd, J = 8.4, 7.4, 1.7 Hz, 1H), 7.57 ppm (dd, J = 7.7, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 28.6, 30.2, 45.0, 45.2, 46.1, 53.4, 58.9, 65.8, 112.5, 121.3, 126.0, 128.4 (4C), 129.0, 130.0, 133.1, 141.4, 156.6, 163.0, 167.8,

201.5 ppm; HRMS (ESI): calcd for $C_{24}H_{28}N_2NaO_4$ m/z 429.1785 $[M+Na]^+$, found m/z 429.1788 $[M+Na]^+$.

2-(2-(5-Methoxy-2-(3-phenylpropanoyl)phenoxy)ethyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (11b): Preparation like **6b** yielded a brown solid—mixture of partially deprotected tert-butyl 2-((2-(5-methoxy-2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)-ethyl)-carbamoyl)pyrrolidine-1-carboxylate (96 mg, yield given for two steps below); HRMS (ESI): calcd for $C_{27}H_{32}N_2NaO_6$ m/z 503.2153 $[M+Na]^+$, found m/z 503.2155 $[M+Na]^+$.

Deprotection like **6b** yielded a yellowish semisolid (66 mg, 25%, 2 steps); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.82–1.94 (m, 1H), 1.96–2.09 (m, 2H), 2.29–2.38 (m, 1H), 3.01 (t, J = 8.1 Hz, 2H), 3.19–3.27 (m, 2H), 3.47–3.61 (m, 3H), 3.83 (s, 3H), 3.86 (d, J = 16.4 Hz, 1H), 3.92 (t, J = 7.9 Hz, 1H), 3.96–4.03 (m, 1H), 4.08–4.17 (m, 1H), 4.23 (d, J = 17.0 Hz, 1H), 4.22–4.28 (m, 1H), 6.43 (d, J = 2.3 Hz, 1H), 6.53 (dd, J = 8.9, 2.2 Hz, 1H), 7.17 (t, J = 7.1 Hz, 1H), 7.23 (d, J = 7.3 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.72 ppm (d, J = 8.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 28.6, 30.4, 44.9, 45.2, 46.1, 53.4, 55.6, 58.9, 65.8, 99.3, 105.8, 121.4, 125.9, 128.4 (2C), 128.5 (2C), 132.6, 159.0, 162.9, 164.1, 167.8, 198.9 ppm; HRMS (ESI): calcd for $C_{25}H_{28}N_2NaO_5$ m/z 459.1890 $[M+Na]^+$, found m/z 459.1894 $[M+Na]^+$.

2-(2-(2-Acetylphenoxy)ethyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (11c): The amine **9c** (200 mg, 1 eq, 0.9 mmol), methyl (2-chloroacetyl)prolinate (184 mg, 1 eq, 0.9 mmol), and Et_3N (0.16 mL, 1.3 eq, 1.2 mmol) were dissolved in 2-ethoxyethanol (6 mL) and heated at reflux for 24 h. The solvent was evaporated, and the residue was redissolved in a mixture of CH_2Cl_2 and toluene (2:1, 100 mL) and extracted once with water (50 mL). The aqueous phase was extracted CH_2Cl_2 (3×50 mL). The combined organic phases were washed once with brine (70 mL), dried over Na_2SO_4 , and the solvent was evaporated. The product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2 :MeOH = 20:1) twice to yield a yellow semisolid (65 mg, 20%).

2-(2-(2-(2-methyl-1,3-dioxolan-2-yl)phenoxy)ethyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione: ^1H NMR (500 MHz, $CDCl_3$): δ = 1.73 (s, 3H), 1.85–1.97 (m, 1H), 1.99–2.12 (m, 2H), 2.36–2.44 (m, 1H), 3.51–3.58 (m, 1H), 3.59–3.67 (m, 2H), 3.77–3.85 (m, 2H), 4.00–4.04 (m, 2H), 4.04–4.08 (m, 1H), 4.09–4.13 (m, 1H), 4.13–4.17 (m, 1H), 4.20 (d, J = 17.0 Hz, 1H), 4.22–4.28 (m, 1H), 4.55 (dd, J = 17.0, 1.9 Hz, 1H), 6.87 (dd, J = 8.2, 0.6 Hz, 1H), 6.94 (td, J = 7.5, 1.1 Hz, 1H), 7.26 (ddd, J = 8.1, 7.4, 1.8 Hz, 1H), 7.51 ppm (dd, J = 7.6, 1.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 25.6, 28.8, 45.2, 46.6, 53.8, 59.0, 64.5 (2C), 66.4, 108.3, 112.8, 120.8, 126.8, 129.4, 130.8, 156.7, 163.7, 167.6 ppm; HRMS (ESI): calcd for $C_{19}H_{24}N_2NaO_5$ m/z 383.1577 $[M+Na]^+$, found m/z 383.1583 $[M+Na]^+$.

The ketal (35 mg, 1 eq, 0.09 mmol) was dissolved in CH_3CN (2 mL); aq. HCl solution (2N, 2 mL) was added, and it was stirred for 4 h at RT. Then it was diluted with water and extracted with EtOAc (4×40 mL), dried over Na_2SO_4 , and evaporated to yield a colorless solid (30 mg, 97%); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.85–1.97 (m, 1H), 1.99–2.12 (m, 2H), 2.35–2.43 (m, 1H), 2.59 (s, 3H), 3.51–3.64 (m, 2H), 3.68–3.76 (m, 1H), 3.99 (d, J = 16.7 Hz, 1H), 3.98–4.05 (m, 1H), 4.10 (t, J = 7.6 Hz, 1H), 4.16–4.23 (m, 1H), 4.25–4.30 (m, 1H), 4.33 (d, J = 16.7 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 7.44 (ddd, J = 7.7, 7.4, 1.8 Hz, 1H), 7.67 ppm (dd, J = 7.7, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.6, 28.7, 31.4, 45.2, 46.1, 53.3, 59.0, 65.8, 112.5, 121.2, 130.3, 133.4, 128.8, 157.0, 163.1, 167.8, 199.6 ppm; HRMS (ESI): calcd for $C_{17}H_{20}N_2NaO_4$ m/z 339.1315 $[M+Na]^+$, found m/z 339.1321 $[M+Na]^+$.

2-(2-(2-Bromoethoxy)phenyl)-2-phenethyl-1,3-dioxolane (13a): Preparation of the ketal from 1-(2-(2-bromoethoxy)phenyl)-3-phenylpropan-1-one like for **1a** yielded a yellow oil (2.79 g, 99%); ^1H NMR (500 MHz, $CDCl_3$): δ = 2.48–2.55 (m, 2H), 2.61–2.68 (m, 2H), 3.68 (t, J = 6.3 Hz, 2H), 3.83–3.92 (m, 2H), 4.02–4.11 (m, 2H), 4.33 (t, J = 6.5 Hz, 2H), 6.89 (d, J = 8.2 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.16 (d, J = 7.6 Hz, 2H), 7.23 (t, J = 7.4 Hz, 2H), 7.27 (t, J = 7.3 Hz, 1H), 7.54 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 29.1, 30.1, 39.6, 64.8 (2C), 68.8, 109.9, 113.6, 121.0, 125.5, 127.7, 128.2 (2C), 128.4 (2C), 129.5, 130.3, 142.4, 155.7 ppm; HRMS (ESI): calcd for $C_{19}H_{22}BrO_3$ m/z 377.0747 $[M+H]^+$, found m/z 377.0747 $[M+H]^+$.

2,2,2-Trifluoro-N-(2-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)-ethyl)-acetamide (14a): The bromide **13a** (1 g, 1 eq, 2.65 mmol), trifluoroacetamide (599 mg, 2 eq, 5.30 mmol), and tetrabutylammonium bromide (85 mg, 0.1 eq, 0.27 mmol) were dissolved in dimethylformamide (DMF, 5 mL), and K_2CO_3 (733 mg, 2 eq, 5.3 mmol) was added. The mixture was heated at 80 °C for 4.5 h, then TLC indicated completion, and it was filtrated and evaporated. The crude product was purified by column chromatography (petrol ether:EtOAc = 30:1 to 4:1) to yield a colorless oil (708 mg, 65%); ^1H NMR (500 MHz, $CDCl_3$): δ = 2.42–2.50 (m, 2H), 2.62–2.69 (m, 2H), 3.76 (dd, J = 9.8, 5.0 Hz, 2H), 3.83–3.94 (m, 2H), 4.07–4.13 (m, 2H), 4.16 (t, J = 5.0 Hz, 2H), 6.93 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 7.12–7.19 (m, 3H), 7.25 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.7 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 8.31 ppm (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 29.9, 39.6, 39.9, 64.8 (2C), 67.1, 110.3, 114.3, 116.0 (q, J = 287.7 Hz), 121.6, 125.6, 127.1, 128.2 (2C), 128.3 (2C), 129.7, 130.4, 142.0, 155.7, 157.4 ppm (q, J = 36.9 Hz); HRMS (ESI): calcd for $C_{21}H_{22}F_3NNaO_4$ m/z 432.1393 $[M+Na]^+$, found m/z 432.1397 $[M+Na]^+$.

2-(2-(2-Phenethyl-1,3-dioxolan-2-yl)phenoxy)ethan-1-amine (9a), Method B: The trifluoroacetamide derivative **14a** (400 mg, 1 eq, 0.98 mmol) was dissolved in MeOH (2 mL) and water (2 mL), and KOH (110 mg, 2 eq, 1.95 mmol) was added and stirred at RT for 2 h. Then the reaction mixture was concentrated and extracted with EtOAc (3×50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to yield **9a** as a colorless oil (305 mg, 99%).

1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)phenyl)-3-phenylpropan-1-one (16a): 1-(2-(Oxiran-2-ylmethoxy)phenyl)-3-phenylpropan-1-one (514 mg, 1.8 mmol) was dissolved in $iPrNH_2$ (10 mL) and heated for 21 h at reflux. The mixture was first concentrated and then redissolved in EtOAc (10 mL) and acidified with 2N HCl (20 mL), which lead to the formation of a precipitate that was separated. The phases of the mother liquor were separated, and the aqueous phase was basified with 2N ammonia (20 mL) and extracted with EtOAc (3×50 mL). The combined extracts were dried over sodium sulfate and evaporated giving a first fraction of product. The formed precipitate was separated, dissolved in CH_2Cl_2 (100 mL) and extracted with 2N ammonia (50 mL). The organic phase was dried over Na_2SO_4 and evaporated, giving a second fraction of product as a yellow oil (combined 353 mg, 57%); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.05 (d, J = 6.3 Hz, 6H), 2.67 (dd, J = 12.1, 7.7 Hz, 1H), 2.74 (septet, J = 6.3 Hz, 1H), 2.83 (dd, J = 12.0, 3.8 Hz, 1H), 3.03 (t, J = 7.7 Hz, 2H), 3.33 (td, J = 7.9, 1.8 Hz, 2H), 3.95–4.01 (m, 1H), 4.07 (d, J = 5.0 Hz, 2H), 6.96 (d, J = 8.5 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 7.18 (t, J = 7.3 Hz, 1H), 7.23 (d, J = 7.6 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.44 (t, J = 7.9 Hz, 1H), 7.66 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.8/23.0, 30.2, 45.1, 48.9, 49.3, 68.1, 71.4, 113.0, 121.0, 125.9, 128.3 (2C), 128.4 (2C), 130.2, 133.4, 141.5,

157.6, 201.5 ppm; HRMS (ESI): calcd for $C_{21}H_{28}NO_3$ m/z 342.2064 $[M+H]^+$, found m/z 342.2070 $[M+H]^+$.

The hydrochloride was prepared like for **5a** and collected by filtration to yield a white solid (181 mg, 83%); HRMS (ESI): calcd for $C_{21}H_{28}NO_3$ m/z 342.2064 $[M+H]^+$, found m/z 342.2068 $[M+H]^+$.

1-(2-(3-((1-Fluoropropan-2-yl)amino)-2-hydroxypropoxy)phenyl)-3-phenylpropan-1-one (16b): The ketal **4a** (501 mg, 1 eq, 1.46 mmol) and $NaBH_3CN$ (368 mg, 4 eq, 5.86 mmol) were dissolved in MeOH (10 mL), and fluoroacetone (357 mg, 3 eq, 4.69 mmol) and acetic acid (0.2 mL) were added. The mixture was heated for 4 h at reflux, then it was diluted with CH_2Cl_2 (40 mL) and with 2 N $NaHCO_3$ solution (40 mL). The organic phase was dried over Na_2SO_4 , filtered, and concentrated to yield a yellow oil (596 mg, quant., mixture of diastereomers) that was used without further purification. 1-((1-fluoropropan-2-yl)amino)-3-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propan-2-ol ^1H NMR (500 MHz, $CDCl_3$): δ = 1.09 (t, J = 7.4 Hz, 3H), 2.44 (t, J = 8.8 Hz, 2H), 2.57–2.72 (m, 2H), 2.79–2.91 (m, 2H), 2.94–3.05 (m, 1H), 3.81–3.93 (m, 2H), 3.94–4.01 (m, 1H), 4.01–4.15 (m, 3H), 4.15–4.46 (m, 3H), 6.90 (d, J = 8.2 Hz, 1H), 6.96 (t, J = 7.6 Hz, 1H), 7.09–7.16 (m, 1H), 7.15 (d, J = 7.6 Hz, 2H), 7.23 (t, J = 7.3 Hz, 2H), 7.28 (t, J = 7.5 Hz, 1H), 7.48 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.2 (d, $^3J(^{13}C-^{19}F)$ = 7.7 Hz), 29.9, 39.9, 49.4, 53.1 (d, $^2J(^{13}C-^{19}F)$ = 18.4 Hz), 64.7, 64.8, 69.2, 72.7, 87.0/87.1 (d, $^1J(^{13}C-^{19}F)$ = 168.5 Hz), 110.3, 114.4, 120.9, 125.6, 127.1, 128.2 (2C), 128.3 (2C), 129.6, 130.1, 142.1, 156.7 ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = –224.5 (dt, J = 47.3, 47.3, 16.9 Hz, 1F), –224.7 (dt, J = 47.5, 47.5, 17.2 Hz, 1F).

The product from the last step (596 mg, 1 eq, 1.48 mmol) was dissolved in EtOAc (50 mL), and aq. HCl (2 N, 50 mL) was added and stirred for 1 h. Then the phases were separated, and the aqueous phase was extracted with EtOAc (2 × 60 mL). The combined organic phases were washed once with 2 N $NaHCO_3$ solution (50 mL), dried over Na_2SO_4 , and concentrated to a yellow oil (451 mg, 86%, mixture of diastereomers); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.10 (t, J = 7.1 Hz, 3H), 2.79 (dd, J = 12.0, 7.6 Hz, 1H), 2.88–2.99 (m, 2H), 3.03 (t, J = 7.7 Hz, 2H), 3.24–3.38 (m, 2H), 3.99–4.14 (m, 3H), 4.20–4.26 (m, 0.5H), 4.29–4.35 (m, 1H), 4.38–4.44 (m, 0.5H), 6.97 (d, J = 8.5 Hz, 1H), 7.02 (t, J = 7.3 Hz, 1H), 7.19 (t, J = 7.3 Hz, 1H), 7.23 (d, J = 7.3 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.45 (t, J = 7.7 Hz, 1H), 7.67 ppm (d, J = 7.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.9/16.1 (d, $^3J(^{13}C-^{19}F)$ = 6.1/7.7 Hz), 30.2, 44.9, 49.2, 53.1/53.2 (d, $^2J(^{13}C-^{19}F)$ = 18.4 Hz), 68.0, 71.2/71.4, 86.4/86.5 (d, $^1J(^{13}C-^{19}F)$ = 170.1/170.2 Hz), 113.1/113.2, 121.1, 126.0, 128.2/128.3, 128.4 (4C), 130.3, 133.5, 141.4, 157.6, 201.5 ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = –225.2 (dt, J = 47.5, 47.5, 17.7 Hz, 1F), –225.3 (dt, J = 47.0, 47.0, 17.7 Hz, 1F); HRMS (ESI): calcd for $C_{21}H_{27}FNO_3$ m/z 360.1969 $[M+H]^+$, found m/z 360.1974 $[M+H]^+$.

Hydrochloride formation like for **13a** yielded a white solid (84 mg, 70%); HRMS (ESI): calcd for $C_{21}H_{27}FNO_3$ m/z 360.1969 $[M+H]^+$, found m/z 360.1969 $[M+H]^+$.

1-(2-(3-((1,1-Difluoropropan-2-yl)amino)-2-hydroxypropoxy)phenyl)-3-phenylpropan-1-one (16c): Reductive amination using 1,1-difluoroacetone was performed like for **16b** yielding a yellow oil (313 mg, quant., mixture of diastereomers), 1-((1,1-difluoropropan-2-yl)amino)-3-(2-(2-phenethyl-1,3-dioxolan-2-yl)phenoxy)propan-2-ol ^1H NMR (500 MHz, $CDCl_3$): δ = 1.16 (dd, J = 6.3, 4.4 Hz, 3H), 2.38–2.48 (m, 2H), 2.56–2.72 (m, 2H), 2.80–3.01 (m, 3H), 3.82–3.92 (m, 2H), 3.92–4.02 (m, 1H), 4.02–4.14 (m, 3H), 4.16–4.24 (m, 1H), 5.63 (td, J = 56.7, 4.0 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 7.09–7.18 (m, 3H), 7.19–7.25 (m, 2H), 7.28 (t, J = 7.2 Hz, 1H), 7.48 ppm (d, J = 7.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =

13.6/14.0 (t, J = 4.6 Hz/t, J = 4.6 Hz), 29.9/30.0, 40.0, 49.5/49.7, 55.2/55.4 (t, J = 21.6 Hz/t, J = 23.1 Hz), 64.7/64.8, 69.1/69.3, 72.6, 110.3, 114.4, 118.0 (t, J = 243.8 Hz), 121.0, 125.6, 127.1, 128.3 (4C), 129.6, 130.1, 142.1, 156.6 ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = (Diastereomer 1) –124.4 (ddd, J = 280.7, 56.8, 10.0 Hz, 1F), –127.9 (ddd, J = 281.0, 56.7, 14.0 Hz, 1F), (Diastereomer 2) –125.0 (ddd, J = 281.2, 56.5, 10.3 Hz, 1F), –127.3 (ddd, J = 281.0, 56.5, 12.8 Hz, 1F).

Deprotection like for **13b** yielded a yellow oil (232 mg, 84%); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.13 (d, J = 6.5 Hz, 3H), 2.71–2.98 (m, 3H), 3.03 (t, J = 7.7 Hz, 2H), 3.23–3.36 (m, 2H), 3.92–4.00 (m, 1H), 4.01–4.15 (m, 2H), 5.59 (td, J = 56.5, 3.8 Hz, 1H)/5.60 (td, J = 56.5, 3.8 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 7.20 (t, J = 6.9 Hz, 1H), 7.23 (d, J = 7.6 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.44 ppm (t, J = 7.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8/14.0 (t, J = 3.9 Hz/t, J = 3.9 Hz), 30.2, 45.0, 49.6, 55.1 (t, J = 22.4 Hz), 68.4/68.6, 71.3/71.4, 113.3, 117.6 (t, J = 244.3 Hz), 121.1, 126.0, 128.3 (2C), 128.4 (2C), 130.0, 130.1, 133.4, 141.5, 157.6, 201.4 ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = (Diastereomer 1) –125.6 (ddd, J = 281.3, 56.7, 10.6 Hz, 1F), –127.6 (ddd, J = 281.4, 56.1 (t, J = 13.4 Hz, 1F), (Diastereomer 2) –125.6 (ddd, J = 281.3, 56.7, 10.6 Hz, 1F), –127.5 (ddd, J = 281.4, 56.4, 13.1 Hz, 1F); HRMS (ESI): calcd for $C_{21}H_{26}F_2NO_3$ m/z 378.1875 $[M+H]^+$, found m/z 378.1878 $[M+H]^+$.

Hydrochloride formation like for **13a** yielded a white solid (123 mg, 53%). HRMS (ESI): calcd for $C_{21}H_{26}F_2NO_3$ m/z 378.1875 $[M+H]^+$, found m/z 378.1882 $[M+H]^+$.

In silico modeling

BCRP model: Data for building the BCRP inhibition prediction model was taken as-is from Ref. [14a]. The dataset contains 433 inhibitors and 545 noninhibitors. Model building is described in Ref. [27]. Briefly, extended connectivity fingerprints (ECFP) were computed for each molecule of the training set using RDKit,^[28] with a bit vector length of 1024 and a radius of 4. Logistic regression was built in Python with the scikit-learn library.^[29] The penalty and C parameters were optimized with GridSearch.

P-gp model: Data for building the P-gp inhibition prediction model was taken from Ref. [14b]. It was curated using the procedure described in^[30] and which can be summarized as follows: inorganic compounds were removed, salts were removed from mixtures, compounds containing rare atoms were removed, chemotypes were normalized using the clean 2D, aromatize, mesomerize, neutralize, tautomerize, and all transform options, duplicated compounds were removed, and permanently charged compounds were removed. MOE 2011.10 and ChemAxon 5.11.3 software were used. The cleaned dataset contains 627 inhibitors and 553 non-inhibitors of P-gp. ECFP fingerprints were computed for each molecule of the training set using RDKit,^[28] with a bit vector length of 1024 and a radius of 4. Support Vector Machine (SVM) with RBF kernel was built in Python with the scikit-learn library.^[16] The gamma and C parameters were optimized with GridSearch.

Model validation: Both models were validated by 10-fold cross-validation. Traditional confusion matrix-related statistics are reported, as well as the area under the receiver operating characteristic (ROC) curve (AUC) that specifically evaluate the ranking capability of the models.

In vitro inhibition studies

Materials: RPMI 1640 media for cell culture was purchased from LifeTechnologies (Rockville, MD, USA). Supplements for cell culture,

including fetal bovine serum (FBS), the antibiotics penicillin and streptomycin, as well as vincristine for selection of CCRF VCR1000 cells were purchased from Sigma (St. Louis, MO, USA). The fluorescent substrates mitoxantrone and daunorubicin as well as verapamil, Ko143, DMSO, PBS (phosphate buffered saline), and all compounds used for HPML buffer preparation were also purchased from Sigma.

Cell culture: The human myeloid leukemia PLB985 parental and stably expressing BCRP cell lines^[31] were kindly provided by B. Sarkadi (Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary) and K. Nemet (Creative Cell Ltd., Budapest, Hungary). Both cell lines were cultured in RPMI 1640 supplemented with 10% FBS and 100 IU mL⁻¹ of penicillin and streptomycin. The human T-lymphoblast cell line CCRF VCR1000, overexpressing P-gp, was obtained by stepwise selection of CCRF-CEM in vincristine-containing medium^[32] and was kindly provided by V. Gekeler (Altana-Pharma formerly Byk-Gulden, Konstanz, Germany). CCRF VCR1000 cells were cultured in RPMI 1640 medium containing 20% FBS and were treated regularly with vincristine (1 µg mL⁻¹) for about 72 h, followed by centrifugation (300 g, 5 min, RT) and resuspension in normal culture medium. All cell lines were maintained at 37 °C in an atmosphere containing 5% CO₂ with 95% relative humidity.

Steady-state accumulation experiments: For BCRP and P-gp inhibition studies, the steady-state accumulation of mitoxantrone (7 µM) and daunorubicin (3 µM), respectively, was performed as previously described.^[33] Briefly, cells were harvested, pelleted (300 g, 5 min, 4 °C), diluted to a concentration of 12 × 10⁶ cells mL⁻¹ in HPML (10 mM Hepes, 120 mM NaCl, 5 mM KCl, 0.4 mM MgCl₂, 0.04 mM CaCl₂, 10 mM glucose, 10 mM NaHCO₃, 5 mM Na₂HPO₄, pH 7.4 with NaOH) and kept on ice for further processing. For each data point, the cell suspension (25 µL) was preincubated for 5 min at 37 °C with 2% DMSO (25 µL) in HPML alone (DMSO control) or containing different concentrations of test compound solutions. Thereafter, cells were preloaded with daunorubicin (50 µL) or mitoxantrone (50 µL) for 30 or 20 min at 37 °C, respectively, to reach a final concentration of 3 or 7 µM, respectively. The final DMSO concentration did not exceed 1.5%. Preloading was stopped by chilling cells on ice for 5 min followed by the addition of 400 µL ice-cold PBS. Cells were pelleted (300 g, 5 min, 4 °C), the supernatant was discarded, and the cell pellet was resuspended in ice-cold PBS (150 µL). Until measurement, cells were kept on ice and in the dark. Immediately before cell fluorescence measurement by flow cytometry (BD Accuri C6 and BD FACSCalibur, Becton Dickinson, San Jose, CA, USA; MACSQuant, MiltenyiBiotec GmbH, Bergisch Gladbach, Germany), 50 µL of 4',6-diamidin-2-phenylindole solution (DAPI, 4 µg mL⁻¹ in PBS) was added to gate out dead cells. 100 µM verapamil and 1 µM Ko143 were used as positive controls for P-gp and BCRP inhibition, respectively.

IC₅₀ value measurements and calculations: For compounds showing an inhibitory effect of 50% inhibition compared with the positive control (100 µM verapamil for P-gp and 1 µM Ko143 for BCRP) at a concentration of 100 µM, IC₅₀ apparent values were estimated measuring at least eight different compound concentrations and performing nonlinear regression analyses (GraphPad Prism 6, "log(Agonist) vs. response—Variable slope"), for which Equation (1) was used, where *X* is the log of compound concentration, *Y* is the response in fluorescent intensity units, "Bottom" and "Top" are the lower and the higher plateaus of the nonlinear fit curve, respectively, IC₅₀ apparent refers to the IC₅₀ apparent value, and HillSlope is a factor that describes the steepness of the curve.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\log(\text{IC}_{50\text{apparent}}) - X) \cdot \text{HillSlope}}} \quad (1)$$

To correct for the expression-level dependency of IC₅₀ apparent values and the pump-leak kinetics as reviewed in W. Stein,^[34] IC₅₀ apparent values were calculated using Equation (2), where the fluorescence intensity at zero inhibitor concentration [I]₀ is according to the "Bottom" level and the fluorescence intensity at infinite inhibitor concentration [I]_∞ is according to the "Top" level given by nonlinear regression analysis.^[35] At least three independent experiments were performed for each compound showing an IC₅₀ value less than or equal to 10 µM.

$$\text{IC}_{50} = \text{IC}_{50\text{apparent}} \cdot \frac{\text{fluorescence intensity at } [I]_0}{\text{fluorescence intensity at } [I]_{\infty}} \quad (2)$$

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