New Inhibitors for the ABCG2 Transporter

Dissertation

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„Wer glaubt etwas
zu sein, hat aufgehört
etwas zu werden."
Philip Rosenthal

Content

1. Palladium- and Copper-Mediated *N*-aryl Bond Formation Reactions for the Synthesis of Biological Active Compounds*

N-Arylated aliphatic and aromatic amines are important substituents in many biological active compounds. In the last years, transition metal mediated *N*-aryl bond formations have become standard procedures for the introduction of amines to aromatic systems. *N*-arylation of simple aromatic halides by simple amines works with many described methods in high yield, the reactions may require detailed optimisation if applied to the synthesis of complex molecules with additional functional groups, such as natural products or drugs. In this review we discuss and compare the three main *N*-arylation methods and their applications in the synthesis of biological active compounds: Palladium-catalysed Buchwald-Hartwig-type reactions, copper-mediated Ullmann-type and Chan-Lam-type *N*-arylation reactions. The discussed examples show that palladium-catalysed reactions are favoured in large scale applications and tolerate sterical demanding substituents on the coupling partners better than Chan-Lam conditions. Chan-Lam *N*-arylations are particular mild and do not require further ligand addition, which facilitates the work-up. However, reaction times can be very long. Ullmann- and Buchwald-Hartwig-type reactions have been used in intramolecular reactions providing access to complex ring structures. All three *N*-arylation methods have specific advantages and disadvantages that should be considered when selecting the reaction conditions for a desired *C-N* bond formation in the course of a total synthesis or drug synthesis.

* Fischer, C, König B, *Beilstein J. Org. Chem*, **2011**, *7*, 59-74.

1.1. Introduction

Palladium- and copper-mediated *N*-arylations are essential tools in organic synthesis. Due to the widespread importance of aryl-*N* bond formation many synthetic methods emerged over the years. Beside the traditional Ullmann[1-2] and Goldberg[3-5] procedures, the palladium-catalysed reaction discovered by Buchwald[6-7] and Hartwig[8-9] has been a major breakthrough in this field. More recently, Chan[10] and Lam[11-12] introduced the copper-mediated arylation of *N*-nucleophiles using stoichiometric copper(II) acetate and boronic acids. Collman improved the procedure using catalytic amounts of $[Cu(OH)TMEDA]_2Cl_2$, omitting the base and working at room temperature.[13-14] Beside palladium and copper, also nickel catalysis allows the arylation of primary and secondary amines.[15-16] However, the three methods (Ullmann-Goldberg, Buchwald-Hartwig and Chan-Lam) have become standard procedures for *N*-aryl bond formation, illustrated by many examples and its wide applications in organic synthesis.

The chelating phosphines BINAP, DPPF[17] and D*t*BPF[18,] commonly used for the Buchwald-Hartwig amination, were recently displaced by the biaryl-(dialkyl)phosphine or arylphosphinepyrrole ligands.[18-20] Industrial upscale of these methods have already been applied in 100 kg scale for arylpiperazines and different diarylamines [21]. In addition, Nolan *et al*. and Organ *et al*. reported Pd-*N*-heterocyclic carbene (NHC) catalysed Buchwald-Hartwig amination protocols that provide access to a range of hindered and functionalized aryl amines.[22-24] Aryl bromides are most frequently applied as substrates for the coupling of primary and cyclic secondary amines.[17] In the presence of a weak base like caesium carbonate many functional groups are tolerated, while NaO^tBu causes limitations for base labile functional groups. Electron neutral- and electron-poor aryl bromides are easily converted as substrates,[17] and ortho-substituents on the aryl halide are tolerated. In contrast, electron-rich aryl bromides give only poor results. Recently, the modular synthesis of indoles by a palladium-catalysed cascade process provided an efficient entry to the synthesis of substituted indoles.[25]

Although copper is less toxic and less expensive than palladium, the required harsh conditions, the limited range of suitable substrates and moderate yields

prevented the use of Ullmann-type reaction at its full potential for a long time. Aryl halides activated by electron-withdrawing groups can only be converted at high temperatures (210 °C) using stoichiometric amounts of copper. The discovery of efficient copper/ligand systems enabled the use of catalytic amounts of metal under milder conditions (90-100 °C) and resulted in good yields.[4, 26] Copper-diamine catalysed *N*-arylation facilitated the arylation of pyrroles, pyrazoles, indazoles, imidazoles, triazoles, benzimidazoles and indoles.[27-29] Beside arylhalides as the aryl donor, arylsiloxanes,[30] aryl stannanes,[31] iodonium salts,[32] aryl lead (IV) triacetates[33] and pentavalent organobismuth reagents[34] were used as aryl donors for copper-mediated *C-N* couplings.

Further improvement of *N*-arylation conditions were achieved by the use of arylboronic acids. The reagents are not sensible to air, the reaction proceeds at room temperature[35-36] and in aqueous solution.[37] However, the reactions are very slow and require several hours or even days to complete.[38]

In general, there is a wide variety of protocols describing the metal-mediated arylation of amines,[17, 37, 39] amides,[38] imides,[38] imidazoles,[14, 37, 40] benzimidazoles,[40-41] sulfonamides,[38] pyrroles[42] and lactams.[43] The three typical methods for *N*-arylation were extensively reviewed concerning scope and limitation of these reactions.[4, 44-48]

However, the application of palladium- and copper-mediated *N*-arylation reactions in the synthesis of complex molecules such as natural products or drugs is, in comparison to standard small molecule *N*-arylation, not always straight forward and requires specially optimized conditions. Since amine- and amide substituted aromatics and heteroaromatics are typical structures in medicinal chemistry and natural product synthesis, a broad application of catalytic *C-N* arylation is highly desirable.

Copper-mediated *C-N* arylation reactions in natural product syntheses were recently reviewed by Evano *et al*. They discussed different examples of total synthesis using the arylation of alkylamines, amides, carbamates, *N*-heterocycles, enamines and intramolecular *N*-arylation reactions.[45]

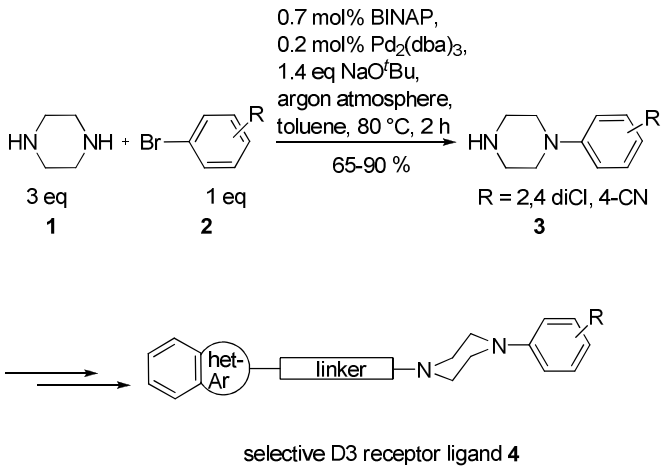
Here we compare the success of the different *C-N* arylation reactions as applied to the synthesis of more complex structures and discuss selected examples of palladium- and copper-mediated reactions for the synthesis of bioactive

compounds in terms of scope and limitation. If available, we will directly compare the different methods and advantages of specific reaction conditions. The review should help synthetic chemists to select the most suitable catalytic *C-N* arylation method for their target molecule.

1.2. Results and Discussion

1.2.1. Pd-Catalysed Synthesis of Biological Active Molecules

A typical example for the application of palladium-catalysed *N*-aryl bond formation, is the synthesis of highly selective D_3 receptor ligands (**4**). Piperazine **1** and a substituted aryl bromide (**2**) (see Scheme 1.1) are coupled in the initial step of the synthesis.

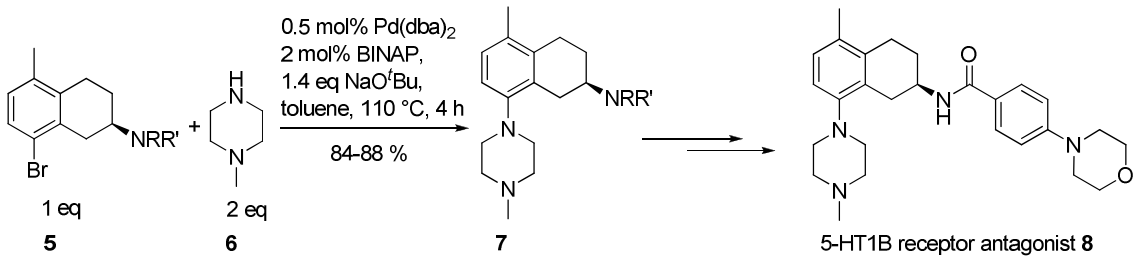


Scheme 1.1: Synthesis of selective D3 receptor ligands.

The electron-withdrawing substituents (nitrile and chloro) on the aryl bromide assisted the reaction and the reported yields of compound **3** are in the range of 65 - 90 %. This method represents conditions of early catalyst generations and allowed the synthesis of a library of 18 compounds, which were investigated to identify a possible structure-activity relationship.[49]

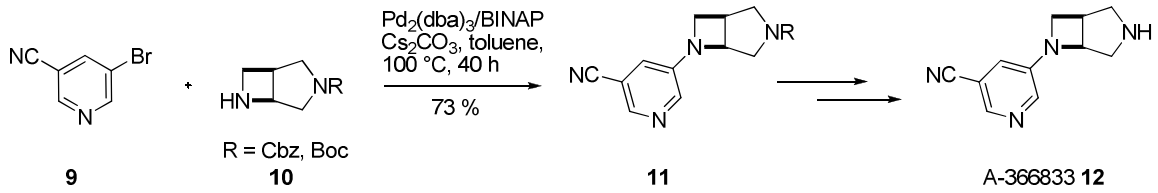
Federsel *et al*. used a piperazine derivative (**6**) and an aryl halide (**5**) for the preparation of a CNS-active substituted chiral aminotetralin (**7**) (see Scheme 1.2) [50]. The 5-HT_{1B} receptor antagonist **8** was developed for the treatment of certain neuronal disorders. Different syntheses have been developed in which

$Pd(OAc)_2$ proved to perform better than the dba complex. BINAP turned out be the privileged ligand without any effect of its enantiomeric purity. The use of $Pd(OAc)_2$ was advantageous on reactions of larger scales due to low cost and easy handling. The choice of base was crucial. NaOMe and NaOEt showed only low conversion in comparison to NaO^{t}Bu. The reaction tolerated up to 0.06 % water. Thus, the conditions could be optimized for the final use in a pilot plant in which batches of 125 kg were synthesised in a robust and reproducible manner.[51]



Scheme 1.2: Synthesis of a novel 5-HT_{1B} receptor antagonist.

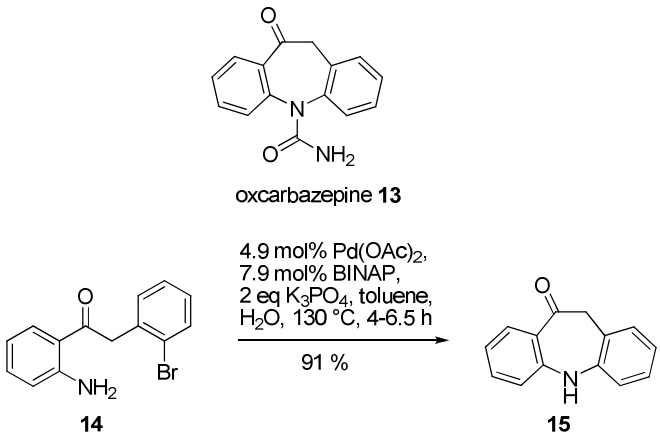
Compound A-366833 (**12**) was found to be a broad-spectrum analgesic having an improved safety profile relative to other pyridine-containing analgesics. The efficient synthesis of this compound used a palladium-catalysed coupling for the final step connecting 3-bromo-5-cyanopyridine (**9**) with the protected (1*R*,5*S*)-3,6-diazabicyclo[3.2.0]heptane **10**. The use of Cs_2CO_3 instead of the conventional base ^{t}BuONa increased the yield of compound **11** from 47 % to 73 % (Scheme 1.3).[52] The enantioselective synthesis of A-366833 allowed large-scale preparation required for preclinical investigations.



Scheme 1.3: Synthesis of A-366833, a selective α4β2 neural nicotinic receptor agonist.

Oxcarbazepine (**13**) (Trileptal) is one the of most prescribed drug for the treatment of epilepsy due to improved tolerability profile compared to carbamazepine. Additionally, its analgesic properties and successful treatment of mood disorders and mania make it an important drug. In 2005, a new method containing a cyclization step by intramolecular *N*-arylation of compound **14** improved the access to compound **15** due to better availability of the starting material and a simple palladium source. Optimized conditions for the palladium catalysis yielded the tricyclic skeleton **15** in 91 % and minimized the amount of dehalogenated byproduct to 4 % (see Scheme 1.4). The beneficial effect of added water may be due to better dissolving of K_3PO_4. Scale-up to gram amounts was possible without significant decrease in yield. The copper-mediated Ullmann-type reactions and heterogeneous palladium catalysis failed for this reaction.[53]

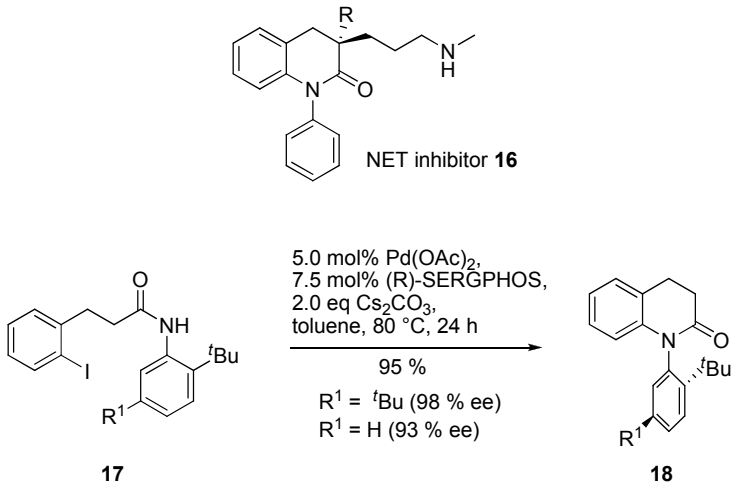
Intramolecular palladium-catalysed *N*-arylations were applied to substituted arenes and thiophenes in good to excellent yields. Electron-rich bromides gave best results, while pyridine derivatives were unreactive.[54]



Scheme 1.4: A new route to oxcarbazepine.

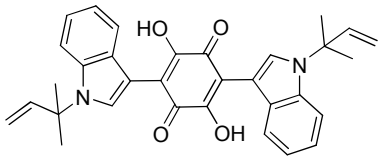
Another intramolecular approach enabled the stereoselective synthesis of atropisomeric *N*-(2-*tert*-butylphenyl)lactam as an intermediate for norepinephrine transporter (NET) inhibitors (**16**). NET inhibitors were developed to treat a variety of mental disorders such as depression and attention deficit hyperactivity disorder (ADHD).

Screening different ligands, SERGPHOS appeared to give the highest stereoselectivity of compound **18** for the *N*-arylation of compound **17** (see Scheme 1.5).[55] Other conditions were not varied in this case.

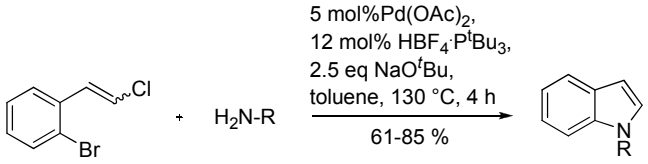


Scheme 1.5: Synthesis of key intermediates for norepinephrine transporter (NET) inhibitors.

Another example for BINAP not being the favoured ligand is the synthesis of the fungal natural product demethylasterriquinone A1 (**19**). Asterriquinones show different biological functions including anti-tumour activity and are used as insulin mimetics. The palladium-catalysed coupling of the styrene **20** with sterical demanding *N*-nucleophiles (**21**) gave the indole building blocks **22** for the natural product synthesis (see Scheme 1.6). In this case, P^{t}Bu$_3$ was the appropriate ligand. As starting materials cyclic and aromatic amino compounds as well as substituted styrenes were tolerated (yields 61 % - 85 %). Only nitro-substituted styrene resulted in poor conversion (34 %).[56]



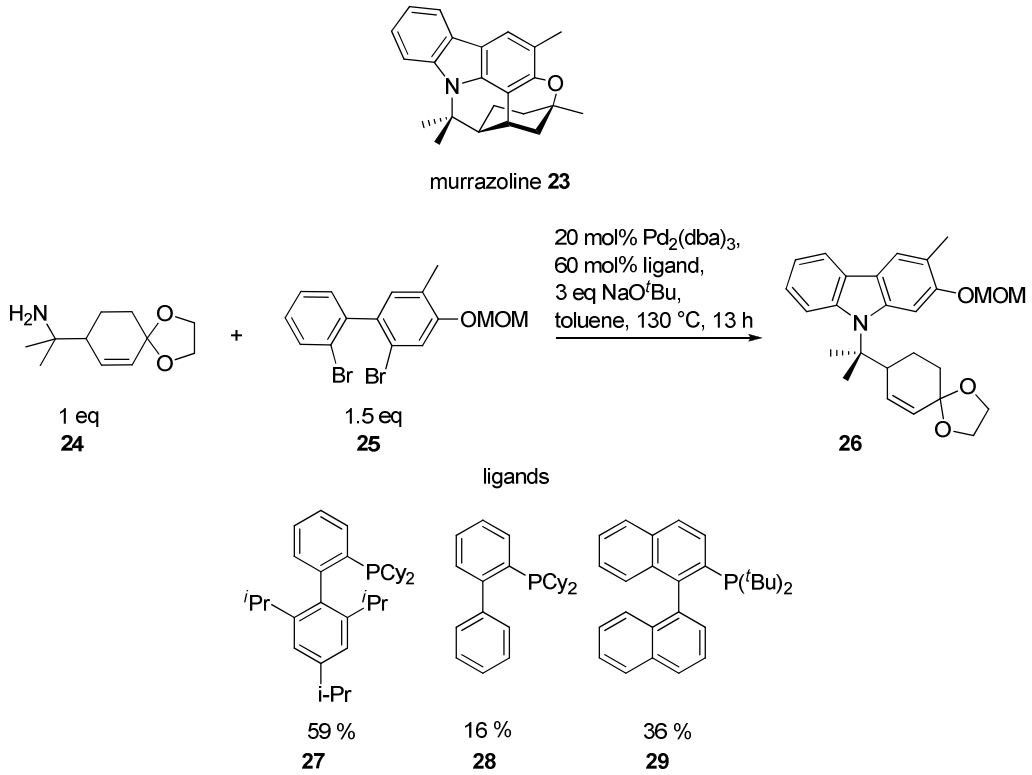
demethylasterriquinone A1 **19**



1 eq **20** + 3 eq **21** → **22**

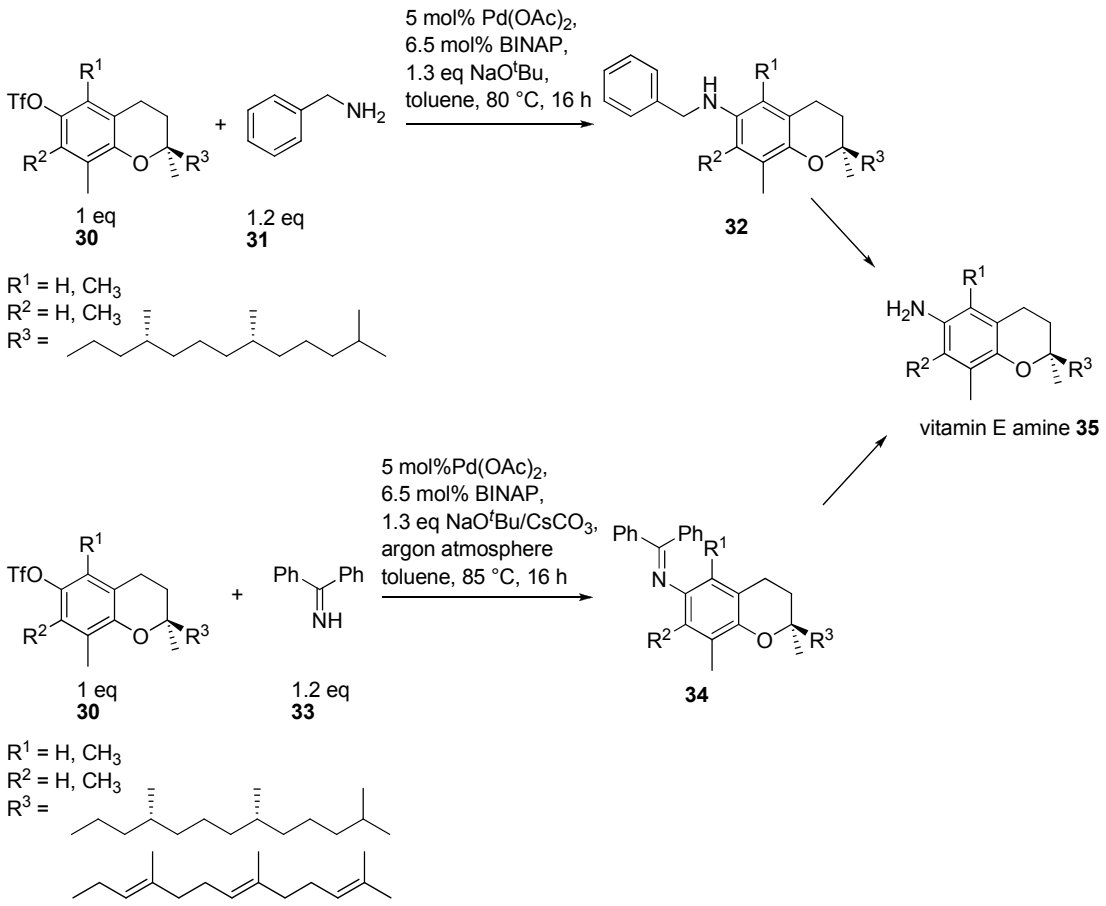
Scheme 1.6: *N*-Annulation yielding substituted indole for the synthesis of demethylasteriquinone A1.

The synthesis of the natural product murrazoline is also an example for connecting a sterical demanding *N*-nucleophile at two positions. Murrazoline (**23**), a carbazole alkaloid isolated from the shrub *Murraya*, is used in folk medicine for the treatment of analgesia and anesthesia, eczema, rheumatics and dropsy. It is known to be a potent platelet aggregation inhibition. The double *N*-arylation of compound **25** was carried out under standard conditions, using compound **24**, and revealed the best yield of compound **26** for the dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine ligand (**27**) (59 %, see Scheme 1.7) compared to the ligands **28** and **29**.[57]



Scheme 1.7: Palladium-catalysed double *N*-arylation contributing to the synthesis of murrazoline.

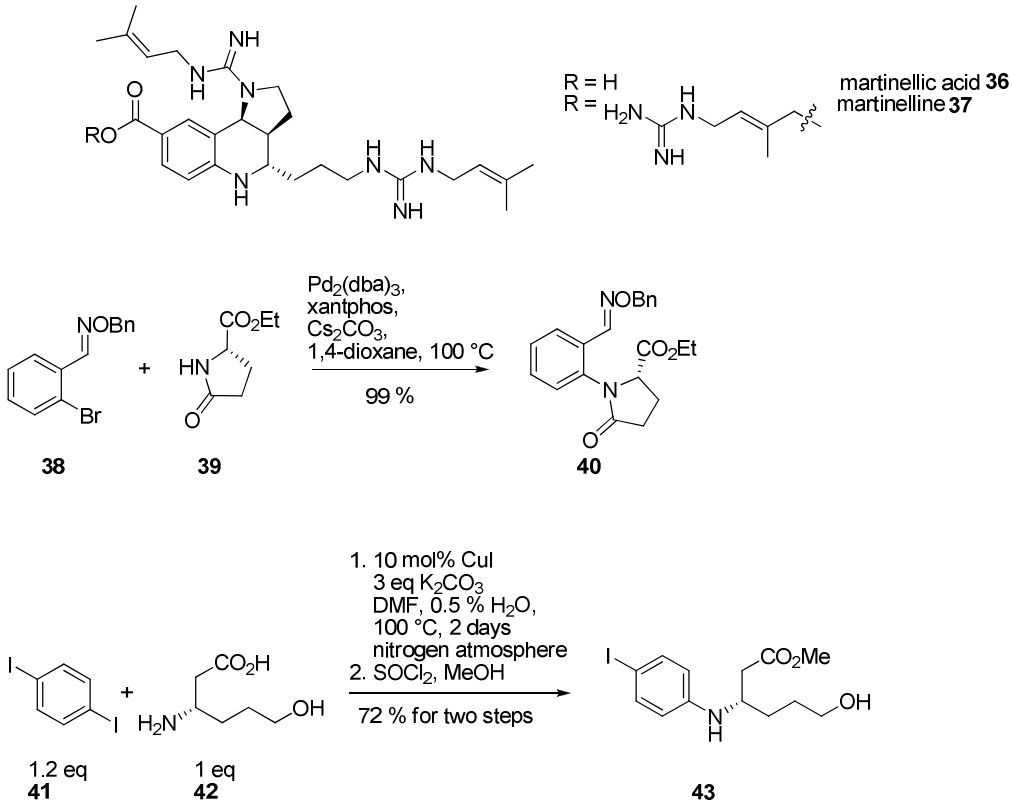
The synthesis of vitamin E amines **35**, possessing antiproliferative activity, could be realized by the easy and efficient procedure by coupling compound **30** or to **31** or to **33** according to Scheme 8. Enantiopure tocopheramines (**32**) and tocotrienamines (**34**) were synthesised using an *N*-aryl amination as key step. Here, the leaving group was triflate instead of the typically used halides. The yields of the reaction range from 10 % to 80 %.[58]



Scheme 1.8: Synthesis of vitamin E amines.

An example showing the application of both methods, the palladium- and the copper-catalysed *N*-arylation, is the synthesis of the natural product martinellic acid (**36**) and its derivative (see Scheme 1.9). The two alkaloids martinellic acid (**36**) and martinelline (**37**) possess antagonist activity towards bradykinin B1 and B2 receptors. The key step of the synthetic sequence is the connection of an amino acid derivative to the aromatic core.

Miyata *et al*. report nearly quantitative yield of compound **40** (99 %) for the palladium strategy converting (*S*)-ethyl 5-oxopyrrolidine-2-carboxylate **39** and 2-bromobenzaldehyde O-benzyl oxime **38** and only moderate yield using CuI (53 %).[59] Targeting the same product, Ma *et al*. used copper-catalysis some years before. The coupling of 1,4-diiodobenzene **41** and (*S*)-ethyl 3-amino-6-hydroxyhexanoate **42**, and subsequent esterfication resulted in an overall yield of 72 % for the two steps to compound **43**.[60-61] The specific yield for the copper-catalysed reaction step was not reported.

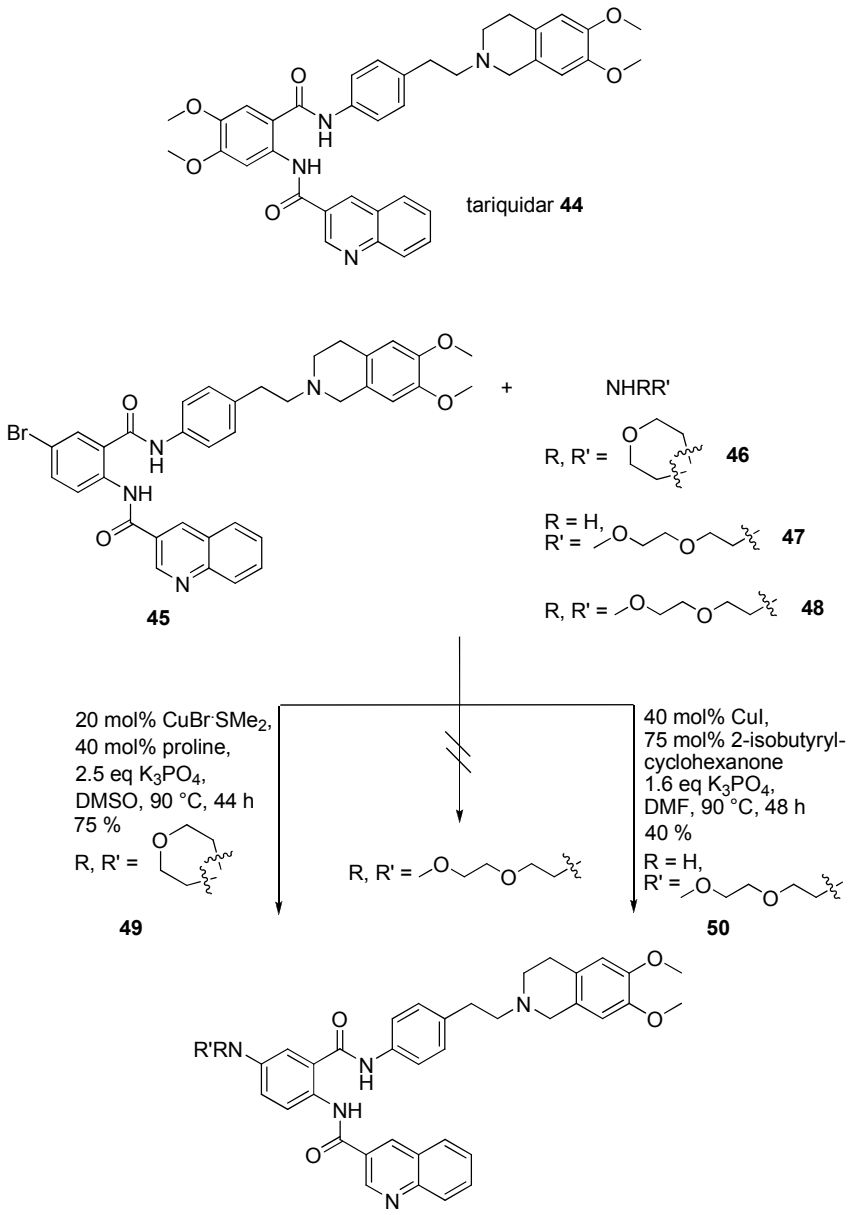


Scheme 1.9: Improved synthesis of martinellic acid.

1.2.2. Cu-Catalysed Synthesis of Biological Active Molecules

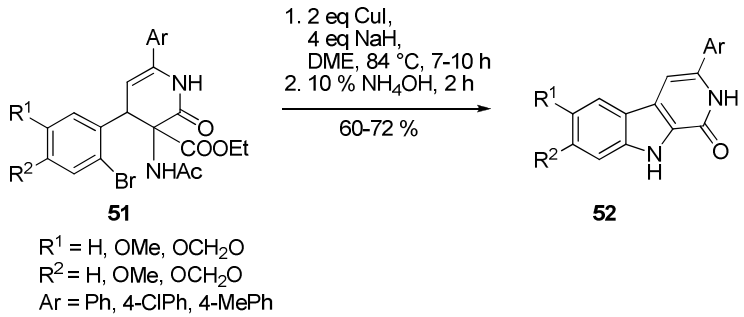
Copper-catalysed *N*-arylation reactions were applied to introduce diversity into new ABCB1 transporter modulators. The tariquidar (**44**) derived compounds showed logP dependant inhibition activity of the ABCB1 transporter, which represents an important component of the blood brain barrier and is a major limitation in cancer chemotherapy.

Primary and cyclic secondary amines were coupled to compound **45** in moderate to good yields of compounds **49** and **50**. Secondary acyclic amines **48** were unreactive although a variety of different conditions was tested, including the variation of base, ligand and copper source. Scheme 1.10 shows the optimized conditions for coupling with morpholine (**46**) and 2-(2-methoxyethoxy)ethanamine (**47**). Palladium-catalysed reactions failed due to the low solubility of the bromide compound in toluene.[62]



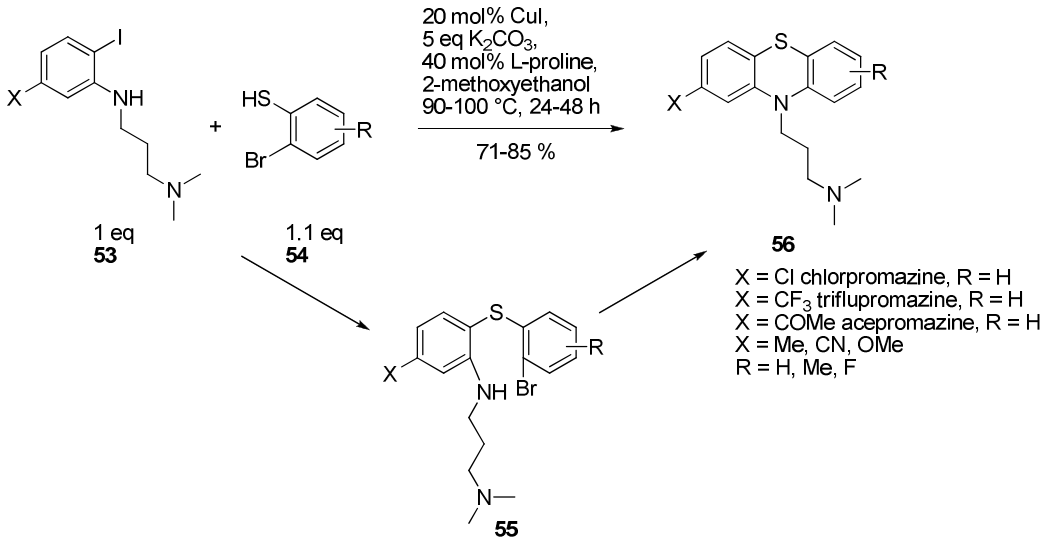
Scheme 1.10: New tariquidar-derived ABCB1 inhibitors.

Another class of compounds related to anti-tumour treatment are β-carbolin-1-ones (**52**) due to their inhibition of cell proliferation. These compounds were accessible using an intramolecular coupling between an aryl bromide and an acylated amine (**51**). In comparison to the Goldberg reaction conditions (CuI, NaH, DMF at 90 °C for two hours), the change of the solvent to DME was crucial for good yields up to 72 % (see Scheme 1.11). Additionally, the reaction was sensible to the amount of NaH. Substituents on the aryl bromine affected the yield marginally.[63]

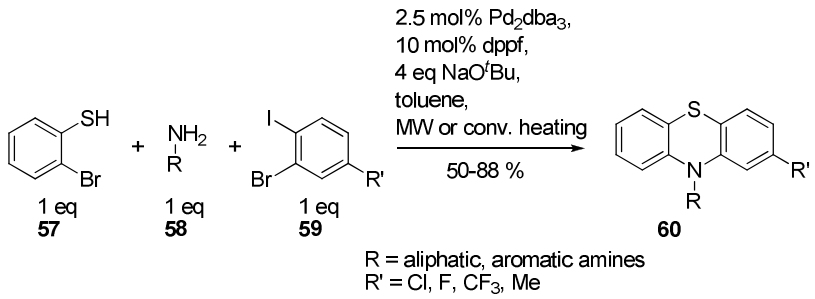


Scheme 1.11: β-Carbolin-1-ones as inhibitors of tumour cell proliferation.

A second example for intramolecular coupling is the synthesis of promazine drugs (**56**) interesting due to their clinical use for psychotropic medication. The CuI/*L*-proline-catalysed cascade process, developed by Ma *et al*., revealed the best yields for 2-methoxyethanol as solvent, compared to DMSO, dioxane and others. The reaction conditions tolerated electron-rich and electron-deficient substituents on the aniline **53** and on the 2-bromobenzenethiol **54** in different substitution patterns and is supposed to run over the intermediate **55**. The catalytic synthetic sequence is an inexpensive and efficient route to the target compounds (see Scheme 1.12).[64] A three component coupling reaction of 2-bromobenzenethiol (**57**), a primary amine (**58**) and 1-bromo-2-iodobenzenes (**59**), also targeting promazine derivatives (**60**) was successfully established using palladium catalysis (see Scheme 1.13). The method benefits from controlled regiochemistry and the applicability for various aliphatic and aromatic amines (**58**). Although the reaction scope was limited to 1-bromo-2-iodobenzenes **59** a scale up to multigram quantities was possible.[65]

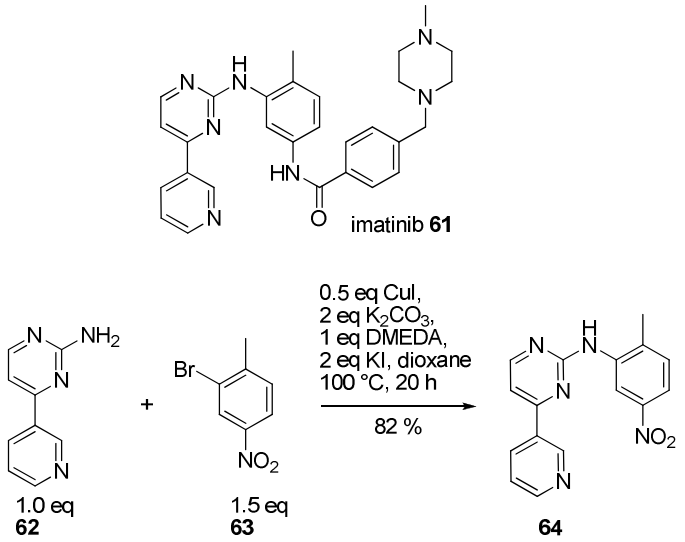


Scheme 1.12: Copper-catalysed synthesis of promazine drugs.



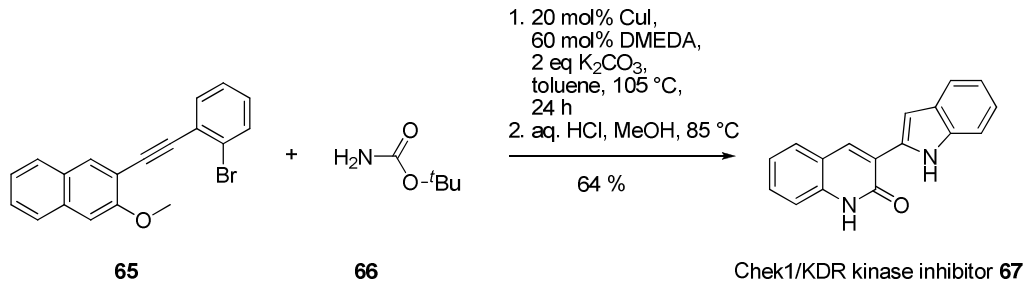
Scheme 1.13: Palladium-catalysed multicomponent reaction for the synthesis of promazine drugs.

A key intermediate **64** of imatinib (**61**), a standard anti-cancer drug treating chronic myelogenous leukaemia and gastrointestinal tumours, was prepared in a copper-mediated *N*-arylation of compounds **62** and **63** in 82 % yield (see Scheme 1.14). Screening different reaction conditions, Cu(I) turned out to be the best copper source compared to Cu(0) and Cu(II). In the series of aryl halides, aryl iodides **63** were most active. The presence of air and water gave only slightly lower yields. Also other heteroarylamines gave good yields.[39]



Scheme 1.14: Key intermediate for imatinib.

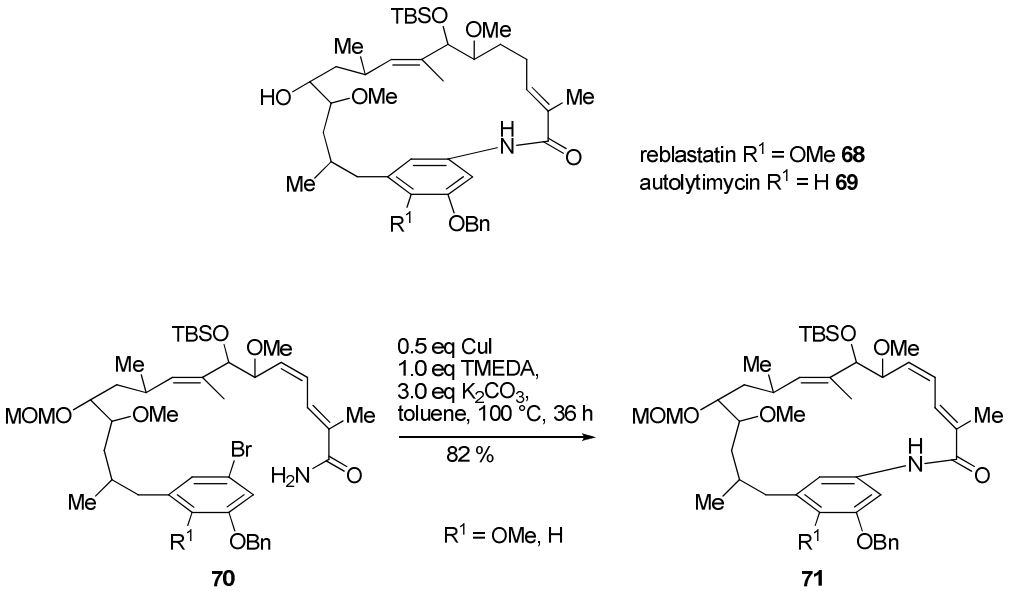
The copper-catalysed domino-indole synthesis facilitated the access to the new Chek1/KDR kinase inhibitor **67** from phenylethynyl naphthalenes (**65**) and *tert*-butyl carbamate (**66**) (see Scheme 1.15). The broad scope of this method improved the synthesis of diversely substituted indoles. Beside *N-H* indoles, *N*-acyl and *N*-aryl indoles were accessible. For this attempt, DMEDA turned out to be the crucial ligand.[66]



Scheme 1.15: Effective Chek1/KDR kinase inhibitor synthesis.

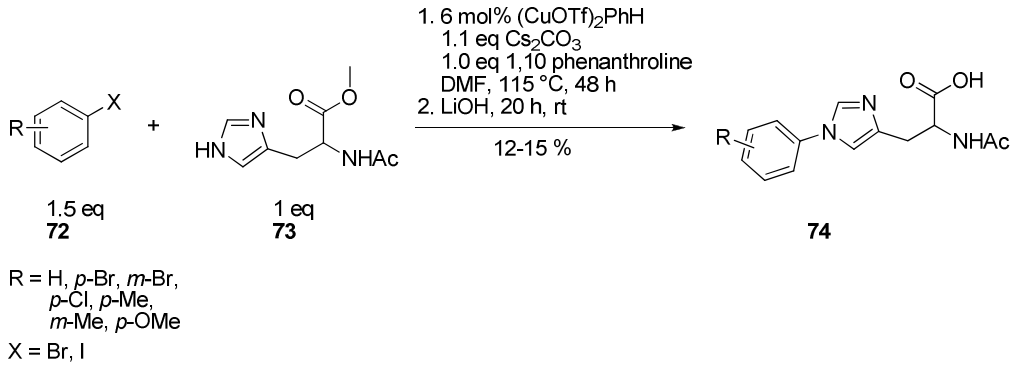
Examples for the synthesis of complex biological active compounds are reblastatin (**68**) and autolytimycin (**69**). The potent inhibitors of heat shock protein 90, an important therapeutic target for cancer treatment, were accessed by a copper-mediated macrocyclisation step of compound **70** to compound **71** in high yield (82 %, Scheme 1.16).[67] The same method was employed in the

total synthesis of glendanamycin and other 8- to 14-membered lactam-containing natural products.[68]



Scheme 1.16: Macrocyclisation as final step of heat shock protein inhibitor synthesis.

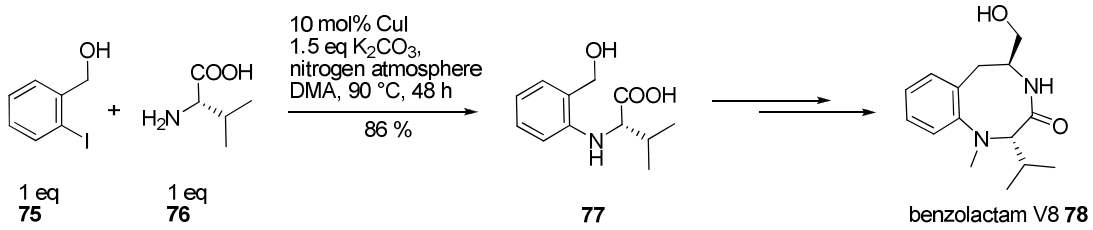
A number of biological targets, such as blocking cytochrome C oxidase, made *N*-aryhistidines (**74**) important drug candidates. The quite simple looking copper triflate catalysed conversion of aryl halides (**72**) with an *N*-acylhistidine (**73**) revealed total regioselectivity on the imidazole ring, but suffered from complicated work-up procedures due to difficult separation from stoichiometric quantities of the ligand. Sufficient reactivity was only observed for aryl iodides, nevertheless resulting in low overall yields including the subsequent ester cleavage step (Scheme 1.17).[69]



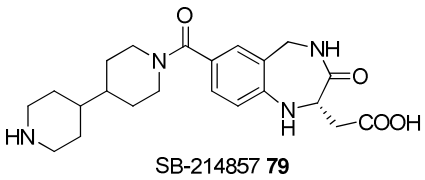
Scheme 1.17: Synthesis of *N*-arylimidazoles.

According to the well-known benzolactam V8 (**78**) synthesis[70] Ma *et al*. proved the accelerating effect of α- (**76**) and β-amino acids (**80**) to aryl aminations (Scheme 1.18 and Scheme 1.19). Lotrafiban (SB-214857) (**79**), a potent GPIIb/IIIa receptor antagonist inhibiting platelet aggregation, was efficiently obtained by an Ullmann-type aryl amination reaction. Despite the disadvantage of long reaction time (2 days), the intermediate **81** was obtained in enantiopure form.[71]

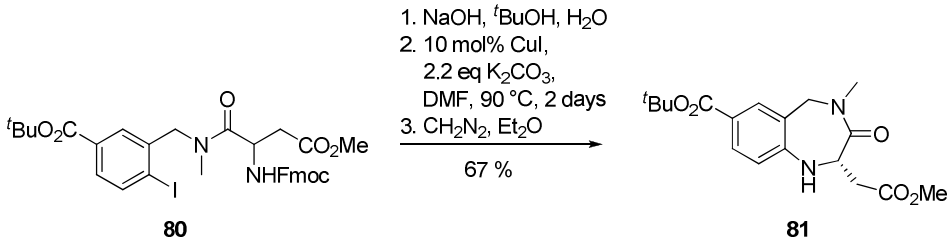
A very similar intermediate **85**, published at the same time, was synthesised by the coupling of L-aspartic acid (**83**) to an aryl bromide (**82**) in 55 % yield and 90 % ee (Scheme 1.20). Under these reaction conditions CuI turned out to be the copper source causing the least racemization.[72]



Scheme 1.18: Benzolactam V8 synthesis.

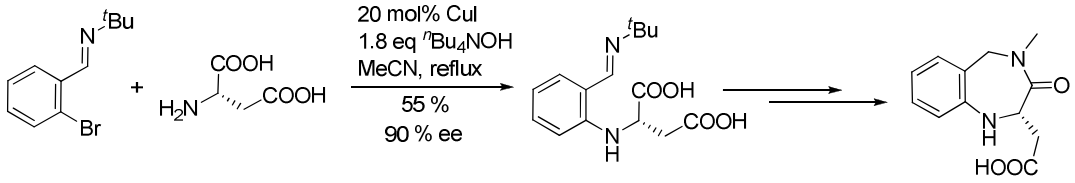


SB-214857 **79**



80 **81**

Scheme 1.19: Synthesis of an intermediate for lotrafiban (SB-214857).

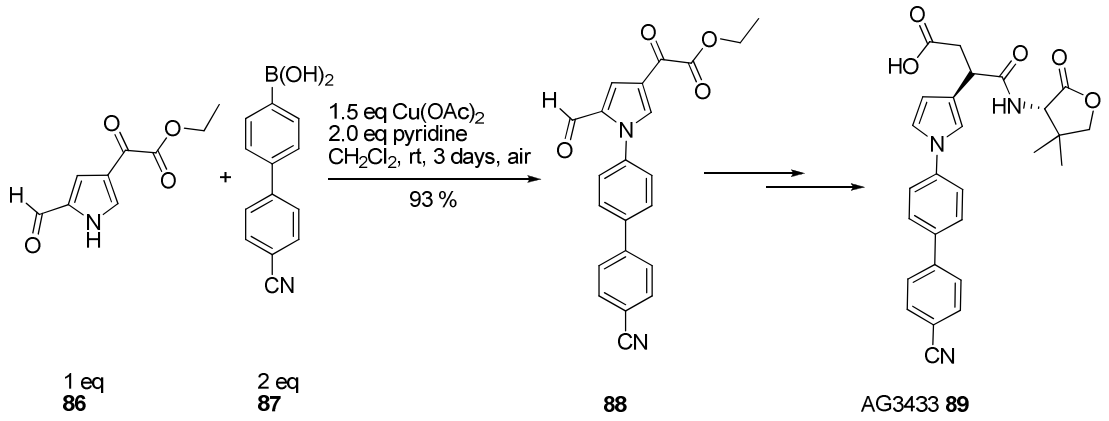


82 **83** **84** **85**

Scheme 1.20: Intermolecular effort towards lotrafiban.

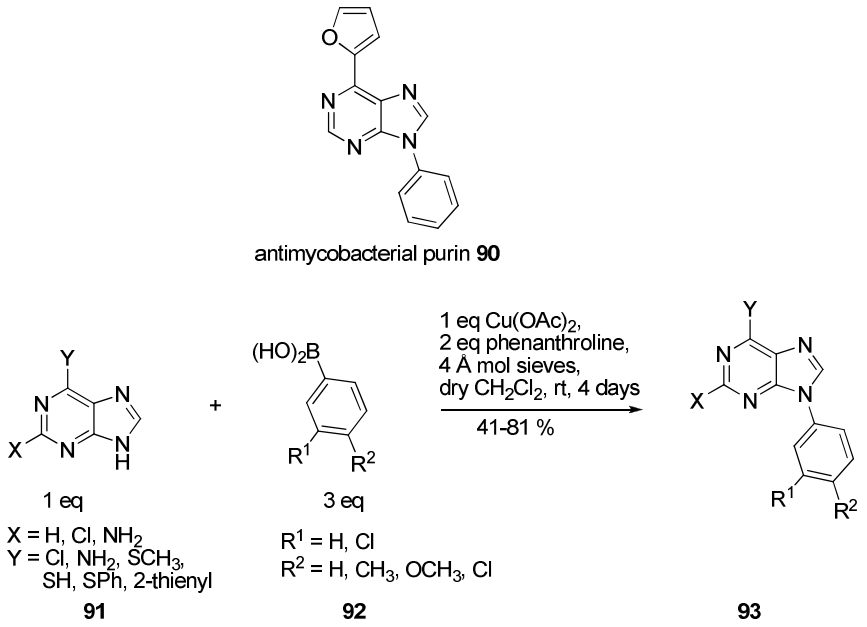
1.2.3. Chan-Lam Arylation Reactions of Biological Active Molecules

A key intermediate for the potent matrix metalloproteases (MMPs) inhibitor AG3433 (**89**) was synthesised by coupling an electron-deficient pyrrole (**86**) with an aryl boronic acid (**87**) in excellent yield of compound **88** (93 %, see Scheme 1.21). Screening numerous boronic acids showed that only boronic acids containing electron-donating or weakly electron-withdrawing substituents were suitable for this purpose. Pyrroles lacking the substituent in the 2-position, which is supposed to support the reaction by a chelating effect with the copper ion, did not succeed in the coupling reaction. Further disadvantages were the required stoichiometric amount of copper and the long reaction time of three days.[42]



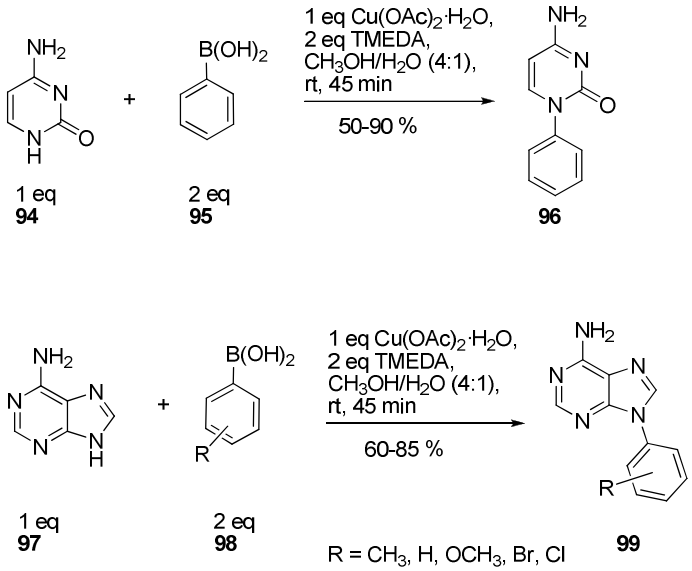
Scheme 1.21: Matrix metalloproteases (MMPs) inhibitor synthesis.

For detailed insight into structural requirements of active antimycobacterial purines (**90**), an easy access to 9-*N*-arylpurines (**93**) was required. Complete regioselectivity and in most cases high chemoselectivity was achieved by reacting 9-*N*-purines (**91**) with an excess of arylboronic acid (**92**) in the presence of copper(II) acetate, molecular sieves and phenanthroline (Scheme 1.22). Bakkestuen *et al.* showed that electron-donating and electron-withdrawing substituents on the aryl boronic acid were tolerated. However, adenine was unreactive under these conditions, probably due to low solubility of the starting material.[73]



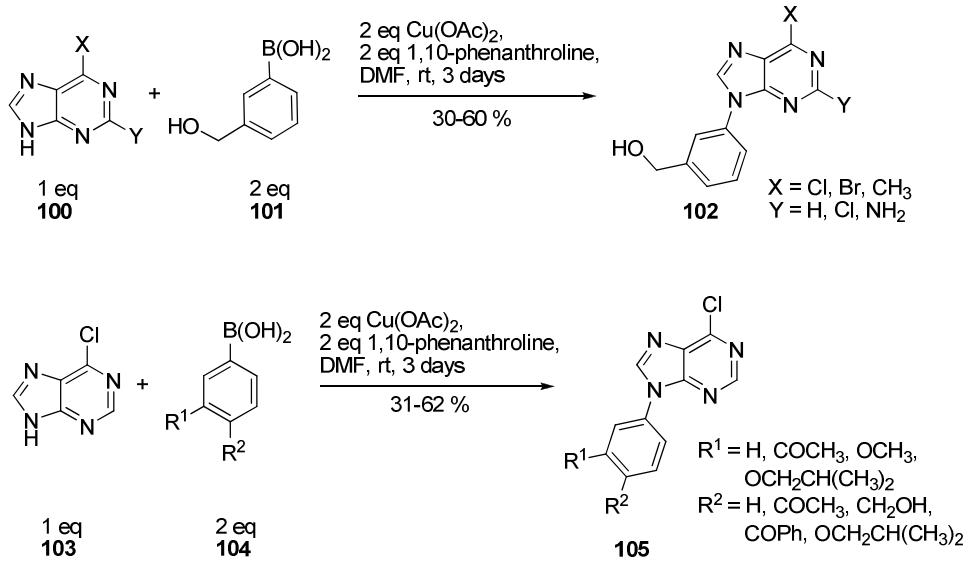
Scheme 1.22: Regioselective 9-*N*-arylation of purines.

Slight changes of the procedure, the use of a protic solvent, enabled the conversion of adenine (**97**) and cytosine (**94**) (Scheme 1.23). These conditions tolerated electron-donating and electron-withdrawing substituents at the *o*-, *m*-, *p*-position of phenylboronic acid **98** and resulted in moderate to excellent yield.[74]



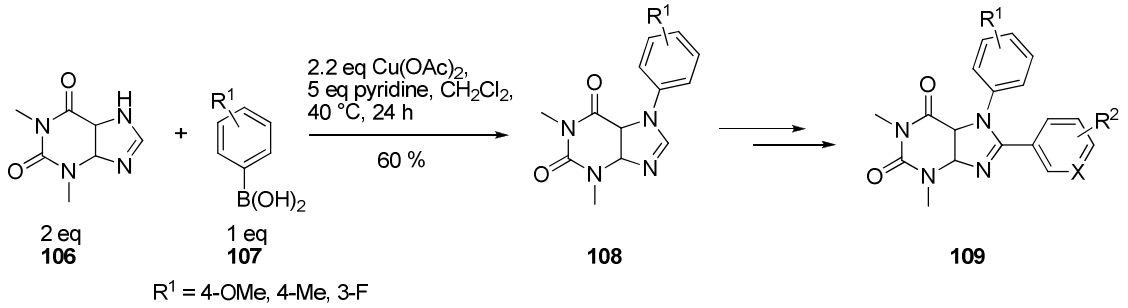
Scheme 1.23: *N*-Arylation of adenine and cytosine.

9-*N*-Arylpurines (**102**, **105**) are used as a new class of inhibitors against enterovirus, which are responsible for a variety of acute human diseases like respiratory infections, meningitis, pancreatitis and others. The conditions reported by Bakkestuen *et al*. [73] gave only low yield (26 %). Here, DMF as solvent and the absence of molecular sieves improved the yield (Scheme 1.24). The reaction conditions were compatible for different purine bases (**100**, **103**) and a variety of functional groups at the arylboronic moiety (**101**, **104**). Thus, a single reaction step from commercial precursors allowed the synthesis of new enterovirus inhibitors with activity in the low μM range.[75]



Scheme 1.24: 9-Arylpurines as enterovirus inhibitors.

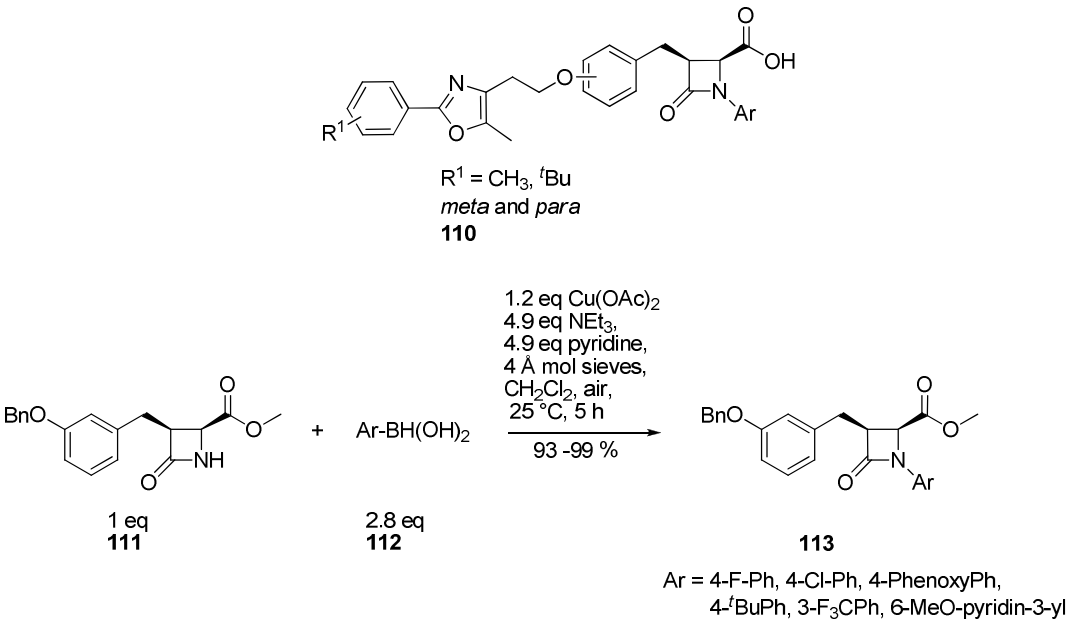
A very recent example of 7-*N*-arylation of purines is the synthesis of highly substituted xanthine derivatives (**109**) as fluorescent and potent kinase inhibitors. The conditions of Bakkestuen *et al*. were modified and resulted in yields up to 60 % for the optimized conditions using pyridine as base and heating the reaction mixture to 40 °C for 24 h (Scheme 1.25). Beside the *p*-methoxy substituent at the boronic acid **107**, *p*-methyl and *m*-fluorine were tolerated. The xanthine compounds showed good antiproliferative activity and exhibited a significant fluorescence response.[76]



Scheme 1.25: Xanthine analogues as kinase inhibitors.

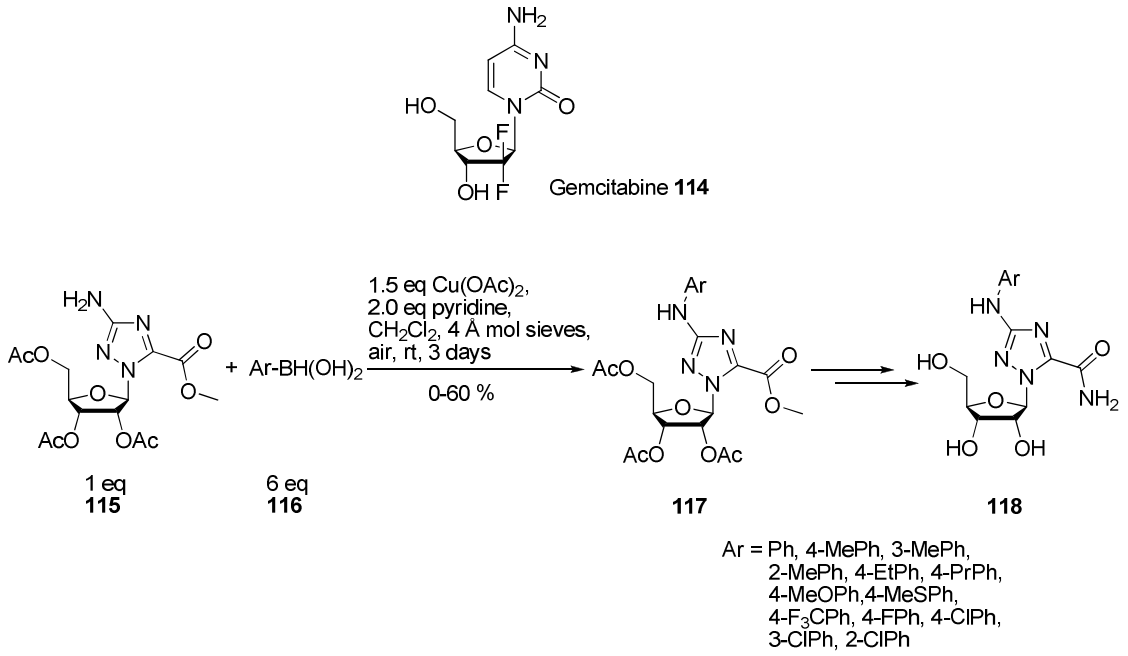
The peroxisome proliferators-activated receptors (PPARs), members of the nuclear hormone receptor super-family, are important targets in the treatment of diabetes and dyslipidemia. Azetidinone acid derivatives **110** were discovered as

new subtype selective PPARα/γ agonists. For detailed structure-activity relationships (SAR) studies, diversity was introduced very efficiently by a copper-mediated *N*-arylation of azetidinones (**111**) with different aryl boronic acids (**112**) in nearly quantitative yields of compound **113** (Scheme 1.26).[77] The palladium-catalysed *N*-arylation of 2-azetidinones was only described for unsubstituted azetidinones before.[78]



Scheme 1.26: Synthesis of dual PPARα/γ agonists.

N-Aryltriazole ribonucleosides (**118**) with potent antiproliferative activity against drug-resistant pancreatic cancer were found by coupling 3-aminotriazole with various boronic acids. Corresponding to the method developed for *N*-aryltriazole acylonucleoside analogues,[79] the *N*-arylation was performed in the presence of stoichiometric $Cu(OAc)_2$, pyridine and freshly activated molecular sieves in CH_2Cl_2 at room temperature and open air for three days (Scheme 1.27). There was no clear trend on the effect of electron-donating or electron-withdrawing substituents of the aryl boronic acid **116** on the course of the reaction. However, sterical hindered, *ortho*-substituted arylboronic reagents were unreactive. Although the coupling resulted only in moderate yield, a new anticancer drug candidate with improved potency on human pancreatic cancer cells, in reference to gemcitabine (**114**) treatment, was found.[80]



Scheme 1.27: *N*-Aryltriazole ribonucleosides with antiproliferative activity.

1.3. Conclusion

Palladium- and copper-mediated *N*-arylation have been shown to be powerful methods for inducing aryl and heteroaryl substituents. However, for substrates of a more complex structure the performance and applicability of the different reactions is often difficult to predict. The three reaction types, palladium-catalysed and copper-catalysed *N*-arylations of aryl halides, and copper-catalysed *N*-arylations using boronic acids show some distinct differences. Palladium-catalysed *N*-arylation reactions typically require reaction temperatures of 80-130 °C and extended reaction times up to 40 h. In most cases, the reactions must be conducted in an inert atmosphere; toluene is a favourite solvent and BINAP a commonly used ligand. Strong bases are required and water is added to dissolve them. Substituents in the starting material, which are labile to basic conditions, are not tolerated. With low catalyst loading and good availability of the palladium sources, reactions on larger scale are possible. Sterical demanding *N*-nucleophiles, as well as cyclic and aromatic amino compounds are suitable coupling partners to aryl bromides.

In contrast to palladium catalysis, Ullmann-type coupling reactions tolerate air oxygen. However, reaction temperatures (90-115 °C) and reaction times (48 h)

are comparable to the palladium-catalysed processes. Ligand-free and ligand-assisted reaction conditions have been applied in the synthesis of biological active compounds. DMEDA, proline and phenantroline are the most commonly used ligands. In some cases, the up to stoichiometric amount of ligand made work-up procedures difficult. In general, CuI is the most efficient catalyst. The reaction conditions tolerate primary, cyclic secondary aliphatic amines, electron-rich and electron-poor anilines and heteroarylamines. Macrocyclisation using amides and aryl bromides are possible. Both, the palladium-catalysed and the Ullmann-type *N*-arylation reactions were successfully applied for inter- and intra-molecular reactions. An advantage of the Ullmann-type reactions is the lower price of catalyst metal salts and ligands.

The mildest conditions for *N*-arylation reactions are provided by the Chan-Lam arylation, but conversion at room temperature resulted in long reaction times up to four days. The general absence of ligands is an additional advantage facilitating the product purification. However, one to two equivalents of $Cu(OAc)_2$ and large excess of the boronic acid are required. Boronic acid reagents tolerated electron-donating and electron-withdrawing substituents in *ortho*-, *meta*- and *para*-position, but sterical demanding substituents were found unreactive. Many commercial precursors for the starting materials used in Chan-Lam arylations are available.

None of the three reactions is clearly superior as method for *N*-arylations. Depending on the substrate that should be converted and the summarized constraints, the preferred method must be selected. In many cases optimization or adaptation of the standard protocols to the specific substrate is required. Nevertheless, the modern *N*-arylation methods have become an indispensable tool in organic chemistry and facilitate the synthesis of complex natural products and drugs significantly.

1.4. References

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2. Fluorescently Labelled ABCG2 Modulators*

The membrane located ABCG2 transporter plays a major role in multi-drug resistance. However, the transport mechanism for this transporter is still unknown. Starting from the recently discovered tariquidar-derived selective ABCG2 modulator **1**, different fluorescent labelled ABCG2 modulators were synthesised. The modified modulators show emission in the red part of the spectrum and reveal quantum yields up to 31.2 %. Moderate potency was obtained by the replacement of the tetrahydroisoquinoline part of the tariquidar-derived lead-structure by a pyrylium label. All synthesised derivatives showed selectivity for ABCG2 over ABCB1. Studies at the physiological expressed ABCG2 concentrations on rat brain capillaries revealed that the parent compound **1** is also influencing the rat ABCG2 transporter, confirming the results of previous *in vitro* experiments with MCF-7/Topo cells and Hoechst 33342 as substrate. Due to lower affinity of the fluorescent labelled compounds this could not be proven for the fluorescent modulators.

* All synthesis and spectroscopical investigations were done by Carolin Fischer, compounds **6** and **8** were a kind gift of Prof. Dr. Otto Wolfbeis, inhibition assays for ABCB1 and ABCG2 were performed by Peter Höcherl and Matthias Kühnle, convocal laser scanning microscopy was done with the help of Nathalie Pop, studies on rat brain capillaries were done by Anne Mahringer (University of Heidelberg).

2.1. Introduction

The large family of ABC (ATP-binding cassette) transporter proteins is involved in numerous physiological processes including absorption, distribution and elimination of xenobiotics.[1] Prominent members of these efflux pumps are ABCB1 (P-gp, MDR1) and ABCG2 (BCRP, MXR, ABCP1), which are physiologically located in the membranes of epithelial and endothelial cells, e. g. in the capillaries of the blood-brain barrier.[2] These transporters use the energy of ATP-hydrolysis to decrease the intracellular concentration of a wide variety of structural different substances.[3] They are responsible for lowering the oral bioavailability and brain penetration of drugs, e.g. in the chemotherapy of malignant brain tumours.[4] Additionally, ABCG2 is considered to be one of the major transporters causing multi-drug resistance (MDR) in cancer cells.[5-7] Overexpression of ABCG2 results in resistance against a large number of different anti-cancer drugs for example mitoxantrone, topotecan, etoposid and flavopiridol.[8] As elevated expression of ABCG2 was also observed in numerous cancer stem cells (CSCs) from different solid tumours they are thereby supposed to withstand chemotherapy.[9-10] According to the tumour stem cell hypothesis these surviving cells give rise to the relapse in cancer.[10-11] Until now a 2D crystal structure is available showing conformational changes upon binding the substrate mitoxantrone.[12] Additionally, several residues within or near the transmembrane helices 1, 2 and 6 were identified to be important for substrate specificity and the overall transport activity of ABCG2.[13-14] Docking-calculations and homology modelling predict TMD 2 of ABCG2, along with other TMDs, to form the large central binding cavity of multiple substrate binding sites.[13] A high resolution 3D structure of ABCG2 is not yet available.[15-16] Since a broad variety of structurally different molecules are known as substrates, no obvious structure-activity relationships are known until now.[17] Very recently, we discovered selective ABCG2 modulators[7] (Figure 2.1) by structural modification of the known ABCB1-preferring inhibitor tariquidar.[18]

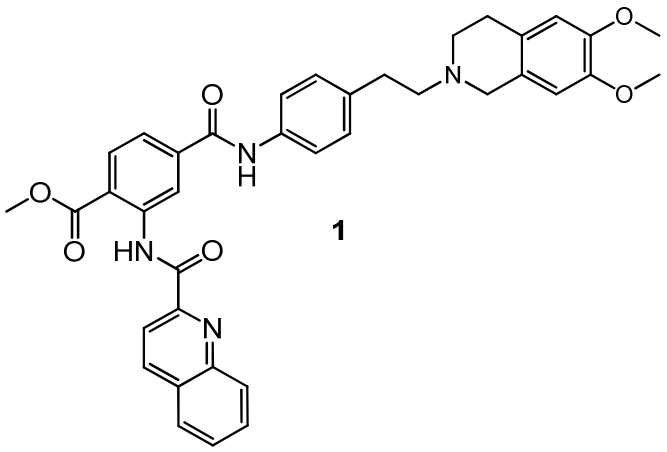

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Figure 2.1: Structure of a selective ABCG2 modulator derived from tariquidar.[7]

The shift of the heteroarylcarbonylamino substituent from the *ortho* to the *meta* position of the benzamide core proved to be crucial for the change in selectivity.[7] The mode of interaction with the membrane located transporters is still unknown. The observed efflux inhibition may be caused by competing with substrates for the same binding side or by allosteric modulation of the transporter activity.

To date, several fluorescent substrates of ABCG2 such as mitoxantrone,[19] pheophorbide a,[20] Hoechst 33342,[21] bodipy-prazosin[22] are known and mainly used for the analysis of active extrusion. Since, by comparably projects, fluorescent pyridinium compounds turned out to be useful pharmacological tools[23], a series of fluorescence-labelled compounds were produced to gain insight into the mechanism of action. With the objective to retain the activity of our previously discovered ABCG2 modulator and to keep the molecular weight low, different substructures of the parent molecule were replaced by fluorescent pyridinium chromophores. These dyes mimic aromatic substructures of the modulator and show spectral properties which are in principle well suited for the analysis with flow cytometers and confocal microscopes. We report the synthesis of eight luminescent potential ABCG2 modulators and the investigation of their spectral and pharmacologic properties.

2.2. Results and Discussion

2.2.1. Synthesis

For the design of the labelled ABCG2 modulators four different pharmacophore substructures **2-5** and three different pyrylium dyes **6-8** (see Figure 2.2) were considered to investigate the contribution of the different moieties of the molecule to the biological activity.

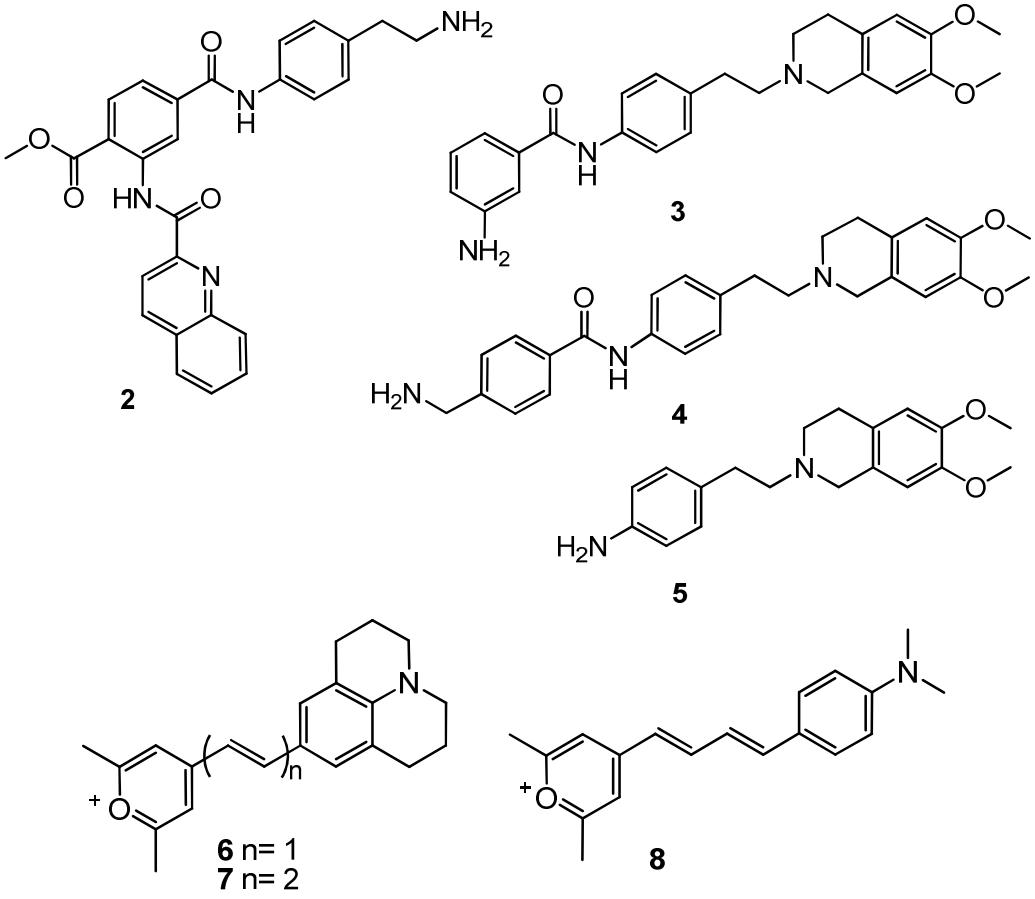


Figure 2.2: Pharmacophore substructures (**2**-**5**) and pyrylium dyes (**6**-**8**) used to design fluorescence-labelled ABCG2 modulators.

On the one hand, the tetrahydroisoquinoline part of the parent structure **1** was exchanged by the pyrylium labels **6-8** giving compounds **9-11**. On the other hand, the quinaldic acid was replaced by the pyrylium labels **6-8** in *meta* and *para* position at the central aromatic core leading to compounds **12-14** and **15**. Finally, 4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-2-yl)ethyl)aniline (**5**) as a building block was linked to pyrylium label **7** to give compound **16** (Figure 2.3). As a reference compound in cell assays and confocal microscopy, the pyrylium dye **7** was conjugated with benzyl amine giving compound **17**.

In order to obtain the pyridinium compounds, the primary amines **2-5** were treated with the pyrylium dyes **6-8** at a pH of 8-9 at room temperature (Table 2.1). The course of the reaction is easily monitored by the typical hypsochromic shift of the absorption maximum by more than 100 nm as demonstrated for the labelling of NPY receptor ligands before.[23] A change in colour from blue to red indicated the end of the coupling reaction after 2.5 h. The labelled ABCG2 modulators were isolated and their purity was determined by analytical HPLC.

Table 2.1: General labelling reaction and obtained yields of synthesised compounds (structure cf. Figure 2.3).

2-5 **6-8 (Pyrylium label)** **9-17** n = 1, 2

Amine (R'-NH_2)	Pyrylium label	Product	Yield (%)
2	**6**	**9**	81
2	**7**	**10**	86
2	**8**	**11**	90
3	**6**	**12**	80
3	**7**	**13**	80
3	**8**	**14**	76
4	**7**	**15**	73
5	**7**	**16**	66
benzyl amine	**7**	**17**	78

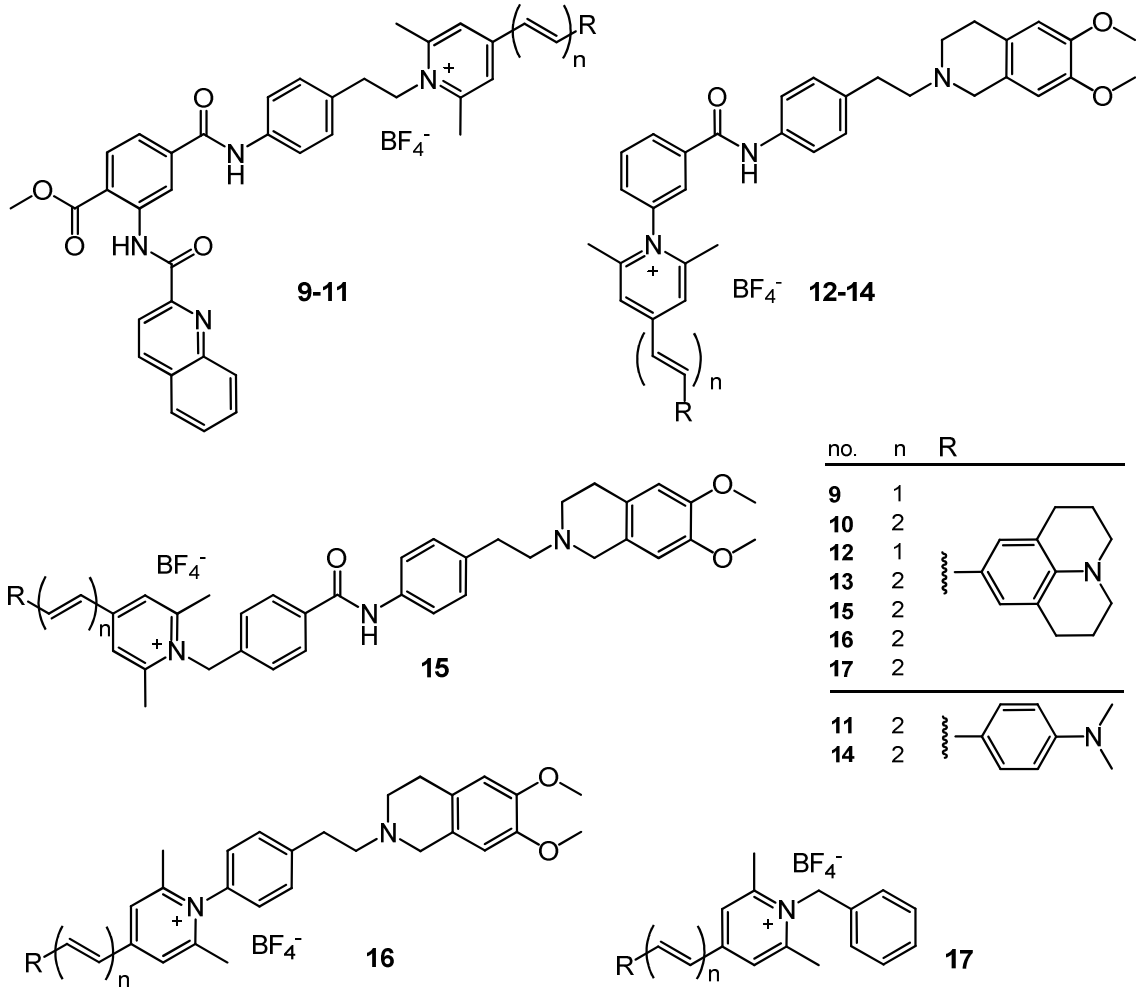
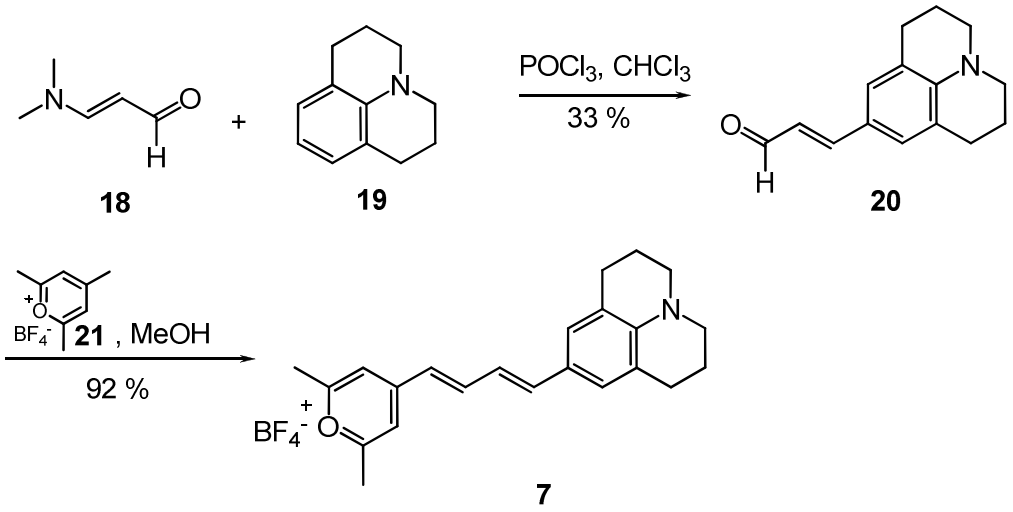


Figure 2.3: Structures of the pyrylium-labelled ABCG2 modulators **9-17**.

The modulator substructures **2**, **3**, **4** and **5** were prepared using the established synthetic route for tariquidar analogues.[7] Primary amines were Boc-protected during the synthesis. The pyrylium dyes **6** and **8** were previously reported for protein staining.[24] Dye **7**, with an extended conjugation length, was synthesised by a Vilsmeier formylation yielding the unsaturated aldehyde **20**, which was converted by a condensation reaction into the pyrylium dye **7** (Scheme 2.1).



Scheme 2.1: Synthetic route of the new pyrylium dye **7**.

2.2.2. Spectral Properties

The spectral properties of the labelled modulators were investigated and the results are summarized in Table 2.2. Absorption maxima of the pyridinium functionalized compounds range between 500 nm and 550 nm. The compounds show emission maxima between 610 nm and 710 nm (Figure 2.4).

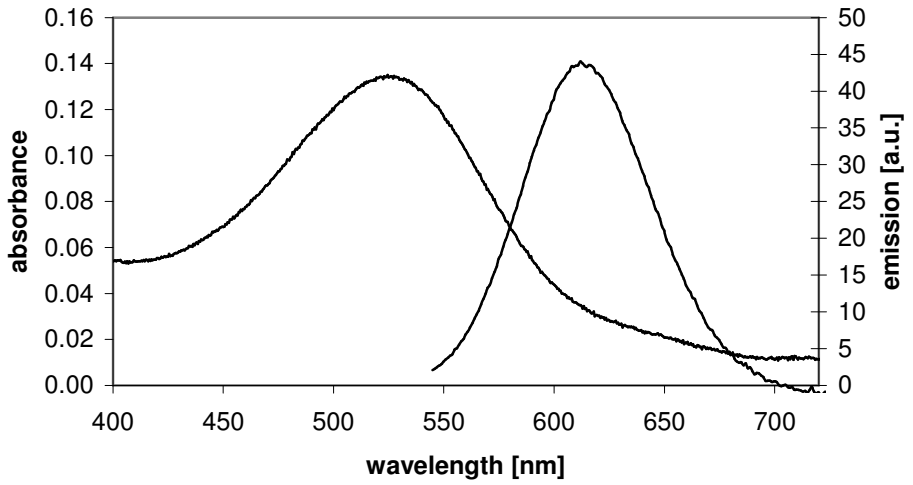


Figure 2.4: Corrected absorption and emission spectra of **9** (10 μM in PBS + 1 % BSA, T = 22 °C), absorption maximum 525 nm, emission maximum 610 nm.

The relative quantum yields were determined using cresyl violet perchlorate. As previously reported, the fluorescence intensity increases significantly in the presence of 1 % bovine serum albumin (BSA) and the quantum yields are given

for these conditions (PBS buffer BSA solution, pH 7.4).[23] The highest quantum yield of Φ = 0.31 was determined for compound **9**.

Table 2.2: Spectral properties of the labelled compounds in PBS buffer with 1 % BSA.

Compound	$\lambda_{abs.}$ [nm]	λ_{em} [nm]	Φ [%]	ε (MeOH) [$cm^{-1}M^{-1}$]
9	525	610	31.2	18199
10	545	707	3.8	1233
11	500	637	8.2	4003
12	520	609	4.1	33457
13	547	695	13.7	3647
14	500	650	3.6	1645
15	546	711	--*	7267
16	523	675	10.8	2388
17	558	706	30.9	1941

* precipitation in buffer.

The absorption and emission properties of the pyridinium labelled compounds are in principle suitable for confocal microscopy of cells or investigations on isolated tissues like brain capillaries.

2.2.3. Inhibition of the ABCG2 Transporter

The fluorescent compounds **9-17**, as well as their amino precursors **2-5** were investigated for inhibition of ABCG2 by the Hoechst 33342 assay[25] using MCF7 cells/topo cells.[7] The overexpression of ABCG2 transporters by topotecan treated MCF7 cells was checked via the flow cytometric (FACS) mitoxantrone-efflux assay.[7] The known potent and selective ABCG2 modulator **1** was used as reference.

Table 2.3: Biological activity of the labelled compounds.

Compound	IC_{50} [μM][a]	Max [%][a,b]
1	0.06 ± 0.01	63 ± 2 (12.5 μM)
2	1.67 ± 0.59	64 ± 3 (15 μM)
3	--	61 ± 2 (50 μM) [c]
4	--	27 ± 1 (50 μM) [c]
5	--[d]	--
9	4.48 ± 2.51	28 ± 1 (35 μM)
10	--	61 ± 4 (15 μM)
11	2.09 ± 1.59	37 ± 4 (50 μM)
12	--	24 ± 7 (5 μM) [e]
13	21.30 ± 9.39	51 ± 2 (100 μM)
14	16.38 ± 13.00	36 ± 1 (15 μM)
15	--	15 ± 1 (100 μM)
16	--	28 (100 μM)

[a] Mean values ± SEM, calculated from 2-3 independent experiments. [b] Maximal inhibitory effects (%) are expressed as inhibition caused by the highest concentration of the compound tested (see parentheses) relative to the inhibitory effect caused by 10 µM fumitremorgin C (100 % inhibition). [c] N = 1; [d] Inactive at concentration up to 50 µM; [e] N = 3 from 1 measurement.

The exchange of significant parts of the molecular structure **1** by the fluorescent labels markedly decreased the activity at the ABCG2 transporter. The inhibition assay shows that the 3-(acylamino)benzoic amide moiety is crucial for biological activity. The IC_{50} values of compounds **9**, **11**, **13** and **14** were in the range from of 2.0 to 21 μM (Table 2.3). The ABCG2 inhibitory potency was less affected by the exchange of the tetrahydroisoquinoline portion compared to the replacement of the quinoline-2-carboxylic acid moiety (cf. **11** versus **14**). This observation is in good agreement with investigations of partial structures of the non-labelled modulators reported before.[7]

Surprisingly, the unconjugated amine **2** was found to be cytotoxic. Compounds **3** and **5** and their pyrylium-labelled derivatives **15** and **16** showed no significant effect on the ABCG2 transporter activity.

With respect to transporter selectivity, the compounds were investigated for ABCB1 modulation in the calcein-AM microtiter assay.[26] Due to low activities of these compounds IC_{50} values could not be calculated (data not shown). The most pronounced ABCB1 inhibition was achieved with compound **10** (14.7 % maximal response at a concentration of 100 μM). Hence, all fluorescence-labelled compounds synthesised in this series show selectivity for ABCG2 compared to ABCB1.

2.2.4. Transport in Rat Brain Capillaries

The active compounds **1** and **9** were used for transport studies in isolated rat brain capillaries to determine the interaction of ABCG2 modulators with the transporter expressed at physiological concentrations. Compound **1** represents the group of the unlabelled ABCG2 modulators and compound **9** represents the group of fluorescent modulators. An increase in luminal fluorescence indicates transport of the applied fluorescent substance. Therefore, brain capillaries of rats were isolated and co-incubated with the modulator **1** and the known ABCG2 substrate prazosin labelled with fluorescent bodipy[27] (Figure 2.5). Compound **1** was applied at three different concentrations (0.001 μM, 0.1 μM and 1 μM), whereas the concentration of the bodipy-prazosin derivative was kept constant at 1 μM. In order to determine relative fluorescence, 4-10 different capillaries were analysed for each concentration. Luminal fluorescence intensity measurements were made of three areas within the central 2 μm (10 pixels) of each segment. The background fluorescence intensity was determined at three background areas and the average pixel intensity for each area was calculated. Luminal fluorescence intensity was corrected by the background intensity.

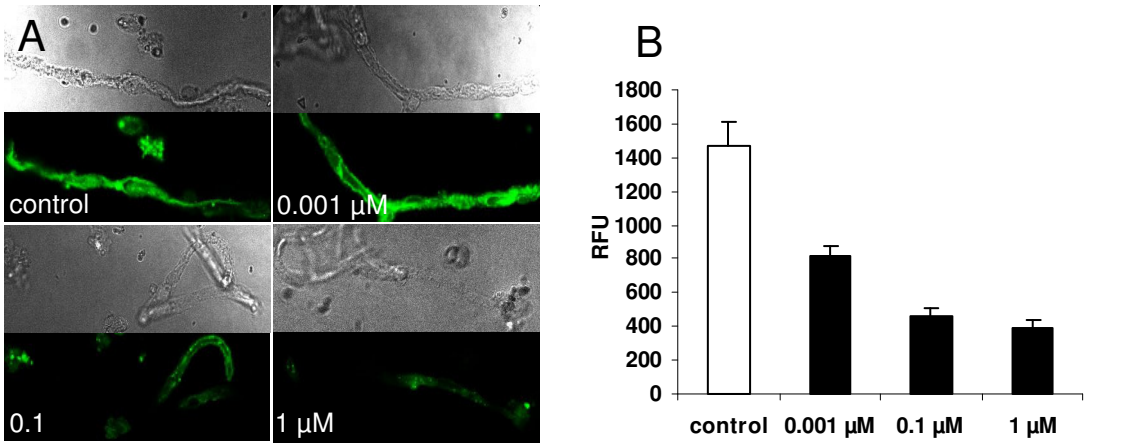


Figure 2.5: Co-incubation experiment on isolated rat brain capillaries of bodipy-prazosin (1 μM) without (control) or with increasing concentrations of compound **1** (0.001 μM, 0.100 μM, 1 μM) in PBS + 1 % BSA after 30 min of incubation; **A**: transmission light and confocal micrograph of decreasing fluorescence of bodipy-prazosin with increasing concentration of compound **1**; **B**: quantitative analysis of the effect of compound **1** on the transport of bodipy-prazosin.

In the presence of compound **1** the luminal fluorescence of co-incubated bodipy-prazosin decreased with increased modulator (**1**) concentrations, i.e. compound **1** inhibits the efflux of the ABCG2 substrate bodipy-prazosin. The results from the brain capillaries confirm the data from the Hoechst 33342 assay. Thus, the modulation of ABCG2 by compound **1** is not restricted to overexpressed transporters as on MCF-7/Topo cells, but is corroborated by the studies on physiologically expressed ABCG2 transporters in rat brain capillaries. Species-dependent differences were not detected in these experiments.

The fluorescent analogue **9** was tested on the same system using three different concentrations (1 μM, 10 μM, 50 μM). Due to lower potency of **9**, the concentrations as high as 50 μM were used for the investigations of the pyrylium-labelled compound on the same system. The luminal fluorescence of bodipy-prazosin stayed constant (Figure 2.6 A), i.e. the transport of the substrate bodipy-prazosin was not affected by compound **9** under these conditions. The luminal fluorescence of compound **9** (Figure 2.6 B) rised with increasing concentration. Additionally, the luminal fluorescence of **9** could not be decreased by co-incubation with the potent ABCG2 inhibitor Ko143, a known ABCG2 inhibitor[28] (Figure 2.6 C).

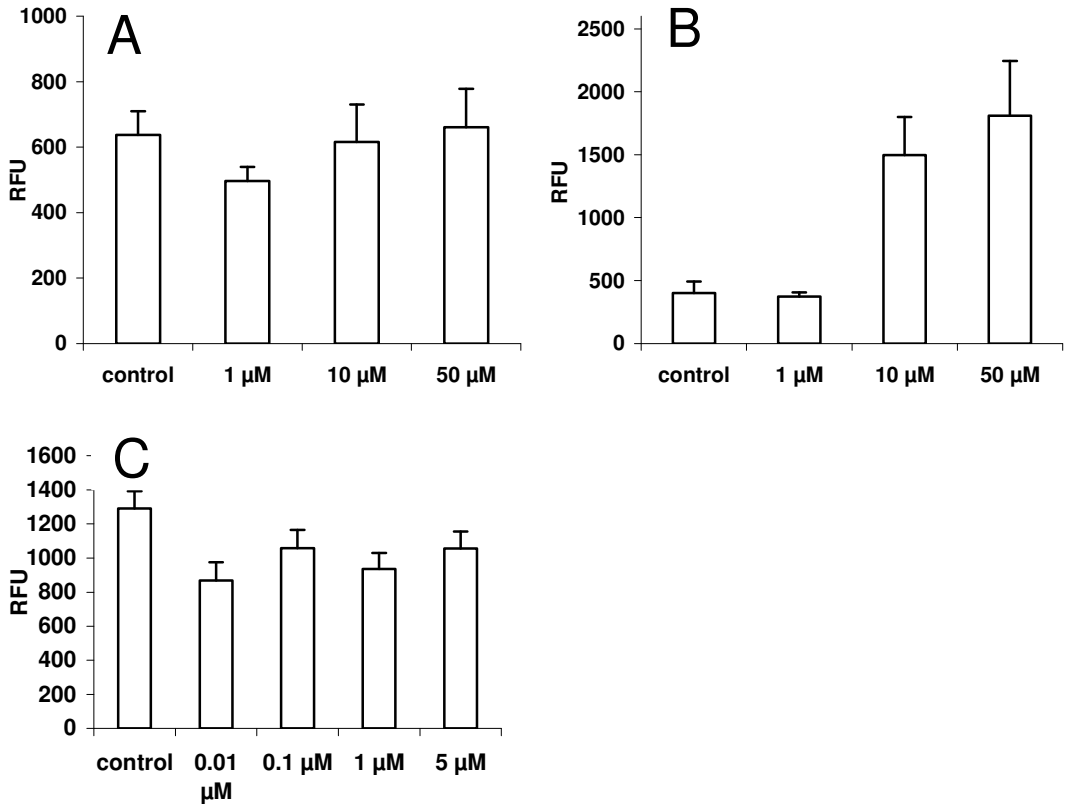


Figure 2.6: Co-incubation experiments on isolated rat brain capillaries, detection of fluorescence after 30 min of incubation; **A**: effect of compound **9** on the transport of bodipy-prazosin (1 μM) without (control) or with increasing concentrations of compound **9** (1 μM, 10 μM, 50 μM) determined by bodipy-prazosin fluorescence; **B**: fluorescence of compound **9** increasing concentrations (1 μM, 10 μM, 50 μM) without (control) or with bodipy-prazosin (1 μM); **C**: effect of Ko143 on the transport of compound **9** (1 μM) without (control) or with increasing concentrations of Ko143 (0.01 μM, 0.1 μM, 1 μM, 5 μM).

The mode of the interaction between the transporter and the modulators could not be identified so far.

2.2.5. Confocal Laser Scanning Microscopy (CLSM) Studies

Additionally, the fluorescence-labelled compounds were investigated by confocal microscopy on MCF7/Topo cells at different incubation periods of time and at different concentrations to monitor the penetration through the cell membrane. Due to weak potency of the compounds, concentrations high as 10 μM were used.

All examined compounds showed significant accumulation in the cytoplasm and gave rinse to intracellular fluorescence. The compounds were not detected in the nuclei. Compounds **15** (Figure 2.7 A) and **16**, which are almost devoid of ABCG2 inhibitory effect, entered the cells within a few minutes, whereas compounds **9** and **11** (Figure 2.7 B) penetrated slower and emitted less intensely. A selective enrichment within the cell membranes, potentially allowing for the localization of ABCG2 transporters, was not observed. Due to the positive charge of the molecules and according to previous results on pyrylium-labelled receptor ligands,[21] the rapid cellular uptake was unexpected and a carrier-mediated process, independent from the interaction with the membrane located ABCG2 transporter, cannot be ruled out. The used high concentrations (10 μM) of the fluorescent compounds may also increase the diffusion through the cell membrane. As a control, MCF-7/Topo cells were incubated with a pyrylium-labelled benzyl amine, which is considered to be neither a substrate nor an inhibitor for ABCG2. We observed for reference compound **17** also a rapid penetration of the membrane (data not shown), showing the same distribution inside the cells. Thus, the label itself contributes at least in part to the cellular uptake.

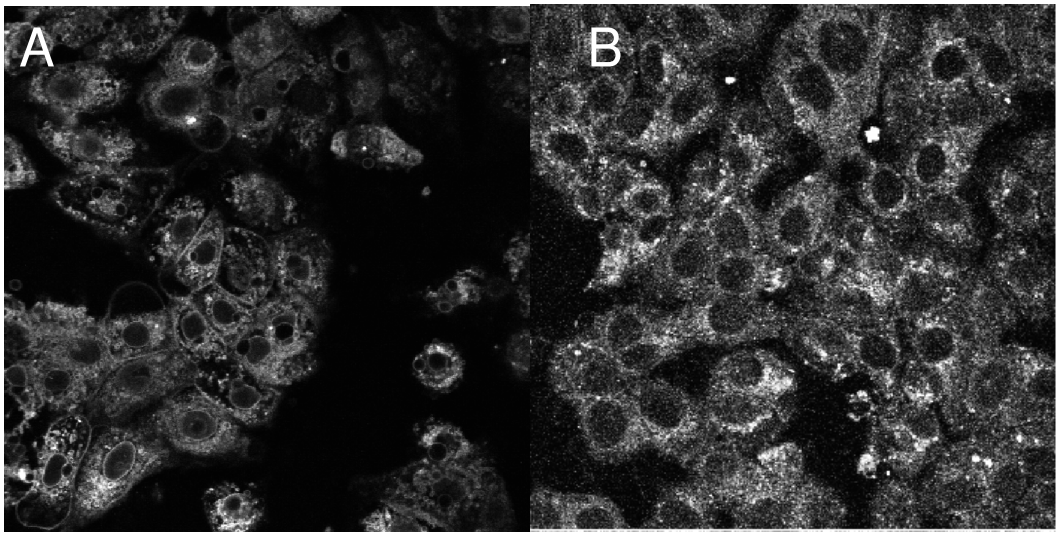


Figure 2.7: A: Compound **15**, 10 µM, incubated 30 min on MCF7/Topo cells, 153th passage; **B**: Compound **11**, 10 µM, incubated 11 min on MCF7/Topo cells, 177th passage; white pseudo-colour images.

2.3. Conclusion

We synthesised fluorescent analogues of the recently developed ABCG2 modulator **1**, which is chemically derived from the ABCB1 inhibitor tariquidar. Structural moieties of the parent structure **1** were exchanged by pyridinium chromophores which are accessible by aminolysis of known and newly synthesised pyrylium dyes. The derivatives showed emission over 600 nm and relative quantum yields up to 31.2 %. The testing for biological activity with the Hoechst 33342 assay revealed some structural activity relationship information. The ability to inhibit ABCG2 transporters of the compounds was least affected by the exchange of the tetrahydroisoquinoline part (compounds **9**-**11**), while the heteroarylcarbonylamino substituent in *meta*-position on the methyl 4-carbamoylbenzoate core was again proved to be essential for an effective interaction with the ABCG2 transporter. All prepared pyrylium derivatives showed selectivity for ABCG2 over ABCB1.

Rat brain capillary studies confirmed biological activity of the unlabelled compound **1** for natural expression levels of ABCG2 in rats, identified by inhibiting the transport of the substrate bodipy-prazosin. Due to lower potency this could not be observed from the pyrylium-labelled compounds. CLSM showed time-dependent unspecific cellular uptake of all fluorescent compounds in MCF-7/Topo cells. Here, we conclude the low potency as reason for weak interactions with the membrane located transporter protein. The hydrophobic character of the pyrylium moiety and an additional net charge make this label less suitable for mechanistic studies on ABCG2.

2.4. Experimental Section

General. Commercial reagents and starting materials were purchased from Aldrich, Alpha Aesar, Fluka or Acros and used without further purification. Flash chromatography was performed on silica gel (Merck silica gel Si 60 40-63 μm); products were detected by TLC on alumina plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm) and visualized by UV-light (λ = 254 nm). Melting points were determined with a Optimelt MPA 100 and are uncorrected. NMR spectra were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300

K) and Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, *T* = 300 K) instruments. Chemical shifts are reported in δ/ppm relative to external standards and coupling constants *J* are given in Hz. Abbreviations for the characterisation of the signals: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, dd = double doublet. The relative numbers of protons is determined by integration. Error of reported values: chemical shift 0.01 ppm (^{1}H-NMR), 0.1 ppm (^{13}C-NMR), coupling constant 0.1 Hz. The used solvent for each spectrum is reported. Mass spectra were recorded with Finnigan MAT TSQ 7000 (ESI) and Finnigan MAT 710 (EI), IR spectra with a Bio-Rad FT-IR-FTS 155 spectrometer and UV/Vis spectra with a Cary BIO 50 UV/Vis/NIR spectrometer (Varian). Analytical HPLC was performed on an Agilent 1100 LC system equipped with a Phenomenex Luna C18 (2) column (particle size 3 µm, pore size 100 A, 150 mm x 2.00 mm). The column temperature was 25 °C. Gradient elution was done with water (0.0059 % w/w TFA) (solvent A) and acetonitrile (solvent B) at a constant flow rate of 0.3 mL/min. A gradient profile with the following proportions of solvent B was applied: 0 min 5 % B, 20 min 98 % B. Compounds were detected with a diode array detector (DAD, 490 nm). The compounds were dissolved in methanol.

Synthesis. Methyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]phenylcarbamoyl}-2-(quinoline-2-carbonylamino)benzoate (**1**),[7] 4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]aniline (**5**),[29] 4-[(*tert*-butoxycarbonylamino)methyl]benzoic acid,[30] 3-(*tert*-butoxycarbonyl-amino)benzoic acid[31] and *tert*-butyl 4-aminophenethylcarbamate[32] were prepared after literature reported procedures.

General procedures: GP 1: Pyridinium formation

The respective amine (1 eq) and NEt_3 (8 eq) were diluted in acetonitrile. A solution of the pyrylium dye (1 eq) in acetonitrile was added. The reaction was monitored by TLC ($CHCl_3$/MeOH 4:1). After 2.5 h stirring at room temperature the solvent was removed yielding a purple solid.

GP 2: Amide coupling over an active ester

The respective carboxylic acid (1.1 eq) was dissolved in CH_2Cl_2. Two equivalents of DIPEA, 1.2 eq of each TBTU and HOBt were added at 0 °C. After 30 minutes the active ester was formed and the respective amine was added. The reaction mixture was stirred for 16 h, taken up with water, diluted with dichloromethane, washed three times with water (3 x 30 mL) and once with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated giving the crude reaction product.

GP 3: Reduction of the nitro function

The respective nitro compound (eq) was dissolved in ethyl acetate and methanol followed by the addition of Pd/C (10 %/g) suspended in ethyl acetate. After stirring the solution under 10 bar of H_2 for 15 h at room temperature, the catalyst was filtered off and the solvent was removed, to obtain the solid product.

GP 4: Boc deprotection

The respective Boc-protected amine (1 eq) was dissolved in dichloromethane and HCl-Ether (15 % /mmol). After stirring 15 h at room temperature the solvent was removed under reduced pressure yielding the crude reaction product.

Methyl 4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenylcarbamoyl}-2-nitro-benzoate

The compound was prepared following GP 2 using 1.5 g (6.7 mmol) of 4-(methoxycarbonyl)-3-nitrobenzoic acid and 1.4 g (6.1 mmol of *tert*-butyl 4-aminophenethylcarbamate. The product was purified by column chromatography (SiO_2, MeOH/EE 1:3, R_f = 0.65). The reaction yielded 2.4 g (5.4 mmol, 88 %) of methyl 4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenyl-carbamoyl}-2-nitrobenzoate

as orange solid, **mp** 135 °C **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.42 (s, 9 H), 2.66 (t, *J* = 6.9 Hz, 2 H), 3.28-3.35 (m, 2 H), 3.94 (s, 3 H), 7.16 (d, *J* = 8.2 Hz, 2 H), 7.57 (d, *J* = 8.5 Hz, 2 H), 7.79 (d, *J* = 7.9 Hz, 1 H), 8.20 (dd, *J* = 7.9 Hz, 1.4 Hz, 1 H), 8.43 (d, *J* = 1.4 Hz, 1 H) **^{13}C-NMR** DEPT 135 (75 MHz, $CDCl_3$): δ = 28.4(+, 3 x CH_3), 38.6 (+, CH_3), 35.7 (-, CH_2), 54.1 (-, CH_2), 91.4 (C_{quat}), 95.9 (C_{quat}), 111.3 (C_{quat}), 115.7 (C_{quat}), 121.0 (+, CH_{arom}), 122.8 (+, CH_{arom}), 129.4 (+, 2 x

CH_{arom}), 129.6 (+, CH_{arom}), 129.9 (C_{quat}), 130.3 (+, CH_{arom}), 131.8 (+, CH_{arom}), 135.7 (C_{quat}), 138.5 (C_{quat}), 148.0 (C_{quat}), 165.3 (C_{quat}) **MS** (-ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 442 (100) $[M-H^+]^-$ **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1509 (N-O), 1659 (C=O), 1685 (C=O), 1738 (C=O), 3362 (N-H), 3389 (N-H) **MF** $C_{22}H_{25}N_3O_7$ **MW** 443.45

Methyl 2-amino-4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenylcarbamoyl}benzoate

Methyl 4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenylcarbamoyl}-2-nitrobenzoate (2.8 g, 6.3 mmol) was reacted according to GP 3, yielding after work-up 2.1 g (5.15 mmol, 94 %) of methyl 2-amino-4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenyl-carbamoyl}benzoate as orange solid, **mp** 165 °C **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H), 2.58-2.68 (m, 2 H), 3.33 (t, *J* = 6.6 Hz, 2 H), 3.88 (s, 3 H), 7.00 (dd, *J* = 1.6 Hz, 8.2 Hz, 1 H), 7.16 (d, *J* = 8.2 Hz, 2 H), 7.19 (d, *J* = 1.64 Hz, 1 H), 7.55 (d, *J* = 8.2 Hz, 2 H), 7.90 (d, *J* = 8.5 Hz, 1 H) **^{13}C-NMR** DEPT (75 MHz, $CDCl_3$): δ = 28.4 (+, 3 x CH_3), 38.6 (+, CH_3), 58.4 (-, CH_2, 2 x), 111.0 (C_{quat}), 112.9 (C_{quat}), 113.6 (+, CH_{arom}), 115.7 (+, CH_{arom}), 155.9 (C_{quat}), 120.7 (+, 2 x CH_{arom}), 121.3 (C_{quat}), 123.7 (C_{quat}), 129.4 (+, 2 x CH_{arom}), 129.6 (C_{quat}), 131.9 (+, CH_{arom}), 150.6 (C_{quat}), 159.1 (C_{quat}), 168.0 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 414 (100) $[MH^+]$ **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1684 (C=O), 3319 (N-H), 3372 (N-H), 3481 (NH_2) **MF** $C_{22}H_{27}N_3O_5$ **MW** 413.47

Methyl 4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenylcarbamoyl}-2-(quinoline-2-carbonylamino)benzoate

Methyl 2-amino-4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]phenylcarbamoyl}-benzoate (1.8 g, 4.5 mmol) was dissolved in dry DMF, then 1.5 eq of NEt_3 (0.67 g, 0.96 mL, 6.7 mmol) and 0.5 eq of DMAP (0.26 g, 2.2 mmol) were added. Under nitrogen atmosphere quinoline-2-carbonyl chloride (1.28 g, 6.7 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. Then the DMF was removed, the residue was taken up with water and extracted with dichloromethane. The remaining white precipitate was collected and washed with acetone. The reaction yielded 0.4 g (0.7 mmol, 15 %) of methyl 4-{4-[2-(*tert*-butoxycarbonylamino)ethyl]-phenylcarbamoyl}-2-

(quinoline-2-carbonylamino)benzoate as white solid, **mp** > 190 °C **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.44 (s, 9 H), 2.80 (t, *J* = 6.7 Hz, 2 H), 3.37-3.39 (m, 2 H), 4.09 (s, 3 H), 7.21 (d, *J* = 8.2 Hz, 2 H), 7.65 (d, *J* = 8.5 Hz, 2 H), 7.83 (t, *J* = 7.7 Hz, 1 H), 7.91 (d, *J* = 8.3 Hz, 2 H), 8.21 (d, *J* = 8.2 Hz, 1 H), 8.29-8.39 (m, 4 H), 9.45 (s, 1 H), 13.35 (s, CONH) **^{13}C-NMR** DEPT 135 (75 MHz, [D_6]-DMSO): δ = 28.2 (+, CH_3, three times), 34.8 (-, CH_2), 35.7 (+, CH_3), 41.5 (-, CH_2), 77.4 (C_{quat}), 118.5 (+, CH_{arom}), 119.4 (+, CH_{arom}), 120.4 (+, 2 x CH_{arom}), 121.8 (+, CH_{arom}), 128.1 (+, CH_{arom}), 128.6 (C_{quat}), 128.7 (+, 2 x CH_{arom}), 129.0 (C_{quat}), 129.2 (+, CH_{arom}), 130.9 (+, CH_{arom}), 131.1 (+, CH_{arom}), 135.0 (C_{quat}), 136.8 (C_{quat}), 138.5 (+, CH_{arom}), 139.7 (C_{quat}), 140.3 (C_{quat}), 140.5 (+, CH_{arom}), 145.6 (C_{quat}), 149.1 (C_{quat}), 162.2 (C_{quat}), 162.7 (C_{quat}), 164.5 (C_{quat}), 166.7 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 569 (100) [MH^+] **IR** $\tilde{\nu}$ (ATR) [cm^{-1}] = 1651 (C=O), 1688 (C=O), 3299 (N-H), 3372 (N-H) **MF** $C_{32}H_{32}N_4O_6$ **MW** 568.62

Methyl 4-[4-(2-aminoethyl)phenylcarbamoyl]-2-(quinoline-2-carbonyl-amino)benzoate (2)

The compound was prepared following GP 4 using methyl 4-{4-[2-(*tert*-butoxy-carbonylamino)ethyl]phenylcarbamoyl}-2-(quinoline-2-carbonylamino)benzoate (0.4 g, 0.7 mmol) and 4.6 mL HCl/ether. The reaction yielded 0.31 g (0.66 mmol, 95 %) of compound **2** as yellow solid, **mp** >200 °C **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 2.97-3.10 (m, 2 H), 3.51-3.60 (m, 2 H), 4.04 (s, 3 H), 7.28 (d, *J* = 8.5 Hz, 1 H), 7.76 (d, *J* = 8.5 Hz, 2 H), 7.80 (s, 1 H), 7.97 (d, *J* = 7.7 Hz, 1 H), 8.07 (s, 2 H), 8.15 (d, *J* = 8.2 Hz, 1 H), 8.20 (d, *J* = 8.2 Hz, 1 H), 8.31 (d, *J* = 8.5 Hz, 1 H), 8.69 (d, *J* = 8.8 Hz, 2 H), 9.37 (s, 1 H) **^{13}C-NMR** DEPT 135 (75 MHz, [D_6]-DMSO): δ = 32.3 (-, CH_2), 35.7 (+, CH_3), 39.9 (-, CH_2), 118.4 (+, CH_{arom}), 119.5 (+, CH_{arom}), 120.6 (+, 2 x CH_{arom}), 121.9 (+, CH_{arom}), 128.1 (+, CH_{arom}), 128.6 (C_{quat}), 128.8 (+, 2 x CH_{arom}), 129.0 (C_{quat}), 129.2 (+, CH_{arom}), 130.9 (+, CH_{arom}), 131.1 (+, CH_{arom}), 132.8 (C_{quat}), 137.4 (C_{quat}), 138.5 (+, CH_{arom}), 139.7 (C_{quat}), 140.1 (C_{quat}), 141.7 (+, CH_{arom}), 145.6 (C_{quat}), 149.0 (C_{quat}), 162.7 (C_{quat}), 164.6 (C_{quat}), 166.7 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 469 (100) [MH^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1570 (C=O), 1655 (C=O), 1688 (C=O), 3300 (N-H), 3406 (N-H) **MF** $C_{27}H_{24}N_4O_4$ **MW** 468.5

***tert*-Butyl 3-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]-phenylcarbamoyl}phenylcarbamate**

The compound was prepared following GP 2 using 3-(*tert*-butoxycarbonylamino)benzoic acid (0.81g, 3.4 mmol) and 4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl]aniline (0.99 g, 3.2 mmol). The product was purified by column chromatography (MeOH/EE 1:3, R_f = 0.58). The reaction yielded 0.61 g (1.1 mmol, 36 %) of *tert*-butyl 3-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]phenylcarbamoyl}phenylcarbamate as yellow solid, **mp** 177 °C **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.50 (s, 9 H), 2.73-2.90 (m, 8 H), 3.66 (s, 2 H), 3.8 (s, 3 H), 3.82 (s, 3 H), 6.51 (s, 1 H), 6.60 (s, 1 H), 7.14 (s, 1 H), 7.18 (d, 8.5 Hz, 2H), 7.32 (t, 8.0 Hz, 1 H), 7.51 (t, 8.4 Hz, 1 H), 7.56 (d, 8.5 Hz, 2 H), 7.97 (s, 1 H) **^{13}C-NMR** DEPT 135 (75 MHz, $CDCl_3$): δ = 28.3 (+, 3 x CH_3), 33.2 (-, CH_2), 36.5 (-, CH_2), 50.9 (-, CH_2), 55.5 (-, CH_2), 55.8 (+, CH_3), 55.9 (+, CH_3), 59.9 (-, CH_2), 80.8 (C_{quat}), 109.4 (+, CH_{arom}), 111.3 (+, CH_{arom}), 117.1 (+, CH_{arom}), 120.7 (+, 2 x CH_{arom}), 121.7 (+, CH_{arom}), 121.9 (+, CH_{arom}), 125.9 (C_{quat}), 126.0 (C_{quat}), 129.2 (+, 2 x CH_{arom}), 129.3 (+, CH_{arom}), 135.9 (C_{quat}), 136.2 (C_{quat}), 136.3 (C_{quat}), 138.9 (C_{quat}), 147.2 (C_{quat}), 147.6 (C_{quat}), 153.0 (C_{quat}), 165.8 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 530 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1667 (C=O), 1716 (C=O), 3238 (N-H), 3353 (N-H) **MF** $C_{31}H_{37}N_3O_5$ **MW** 529.67

3-Amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]-phenyl}benzamide (3)

The compound was prepared following GP 4 using *tert*-butyl 3-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]phenylcarbamoyl}phenylcarbamate (0.5 g, 0.9 mmol) and 6 mL HCl/ether. The reaction gave 0.37 g (0.85 mmol, 98 %) of compound **3** as yellow solid, **mp** 160 °C **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 3.00-3.38 (m, 10 H), 3.72 (s, 3 H), 3.73 (s, 3 H), 6.78 (s, 1 H), 6.81 (s, 1 H), 7.30 (d, *J* = 8.5 Hz, 2 H), 7.60-7.66 (m, 2 H), 7.76 (d, *J* = 8.5 Hz, 2 H), 7.92 (d, *J* = 7.1 Hz, 1 H), 8.03 (d, *J* = 7.4 Hz, 1 H) **^{13}C-NMR** DEPT 135 (75 MHz, [D_4]-methanol): δ = 20.7 (-, CH_2), 26.2 (-, CH_2), 31.1 (-, CH_2), 51.5 (-, CH_2), 56.6 (+, CH_3), 56.8 (+, CH_3), 58.34 (-, CH_2), 110.9 (+, CH_{arom}), 112.7 (+, CH_{arom}), 120.9 (C_{quat}), 122.8 (C_{quat}), 122.9 (+, CH_{arom}), 123.0 (+, CH_{arom}), 123.9 (+, CH_{arom}), 124.6 (C_{quat}), 127.7 (C_{quat}), 129.1 (C_{quat}), 129.3 (+, CH_{arom}), 131.0

(+, CH_{arom}), 131.3 (+, CH_{arom}), 131.6 (+, CH_{arom}), 131.7 (+, CH_{arom}), 132.6 (C_{quat}), 149.8 (C_{quat}), 150.6 (C_{quat}), 165.5 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 432 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 3348 (N-H), 3233 (N-H), 1667 (C=O) **MF** $C_{26}H_{29}N_3O_3$ **MW** 431.53

tert-Butyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]-phenylcarbamoyl}benzylcarbamate

The compound was prepared following GP 2 using 4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]aniline (0.9 g, 2.9 mmol) and 4-[(*tert*-butoxycarbonylamino)methyl]benzoic acid (0.8 g, 3.2 mmol). The reaction gave 1.35 g (2.4 mmol, 85 %) of *tert*-butyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl]phenylcarbamoyl}benzylcarbamate as orange oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.33 (s, 9 H), 2.63-2.76 (m, 8 H), 3.52 (s, 2 H), 3.66 (s, 3 H), 3.68 (s, 3 H), 4.11 (d, *J* = 5.7 Hz, 2 H), 6.38 (s, 1 H), 6.45 (s, 1 H), 7.02-7.07 (m, 4 H), 7.54 (d, *J* = 8.23 Hz, 2 H), 7.66 (d, *J* = 7.68 Hz, 2 H) **^{13}C-NMR** DEPT 135 (75 MHz, $CDCl_3$): δ = 28.4 (+, 3 x CH_3), 33.1 (-, CH_2), 43.9 (-, CH_2), 50.8 (-, CH_2), 55.4 (-, CH_2), 55.8 (+, CH_3), 55.9 (+, CH_3), 59.9 (-, CH_2), 109.4 (+, CH_{arom}), 111.3 (+, CH_{arom}), 120.8 (+, CH_{arom}), 125.9 (C_{quat}), 126.9 (+, 2 x CH_{arom}), 127.5 (+, 2 x CH_{arom}), 129.0 (+, 2 x CH_{arom}), 130.8 (+, CH_{arom}), 133.6 (C_{quat}), 135.8 (C_{quat}), 136.7 (C_{quat}), 143.4 (C_{quat}), 147.1 (C_{quat}), 147.5 (C_{quat}), 156.2 (C_{quat}), 162.6 (C_{quat}), 165.6 (C_{quat}), 166.0 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 546 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1513 (N-H), 1612 (C=O), 1663 (C=O) **MF** $C_{32}H_{39}N_3O_5$ **MW** 545.29

4-Aminomethyl-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)-ethyl]phenyl}benzamide (4)

The compound was prepared following GP 4 using *tert*-butyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl]phenylcarbamoyl}benzylcarbamate (0.3 g, 0.5 mmol) and 3.3 mL HCl/ether. The resulting pale orange solid was recrystallised from acetone and water to yield 0.2 g (0.44 mmol, 90 %) of compound **4** as yellow solid, **mp** >190 °C **^{1}H-NMR** (300 MHz, [D_4]-methanol): δ = 2.95-3.39 (m, 10 H), 3.81 (s, 3 H), 3.82 (s, 3 H), 4.22 (s, 2 H), 6.80 (s, 1 H), 6.83 (s, 1 H), 7.37 (d, *J* = 8.5 Hz, 2 H), 7.63 (d, *J* = 8.2 Hz, 2 H), 7.72 (d, *J* = 8.5 Hz, 2 H), 8.02 (d, *J* = 8.2 Hz, 2 H) **^{13}C-NMR** DEPT 135 (75 MHz, [D_4]-methanol):

δ = 26.2 (-, CH_2), 31.0 (-, CH_2), 43.9 (-, CH_2), 51.5 (-, CH_2), 54.1 (-, CH_2), 56.5 (+, CH_3), 56.6 (+, CH_3), 58.3 (-, CH_2), 110.8 (+, CH_{arom}), 112.7 (+, CH_{arom}), 120.8 (C_{quat}), 122.9 (+, 2 x CH_{arom}), 124.4 (C_{quat}), 129.5 (+, 2 x CH_{arom}), 130.3 (+, 2 x CH_{arom}), 130.4 (+, 2 x CH_{arom}), 133.9 (C_{quat}), 136.8 (C_{quat}), 138.2 (C_{quat}), 139.0 (C_{quat}), 146.9 (C_{quat}), 150.8 (C_{quat}), 168.2 (C_{quat}) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 446 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1515 (N-H), 1612 (C=O), 3380 (NH_2) **MF** $C_{27}H_{31}N_3O_3$ **MW** 445.24

4-[(1*E*,3*E*)-4-(1,2,3,5,6,7-Hexahydropyrido[3,2,1-ij]quinolin-9-yl)buta-1,3-dienyl]-2,6-dimethylpyrylium tetrafluoroborate (7)

(*E*)-3-(2,3,6,7-Tetrahydro-1*H*,5*H*-pyrido[3,2,1-ij]quinolin-9-yl)propenal (**20**) (50 mg, 0.21 mmol) and 2,4,6-trimethylpyrylium (60 mg, 0.29 mmol) were diluted in methanol with a small amount of chloroform for better solubility. After the solution has turned green, the mixture was refluxed for 10 min and then the solvent was removed. The crude product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH 4:1, R_f = 0.65). The reaction yielded 80 mg (0.19 mmol, 92 %) of compound **7** as dark blue solid, **mp** > 200 °C **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.94-2.00 (m, 4 H), 2.07 (s, 3 H), 2.16 (s, 3 H), 2.75 (t, *J* = 6.6 Hz, 4 H), 3.12 (t, *J* = 5.6 Hz, 4 H), 6.48-6.56 (m, 1 H), 6.77 (d, *J* = 7.4 Hz, 1 H) 6 aromatic protons exchanged to the solvent **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 332 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 2927 (C-H) **UV** λ_{max} = 500 nm, ε = 4069 L*$mol^{-1}cm^{-1}$ (in PBS-buffer) **Fluorescence**: λ_{em} = 679 nm, Φ = 12.1 % (in PBS-buffer) **MF** $C_{23}H_{26}BF_4NO$ **MW** 419.26

(*E*)-4-[2-(1,2,3,5,6,7-Hexahydropyrido[3,2,1-ij]quinolin-9-yl)vinyl]-1-{4-[4-(methoxycarbonyl)-3-(quinoline-2-carboxamido)benzamido]phenethyl}-2,6-dimethylpyridinium tetrafluoroborate (9)

The compound was prepared following GP 1 using 4 mg (0.01 mmol) of compound **2** and 3 mg (0.01 mmol) of compound **6**. The reaction yielded 5 mg (0.006 mmol, 81 %) of compound **9** as purple solid. Due to the small scale, purity was checked by HPLC (86 %, t_r = 16.8 min), **mp** 152 °C **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 756 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1519 (N-H), 1573 (N-H), 2501 (N^+-$(C)_3$) **UV** λ_{max1} = 512 nm (MeOH), ε = 18199

$L^*mol^{-1}cm^{-1}$, λ_{max2} = 622 nm (MeOH), ε = 11672 $L^*mol^{-1}cm^{-1}$ **MF** $C_{48}H_{46}N_5O_4^+$ BF_4^- **MW** 843.91

4-[(1*E*,3*E*)-4-(1,2,3,5,6,7-Hexahydropyrido[3,2,1-ij]quinolin-9-yl)buta-1,3-dienyl]-1-{4-[4-(methoxycarbonyl)-3-(quinoline-2-carboxamido)-benzamido]phenethyl}-2,6-dimethylpyridinium tetrafluoroborate (10)

The compound was prepared following GP 1 using 17 mg (0.04 mmol) of compound **2** and 15 mg (0.04 mmol) of compound **7**. The reaction yielded 10 mg (0.01 mmol, 86 %) of compound **10** as purple solid. Due to the small scale, purity was checked by HPLC (80 %, t_r = 17.5 min), **mp** 160 °C **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 782 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1474 (N-H), 1573 (N-H), 2457 (N^+-$(C)_3$), 2498 (N^+-$(C)_3$) **UV** λ_{max} = 490 nm (MeOH), ε = 4004 $L^*mol^{-1}cm^{-1}$ **MF** $C_{50}H_{48}N_5O_4^+$ BF_4^- **MW** 869.95

4-{[1*E*,3*E*]-4-[4-(Dimethylamino)phenyl]buta-1,3-dienyl}-1-{4-[4-(methoxy-carbonyl)-3-(quinoline-2-carboxamido)benzamido]phenethyl}-2,6-dimethylpyridinium tetrafluoroborate (11)

The compound was prepared following GP 1 using 4 mg (0.01 mmol) of compound **2** and 5 mg (0.01 mmol) of compound **8**. The reaction gave 4 mg (0.01 mmol, 90 %) of compound **11** as red solid. Due to the small scale, purity was checked by HPLC (85 %, t_r = 16.1 min), **mp** 145 °C **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 730 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 2359 (N-$(C)_3$), 2498 (N^+-$(C)_3$), 2603 (N^+-$(C)_3$) **UV** λ_{max} = 530 nm (MeOH), ε = 1233 $L^*mol^{-1}cm^{-1}$ **MF** $C_{46}H_{44}N_5O_4^+$ BF_4^- **MW** 817.87

(*E*)-1-{3-[4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenylcarbamoyl]phenyl}-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium tetrafluoroborate (12)

The compound was prepared following GP 1 using 5 mg (0.01 mmol) of compound **3** and 4 mg (0.01 mmol) of compound **6**. The reaction yielded 6 mg (0.008 mmol, 80 %) of compound **12** as red solid. Due to the small scale, purity was checked by HPLC. (81 %, t_r = 12.4 min), **mp** >190 °C **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 720 (100) [MH^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}]

= 1518 (N-H), 2340 (N-$(C)_3$), 2356 (N-$(C)_3$), 2944 (N^+-H), 2605 (N^+-H) **UV** λ_{max} = 520 nm (MeOH), ε = 401 L*$mol^{-1}cm^{-1}$ **MF** $C_{47}H_{51}BF_4N_4O_3$ **MW** 822.97

1-{3-[4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-phenylcarbamoyl]phenyl}-4-[(1*E*,3*E*)-4-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)buta-1,3-dienyl]-2,6-dimethylpyridinium tetrafluoroborate (13)

The compound was prepared following GP 1 using 6 mg (0.01 mmol) of compound **3** and 6 mg (0.01 mmol) of compound **7**. The reaction yielded 10 mg (0.01 mmol, 80 %) of compound **13** as purple solid. Due to the small scale, purity was checked by HPLC (74 %, t_r = 12.0 min), **mp** 187 °C **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 746 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1668 (C=O), 2342 (N-$(C)_3$), 2360 (N-$(C)_3$) **UV** λ_{max} = 551 nm (MeOH), ε = 3647 L*$mol^{-1}cm^{-1}$ **MF** $C_{49}H_{53}BF_4N_4O_3$ **MW** 832.97

1-{3-[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl)phenyl-carbamoyl]phenyl}-4-[(1*E*,3*E*)-4-[4-(dimethylamino)phenyl]buta-1,3-dienyl]-2,6-dimethylpyridinium tetrafluoroborate (14)

The compound was prepared following GP 1 using 5 mg (0.01 mmol) of compound **3** and 3 mg (0.01 mmol) of compound **8**. The reaction yielded 6 mg (0.008 mmol, 76 %) of compound **14** as red solid. Due to the small scale, purity was checked by HPLC. (83 %, t_r = 12.6 min), **mp** >190 °C **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 693 (100) [MH^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1518 (N-H), 1652 (C=O), 2263 (N-$(C)_3$), 2356 (N-$(C)_3$), 2463 (N^+-H) **UV** λ_{max} = 490 nm (MeOH), ε = 1654 L*$mol^{-1}cm^{-1}$ **MF** $C_{45}H_{49}BF_4N_4O_3$ BF_4^- **MW** 780.9

1-{4-[4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-phenylcarbamoyl]benzyl}-4-[(1*E*,3*E*)-4-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)buta-1,3-dienyl]-2,6-dimethylpyridinium tetrafluoroborate (15)

The compound was prepared following GP 1 using 50 mg (0.11 mmol) of compound **4** and 51 mg (0.11 mmol) of compound **7**. The reaction gave 70 mg (0.08 mmol, 73 %) of compound **15** as purple solid. Due to the small scale, purity was checked by HPLC (82 %, t_r = 12.0 min), **mp** >190 °C **MS** (+ESI,

DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 760 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1686 (C=O), 3326 (N^+-H), 3362 (N-H), 3477 (N-H) **UV** λ_{max} = 546 nm (MeOH), ε = 7267 L*$mol^{-1}cm^{-1}$ **MF** $C_{50}H_{55}N_4O_3^+$ BF_4^- **MW** 846.43

1-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]phenyl}-4-[(1*E*,3*E*)-4-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)buta-1,3-dienyl]-2,6-dimethylpyridinium tetrafluoroborate (16)

The compound was prepared following GP 1 using 4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)benzenamine (20 mg, 0.06 mmol), NEt_3 (30 mg, 0.3 mmol) and the pyrylium dye **7** (25 mg, 0.06 mmol) in acetonitrile. The reaction yielded 25 mg (0.04 mmol, 66 %) of compound **16** as violet solid. HPLC purity (78 %, t_r = 11.9 min), **mp** > 200 °C **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.91-1.99 (m, 4 H), 2.72-2.76 (m, 4 H), 2.80-2.85 (m, 8 H), 2.86 (s, 3 H), 2.97 (s, 3 H), 3.67 (s, 2 H), 3.82 (s, 3 H), 3.83 (s, 3 H), 6.52 (s, 1 H), 6.59 (s, 1 H), 6.63 (d, Hz, 2 H), 6.76 (d, Hz, 1 H), 7.02 (d, Hz, 2 H), 8 aromatic protons exchanged to the solvent **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 626 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 2828 (C-H), 1609 (C-N), 1258 (C-O) **UV** λ_{max} = 523 nm, ε = 2606 L*$mol^{-1}cm^{-1}$ (in PBS-buffer) **Fluorescence**: λ_{em} = 673 nm, Φ = 10.8 % (in PBS-buffer) **MF** $C_{42}H_{48}N_3O_2^+$ BF_4^- **MW** 713.85

1-benzyl-4-[(1*E*,3*E*)-4-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)buta-1,3-dienyl]-2,6-dimethylpyridinium tetrafluoroborate (17)

The compound was prepared following GP 1 using 25 mg (0.2 mmol) of benzyl amine and 8 mg (0.02 mmol) of compound **7**. The reaction yielded 8 mg (0.01 mmol, 78 %) of compound **17** as purple solid. Due to the small scale, purity was checked by HPLC. (85 %, t_r = 14.5 min), **mp** 59 °C (decomposition) **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 421 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 2979 (N^+-H), 2923 (N^+-H) **UV** λ_{max} = 542 nm (MeOH), ε = 1941 L*$mol^{-1}cm^{-1}$ **MF** $C_{30}H_{33}N_2^+BF_4^-$ **MW** 508.6

(*E*)-3-(2,3,6,7-Tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)propenal (20)

To a solution of 1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinoline (0.2 g, 1.2 mmol) and 3-dimethylaminoacrolein (0.1 g, 0.1 mL, 1.2 mmol) in chloroform a solution of phosphorylchloride (0.18 g, 0.11 mL, 1.2 mmol) in chloroform was added

dropwise. The red reaction mixture was stirred for 15 h at room temperature turning blue and was subsequently heated for two hours to 60 °C before the solvent was removed. Under ice-cooling the residue was dissolved in methanol, hydrolysed with ice and quenched with 5 mL of a 5 N NaOH. Subsequently the product was extracted with chloroform. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The dark red crude product was further purified by recrystallisation from acetone and hexane. The reaction yielded 0.1 g (0.4 mmol, 33 %) of compound **20** as red oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.95 (m, 4 H), 2.73 (t, *J* = 6.3 Hz, 4 H), 3.25 (t, *J* = 5.7 Hz, 4 H), 6.45 (dd, *J* = 15.6 Hz, *J* = 7.9 Hz, 1 H), 7.00 (s, 2 H), 7.26 (d, *J* = 15.4 Hz, 1 H), 9.52 (d, *J* = 7.9 Hz, 1 H) **^{13}C-NMR** DEPT 135 (75 MHz, $CDCl_3$): δ = 20.4 (-, two times CH_2), 26.6 (-, 2 x CH_2), 48.9 (-, 2 x CH_2), 119.6 (2 x C_{quat}), 119.9 (C_{quat}), 121.2 (+, CH), 127.2 (+, 2 x CH_{arom}), 144.7 (C_{quat}), 153.5 (+, CH), 192.6 (+, CHO) **MS** (EI): m/z (%) = 227.2 (100) [M^+] **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 2929 (C-H), 2839 (C-H), 1637 (C=O) **UV** ($CHCl_3$) λ_{max} = 412 nm, ε = 15920 L*$mol^{-1}$$cm^{-1}$ **MF** $C_{15}H_{17}NO$ **MW** 227.30

Fluorescence and UV measurements of pyrylium dyes. The determination of quantum yields and the recording of fluorescence spectra were performed with a Cary Eclipse spectrofluorimeter (Varian Inc., Mulgrave, Victoria, Australia). The excitation spectrum of antagonist 4 was scanned from 400 nm to 650 nm (slit = 5 nm), the emission was detected at 660 nm (slit = 10 nm). The emission spectrum of antagonist 4 was scanned from 540 nm to 860 nm (slit = 5 nm), the excitation wavelength was set to 530 nm (slit = 10 nm). For both, excitation and emission spectra, the photomultiplier voltage was set to 400 V. For the determination of quantum yields, cresyl violet perchlorate was used as a standard fluorophore, for which a quantum yield of 54 % in ethanol was reported.[33] It is important to take the perchlorate, because only this salt is provided with sufficient purity.

All spectra were recorded in acryl cuvettes. The stock solutions (1 mM) of the fluorescent probes and the standard cresyl violet were prepared in DMSO. These solutions were diluted to yield a final concentration of 2 μM for **7**, 1 μM for **9**, 50 μM for **10**, 10 μM for **11**, 50 μM for **12**, 50 μM for **13**, 50 μM for **14**, 100 μM for **15**, 50 μM for **16**, 10 μM for **17** and 2.5 μM for the standard. Two

solutions were prepared of every fluorescent ligand: one in PBS buffer and the other one in PBS and an additional 1 % of BSA in order to determine the influence of serum albumin on the quantum yield. The cresyl violet standard was diluted in ethanol. For the determination of the reference spectra the pure solvents were used with the same protein and DMSO content, but without fluorescent compounds. Every solution was maintained in the dark and thermo stated to 22 °C.

The emission spectra were recorded at a temperature of 22 °C using a medium scan rate and a photomultiplier voltage of 400 V. The filter settings were “auto” for the excitation and “open” for the emission filter. Excitation wavelengths were set to 500 nm for **7**, 525 nm for **9**, 545 nm for **10**, 500 nm for **11**, 520 nm for **12**, 547 nm for **13**, 500 nm for **14**, 523 nm for **16**, 558 nm for **17** and 600 nm for cresyl violet. The excitation slit was set to 10 nm and the emission slit was set to 5 nm. A wavelength near the excitation maximum or at a plateau of the excitation spectrum was used for excitation. The excitation of the fluorescent compounds in the steep part of the excitation spectrum was strictly avoided. In addition to every emission spectrum the corresponding reference spectrum was subtracted to yield the net spectra, which were multiplied with the corresponding lamp correction spectra. The resulting corrected net spectra were integrated in the ranges described above. The absorbance at the excitation wavelength was determined by recording absorption spectra using a Cary 100 (Varian) UV/Vis photometer. Baselines were recorded by using reference solutions and subtracted from the raw spectra. The quantum yield was calculated according to the following formula:

$$\Phi_{F(X)} = \left(\frac{A_S}{A_X}\right) \cdot \left(\frac{F_X}{F_S}\right) \cdot \left(\frac{n_X}{n_S}\right)^2 \Phi_{F(S)}$$

A_S and F_S are the absorbance and the integral of the emission spectrum of the cresyl violet standard solution. A_X and F_X stand for the absorbance and the integral of the emission spectrum of the fluorescent ligand. The refraction indices of the solvents for the fluorescent ligands and the cresyl violet standard are symbolized by n_X and n_S, respectively. $\Phi_{F(S)}$ is the reported quantum yield of cresyl violet, in this case 54 %.

Drugs and chemicals used for assays. A Milli-Q system (Millipore, Eschborn, Germany) was used for the purification of water in aqueous drug solutions. All chemicals used were of analytical grade, if not otherwise mentioned. Hoechst 33342 (Invitrogen, Karlsruhe, Germany) was dissolved in sterile water to produce a 1.6 mM working solution. Test compounds were dissolved in DMSO (Merck, Darmstadt, Germany) at a concentration of 10 mM. All stocks were stored at -20 °C. Topotecan stocks were prepared by diluting Hycamtin (GlaxoSmithKline, Munich, Germany) in 70 % ethanol to a concentration of 0.1 mM and stored at 4 °C. PBS (phosphate buffered saline) was made of 8.0 g/L NaCl, 1.0 g/L $Na_2HPO_4 \cdot 2\ H_2O$, 0.20 g/L KCl, 0.20 g/L KH_2PO_4 and 0.15 g/L $NaH_2PO_4 \cdot H_2O$. The pH-value was adjusted to 7.3 - 7.4 by using a 1 M NaOH or HCl solution. Phosphate buffered saline with calcium and magnesium was made by dissolving 0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8.0 g/L NaCl, 1.15 g/L $Na_2HPO_4 \cdot 2\ H_2O$ in water followed by adding 0.132 g/L of $CaCl_2 \cdot 2\ H_2O$ and 0.10 g/L of $MgCl_2 \cdot 6\ H_2O$. Adjusting the pH-value to 7.3 was performed by the dropwise addition of a 1 M NaOH solution. Fumitremorgin C (FTC, gift of Dr. Susan Bates, NIH) was also dissolved in DMSO and diluted to a concentration of 1 mM. A solution of 3 % (m/m) of paraformaldeyde (Merck, Darmstadt, Germany) in Ca^{2+}/Mg^{2+} containing PBS was made by stirring 1.5 g of paraformaldehyde per 50 g total solution while heating on a magnetic stirrer for approximately 30 min.

Cell lines and culture conditions. MCF-7/Topo cells, an ABCG2 overexpressing subclone of MCF-7 breast cancer adenocarcinoma cells (ATTC HTB-22), were obtained by passaging the MCF-7 cells with increasing concentrations of topotecan in the culture medium to a maximum concentration of 0.55 μM. Having reached the final concentration of topotecan, the cells were passaged after trypsinization using 0.05 % trypsin/0.02 % EDTA (PAA Laboratories, Pasching, Austria) every 3-5 days. The treated cells showed sufficient quantities of the ABCG2 transporter after three passages in Eagle's minimum essential medium (Sigma, Deisenhofen, Germany) containing L-glutamine, 2.2 g/L $NaHCO_3$ (Merck, Darmstadt, Germany), 0.11 g/L sodium pyruvate (Serva, Heidelberg, Germany), 5 % fetal calf serum (Biochrom, Berlin, Germany), and topotecan at a concentration of 0.55 μM.

Modulation of ABCG2 in the Hoechst 33342 assay. The standard protocol for the performance of Hoechst 33342 was as follows: 3-5 days after passaging (70-90 % confluency), MCF-7/Topo cells were seeded into 96-well plates at a density of 20000 cells/well (total volume 100 µL). Subsequently, the cells were allowed to attach to the surface of the microplates in a water saturated atmosphere (95 % air, 5 % carbondioxide) at 37 °C for at least 3 h. In 1.5 mL reaction vessels pre-mixtures of the test compounds were prepared by the following procedure: 1000 µL of pre-heated (37 °C) EMEM (Eagle's Minimum Essential Medium, Sigma, Munich, Germany) containing L-glutamine, 2.2 g/L of $NaHCO_3$ and 0.55 g/L of sodium pyruvate, supplemented with 5 % FCS, were transferred into the cups. Subsequently, 10 µL of a 1.6 mM Hoechst 33342 dye solution was added to the samples. Afterwards 10 µL of DMSO, FTC (10 mM) or the test compounds were added to the mixture at increasing concentrations, respectively. The samples were immediately vortexed and transferred into 50 mL polystyrene reagent reservoirs™ (BD, Heidelberg, Germany). By means of a multichannel pipette, 100 µL of the pre-mixtures were added to each well of the microplate, achieving a final concentration of 50 µM fumitremorgin C (positive control) and 8 µM of Hoechst 33342 or 1 µM, respectively. In order to get homogenous solutions in each well, the microplates were slightly shaken horizontally for a couple of times and placed into an incubator (37 °C, 5 % carbondioxide) for 120 min. The supernatants were drained, and the cells were fixed for 30 min using 100 µL per well of a 3 % paraformaldehyde solution (plates were protected from light by covering with aluminium foil). Finally, MCF-7/Topo cells were washed twice with 250 µL of calcium and magnesium containing PBS for each well. Thereby, the supernatant of the first washing step was immediately removed, whereas the second portion of washing buffer was added to the cells for another 10 min in order to get rid of residual dye. Afterwards, cells were overlaid with 100 µL / well of Ca^{2+}/Mg^{2+}-containing PBS, and the relative fluorescence intensities were determined via microplate reader. Instruments were set as follows: Measurement mode: fluorescence top; excitation filter (Hoechst 33342): 340/35, emission filter (Hoechst 33342): 485/20, number of reads: 10; integration time: 40 µs; lag time: 0 µs; mirror selection: automatic; plate definition file GRE96ft.pdf or GRE96fb.pdf; multiple reads per well (circle): 4x4; time between move and flash: 50 ms. On each

plate, the optimal gain was calculated by determination of the fluorescence intensity in the presence of the control substance, fumitremorgin C. After measurements, the microtiter plates were stored at 4 °C for the following cell quantification procedures. As a loss of cells and unspecific toxic effects of the test compounds during the incubation phase have to be considered, the obtained fluorescence values had to be normalized to the cell number of each well. Therefore, the microplates were processed with a 0.02 % aqueous crystal violet solution (100 µL / well) for 20 min. Excess dye was removed by rinsing the trays with water for 20 min. Crystal violet bound by the cells was re-dissolved in 70 % ethanol (180 µL / well) while shaking the microplates for 2-4 h. Subsequently, the absorbance was messured as a parameter proportional to cell mass at the TECAN plate reader. Normalization of the fluorescence intensities to the cell mass was achieved by dividing the detected fluorescence values were through the obtained absorbance data of each well. All values were corrected to the unspecific uptake of the dye (DMSO control value) and the data were referred to the maximal signal caused by 50 µM of the reference compound fumitremorgin C. Addition of increasing concentrations of the modulators led to sigmoidal concentration response curves. IC_{50} values were calculated using SIGMA PLOT 9.0, four parameter logistic curve fitting. Errors were expressed as standard error of the mean (SEM). The required concentration of 50 µM fumitremorgin C in the final assay protocol, as a reference value for maximal transporter inhibition, was determined via the performance of the H33342 assay according to the standard protocol.

Confocal laser scanning microscopy (CLSM) of MCF-7/Topo cells. After trypsinization, ABCG2-overexpressing MCF-7/Topo cells were resuspended in culture medium supplemented with 5 % fetal calf serum (FCS) and 0.55 µM topotecan. 250 µL of the cell suspension were seeded into each well of a sterile 1 µ-slide 8-well ibiTreat™ microscopy chamber (ibidi, Martinsried, Germany). Having reached the appropriate confluency of 70 % (approximately 48 h later), the medium was removed by suction, and the cells were washed with 280 µL of Leibovitz´s L15 medium containing L-glutamine (Invitrogen, Karlsruhe, Germany). Subsequently, the cells were overlaid with 240 µL of Leibovitz´s L15 medium. On the eve of the experiment, 40 µL of a 7-fold concentrated stock

solution of the test compound were pipetted to the cells. Fluorescence imaging was performed with a Carl Zeiss Axiovert 200M LSM 510 confocal-laser scanning microscope (CLSM) encased by a tempered incubation chamber. Instrument settings were as follows: pyrylium-labelled compounds: excitation wavelength 543 nm (laser intensity 10 %), emission filter: 659 LP, magnification 400-fold, (Apochromat 40 x / NA 1.2 W); for bodipy-prazosin: excitation wavelength 488 nm (laser intensity 3 %), emission filter: BP 530-600, magnification 400-fold, (Apochromat 40 x / NA 1.2 W). For image processing the Carl Zeiss LSM Image Examiner and the LSM image browser software were used.

Isolation of rat brain capillaries and confocal lacer scanning microscopy (CLSM). Rat brain capillaries isolation and confocal microscopy measurements were performed as described by Miller *et al*. (2000).[34] In brief: Capillaries from rat (3–6 animals per preparation) brain were isolated using a modification of the procedure of Pardridge *et al*. (1985).[35] All steps in the isolation procedure were carried out at 4 °C in pregassed (95 % O_2/5 % CO_2) solutions. Keeping the tissue on ice and in wellgassed buffers was essential for preservation of transport function. Briefly, pieces of gray matter were gently homogenized in three volumes (v/w) of buffer A (artifical cerebral spinal fluid, commercial ACSF) and after addition of dextran (final concentration 30 %), the homogenate was centrifuged at low speed (15 min, 7200 mrp). The resulting pellet was resuspended in (ACSF+0.5 % BSA) and then filtered through a 150 μM nylon mesh. The filtrate was passed over a glass bead column and, after washing with 500-mL ACSF + 0.5 % BSA solution, the capillaries adhering to the beads were collected by gentle agitation. Capillaries were centrifuged, the pellet resuspended in ice-cold ACFS, gassed, BSA-free Krebs-Henseleit buffer and immediately used for transport experiments. To measure transport, 10 μL of capillary suspension was transferred to a covered, Teflon incubation chamber (containing 1.5 mL of pregassed Krebs-Henseleit buffer with fluorescent compound and added effectors.) The chamber floor was a glass cover slip to which the capillaries adhered and through which capillaries could be viewed by means of an inverted confocal laser microscope. Fluorescent compounds and inhibitors were added (490 μL) as thinned solutions in PBS + 1 % BSA in

different concentrations. The dilution series was prepared from stock solutions (in DMSO) and PBS + 1 % BSA. Preliminary experiments showed that the concentrations of dimethylsulfoxide used (< 0.5 %) had no significant effects on the uptake and distribution of the fluorescent labelled test compounds in brain capillaries as measured by confocal microscopy (D.S. Miller, unpublished data). Incubation time was set to 30 min. All transport experiments were conducted at room temperature (18–20°C). Final concentrations for the test compounds were as follows: co-incubation bodipy-prazosin (1 μM) and compound **1** (0.001 μM, 0.1 μM, 1 μM), co-incubation bodipy-prazosin (1 μM) and compound **9** (1 μM, 10 μM, 50 μM), co-incubation compound **9** (1 μM) and Ko143 (0.01 μM, 0.1 μM, 1 μM, 5 μM), co-incubation compound **9** (1 μM) and compound **1** (0.01 μM, 0.1 μM, 1 μM, 10 μM).

For image processing and data evaluation Nikon EZ-C1 3.90 and ImageJ software were used. Luminal fluorescence intensity measurements were made of areas within the central 2 mm (10 pixels) of each segment. The background fluorescence intensity was subtracted and the average pixel intensity for each area was calculated. Three fluorescent samples and three background samples were analysed per capillary, using 4-10 capillaries per concentration.

2.5. References

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3. Synthesis and Metabolic Stability of New Tariquidar-derived ABCG2 Inhibitors*

Breast cancer resistance protein (ABCG2) has been studied intensely since its discovery one decade ago. The modulation of the membrane located ABC-transporters is an effective tool for the treatment of multi-drug resistant cancer cells. Recently, a new class of tariquidar-like selective ABCG2 inhibitors was discovered. By structural variations of this lead structure an improvement of the drug-like properties and the potency was attempted. Incubation in mouse plasma proved the stability of the ester functionality, but one of the peptide bonds was cleaved, also when *N*-methylated. Structural changes of different parts of the molecule decreased the potency only moderately as long as there was no negative charge introduced under physiological conditions. The phenylbenzamide group is essential for the interaction with the transporter.

* All synthesis were done by Carolin Fischer, inhibition assays for ABCB1 and ABCG2 and plasma stability experiments were performed by Peter Höcherl, Matthias Kühnle and Kira Bürger.

3.1. Introduction

The ATP-binding cassette (ABC) transporter G2 (BCRP) plays a major role in the protection from xenobiotics. Physiological high concentrations are found in the blood brain barrier,[1] the gastrointestinal tract, the blood-testis barrier[2] and the maternal-fetal[3] barrier to protect the organism and to limit the absorption of drugs.[4, 5] ABCG2 overexpression has also been demonstrated in cancer cells.[6] On the one hand, cancer cells develop high levels of ABCG2 after the treatment with cytostatic agents. On the other hand, cancer stem cells are known to express naturally high concentrations of the ABCG2 transporter.[7, 8] Here, ABCG2 is supposed to protect the cancer cells against chemotherapeutic agents.[9] These properties make the membrane located protein an important target to address multi-drug-resistance of cancer cells. In the last few years, the list of ABCG2 substrates and inhibitors increased rapidly.[10, 11] Most of the identified substances were used in cancer chemotherapy and are large and lipophilic molecules, such as mitoxantrone, topotecan, doxorubicin and others.[12] Also tyrosine kinase inhibitors (imatinib and gefitinib) and fluorescent dyes (Hoechst 33342 and rhodamine 123) have been shown to be transported.[13, 14] As the substrate spectrum is highly overlapping with other ABC-transporters like ABCB1 (p-glycoprotein) and ABCC1 (MRP1), also many inhibitors block more than one ATP-binding cassette transporter.[15] Only few specific inhibitors are known for ABCG2. The fungal toxin fumitremorgin C (FTC)[16] and its analogue Ko143 are the most commonly used selective inhibitors.[17] Novobiocin also displays a selective, but weak inhibitor.[18]

Structural variation of the ABCB1 inhibitor tariquidar (XR 9576) mostly resulted in broad spectrum inhibitors or even preferring ABCB1 inhibition.[19-23] Recently, selective ABCG2 inhibitors **1a** and **1b** (Figure 3.1) were found by changing the substitution pattern of the carboxylic acid and the amine functionalities on the central aromatic core.[24] Instead of anthranilic acid, 3-aminobenzoic acid was used. Additionally, a bicyclic hetero arene gave better inhibitory activity compared to monocyclic arenes. In 4-position on the central aromatic core the methyl ester was the preferred substituent concerning the potency to modulate ABCG2, in comparison to methoxy- and methyl-substituents.

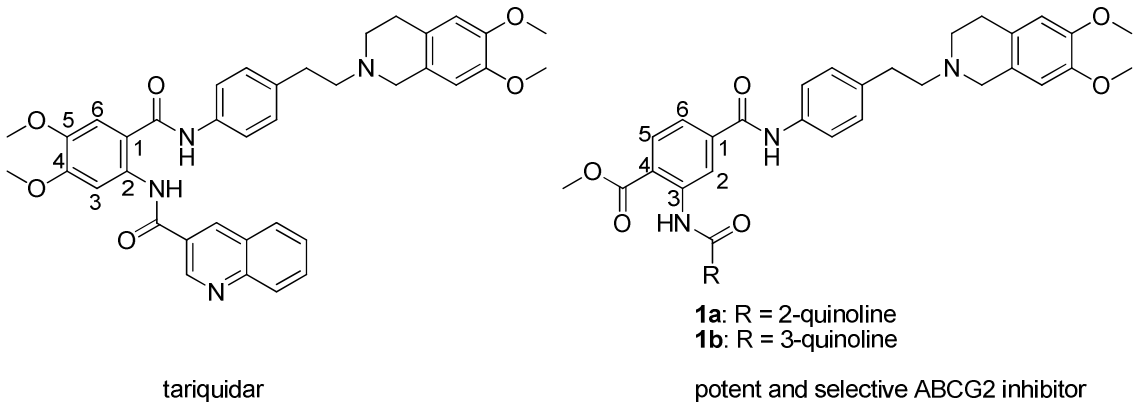


Figure 3.1: The parent compound tariquidar and structural modified derivatives as specific ABCG2 inhibitors.

In an attempt to improve the potency of the compounds and to identify essential structural elements for their inhibitory activity, modifications in the little explored tetrahydroisoquinoline part of **1** were made and substructures were synthesised and tested.

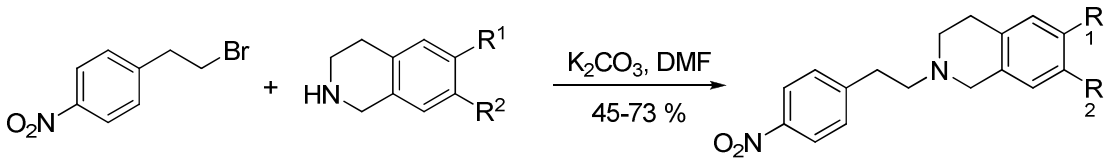
Furthermore, with respect to *in vivo* studies, the metabolic stability of these compounds should be high. The ester derivatives **1a** and **1b** are supposed to be cleaved under physiological conditions yielding the corresponding carboxylic acid.[25] Therefore, the carboxylic acid and analogues owing a different functionality in 4-position on the central aromatic core were synthesised. The goal was to retain the activity and at the same time improve the metabolic stability. Derivatives bearing hydrogen, a methyl ketone, methyl amide and propenyl as substituents were therefore prepared.

Moreover, amide bonds are also susceptible to enzymatic degradation.[26] The quinoline amide bond should be stabilized and protected by intramolecular hydrogen bonding in a six-membered ring between the carbonyl oxygen of the ester and the NH. The second amide bond was stabilized by *N*-methylation. The method is commonly used to improve the physiological stability of drugs.[27, 28]

3.2. Results and Discussion

3.2.1. Synthesis

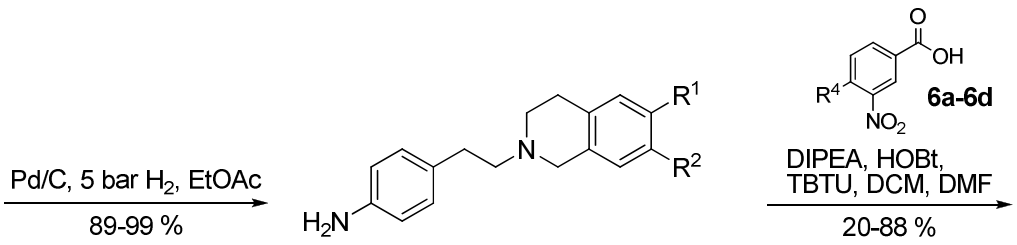
Following the well-established procedures[24, 29] (Scheme 3.1), a library of different compounds was synthesised to improve enzymatic stability and to investigate the structure-activity-relationship of tariquidar-derived ABCG2 modulators. The synthetic sequence started with an S_N2 reaction converting 1-(2-bromoethyl)-4-nitrobenzene **2** with different tetrahydroisoquinolines **3a-c**. The residues on the tetrahydroisoquinolines were hydrogen, methoxy or amine. The reaction gave 45-73 % yield of **4a-d**. Boc-protection of compound **4b** gave compound **4c**. Consequently, the nitro functionality was reduced with Pd/C and hydrogen to the corresponding amine (**5a-c**). Here, the conversion was nearly complete. At this step of the synthesis, **5c** was also modified to the acyl (**5d**), the formyl (**5e**) and the *N*-methyl derivative (**5f**). The amide bond formation between the 3-nitrobenzoic acid derivatives (**6a-c**) and compounds **5a-d** was achieved using classical peptide coupling reagents as HOBt and TBTU, yielding 20-88 % of **7a-g**. Again, the nitro functionality was converted with the help of Pd/C and hydrogen gas in good to excellent yields to compounds **8a-h**. To obtain **8a** und **8e**, reduction with $SnCl_2$ was necessary. The most sensitive reaction was the formation of the second amide bond using the acid chloride either of the quinoline-2- or the quinoline-3 carboxylic acid with yields varying between 3 % and 69 % for compounds **9a-j**. Compound **9c** was the product of Boc-deprotected derivative **9d**, and compound **9f** was derived from ester cleavage of compound **1b** using LiOH.



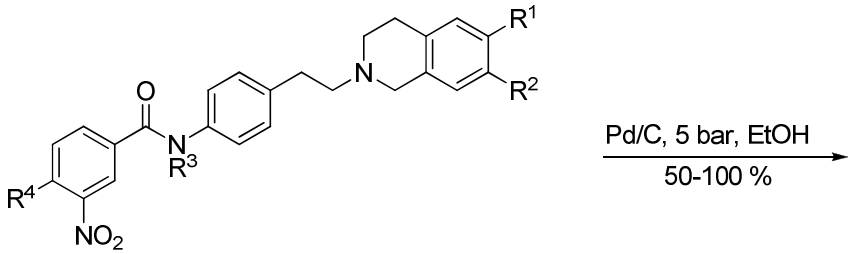
2

3a: R^1 = H, R^2 = H
3b: R^1 = H, R^2 = NH_2
3c: R^1 = OCH_3, R^2 = OCH_3

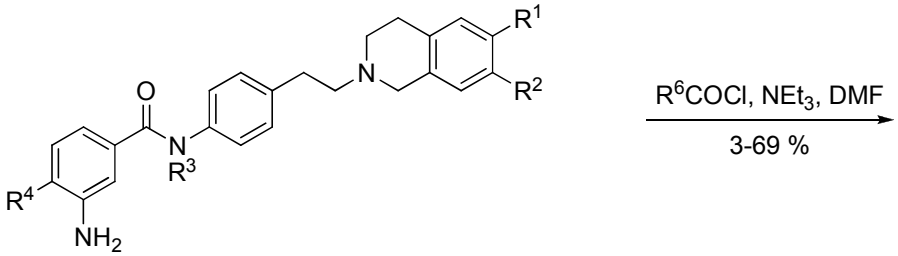
4a: R^1 = H, R^2 = H
a) **4b**: R^1 = H, R^2 = NH_2
4c: R^1 = H, R^2 = NHBoc
4d: R^1 = OCH_3, R^2 = OCH_3



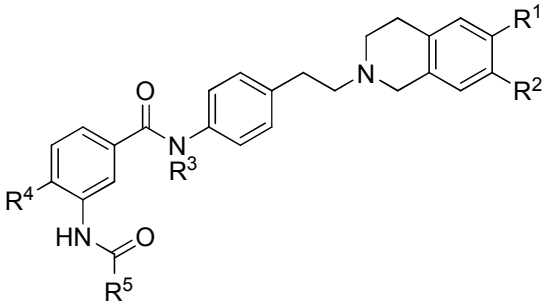
5a: R^1 = H, R^2 = H
5b: R^1 = H, R^2 = NHBoc
d) b) **5c**: R^1 = OCH_3, R^2 = OCH_3
5d: R^1 = OCH_3, R^2 = OCH_3, NHCOH
c) **5e**: R^1 = OCH_3, R^2 = OCH_3, $NHCH_3$
5f: R^1 = OCH_3, R^2 = OCH_3, $NHCOCH_3$



7a: R^1 = H, R^2 = H, R^3 = H, R^4 = $CONHCH_3$
7b: R^1 = H, R^2 = H, R^3 = H, R^4 = CO_2CH_3
7c: R^1 = H, R^2 = NHBoc, R^3 = H, R^4 = CO_2CH_3
7d: R^1 = OCH_3, R^2 = OCH_3, R^3 = CH_3, R^4 = CO_2CH_3
7e: R^1 = OCH_3, R^2 = OCH_3 R^3 = H, R^4 = $CONHCH_3$
7f: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = $COCH_3$
7g: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = $CHOHCH_2CH_3$



8a[e)]: R^1 = H, R^2 = H, R^3 = H, R^4 = $CONHCH_3$
8b: R^1 = H, R^2 = H, R^3 = H, R^4 = CO_2CH_3
8c: R^1 = H, R^2 = NHBoc, R^3 = H, R^4 = CO_2CH_3
8d: R^1 = OCH_3, R^2 = OCH_3, R^3 = CH_3, R^4 = CO_2CH_3
8e[e)]: R^1 = OCH_3, R^2 = OCH_3 R^3 = H, R^4 = $CONHCH_3$
8f: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = $COCH_3$
8g: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = $CHOHCH_2CH_3$
8h[f)]: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = H

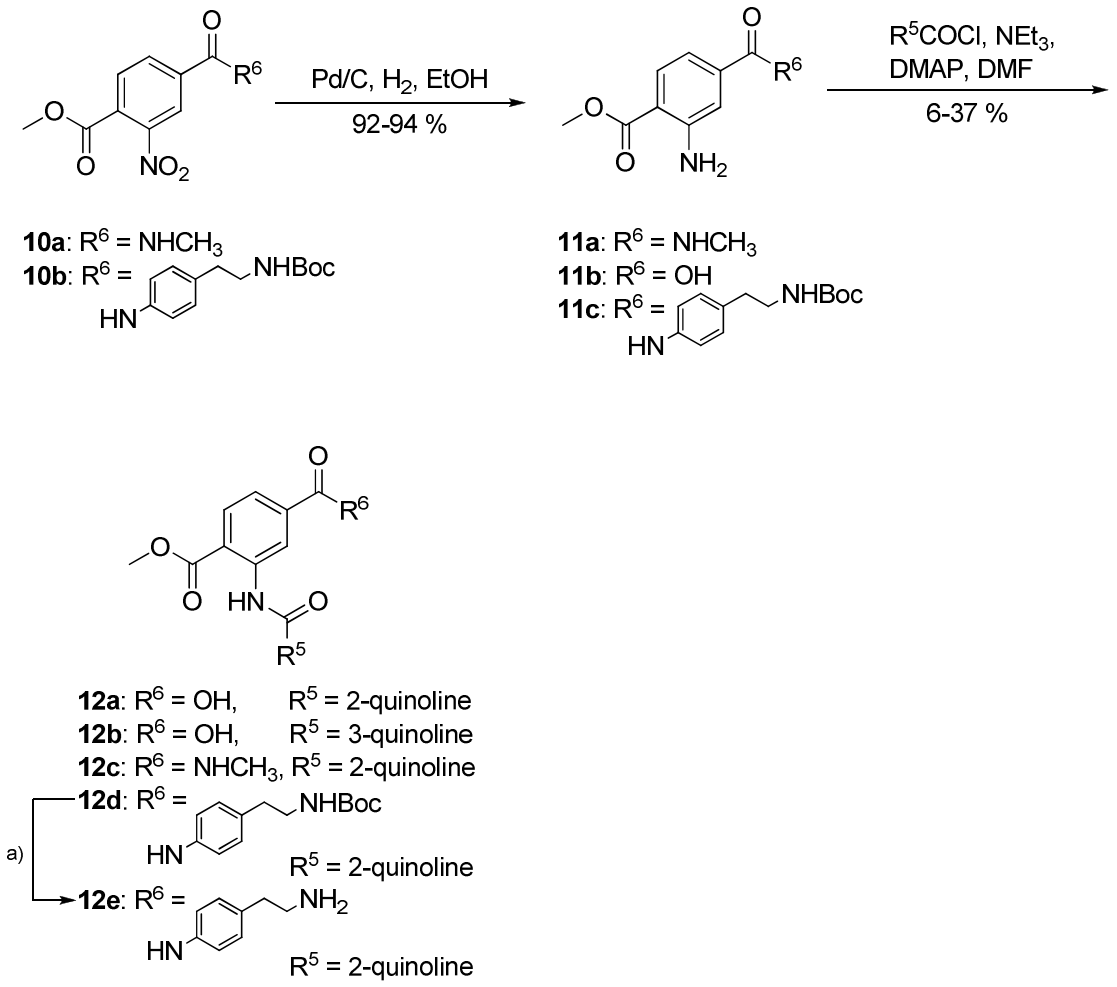


9a: R^1 = H, R^2 = H, R^3 = H, R^4 = CO_2CH_3, R^5 = 2-quinoline
9b: R^1 = H, R^2 = H, R^3 = H, R^4 = $CONHCH_3$, R^5 = 2-quinoline
g) **9c**: R^1 = H, R^2 = NHBoc, R^3 = H, R^4 = CO_2CH_3, R^5 = 2-quinoline
9d: R^1 = H, R^2 = NH_2, R^3 = H, R^4 = CO_2CH_3, R^5 = 2-quinoline
9e: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = H, R^5 = 2-quinoline
h) **1b**: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = CO_2CH_3, R^5 = 3-quinoline
9f: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = CO_2H, R^5 = 3-quinoline
9g: R^1 = OCH_3, R^2 = OCH_3, R^3 = CH_3, R^4 = CO_2CH_3, R^5 = 2-quinoline
9h: R^1 = OCH_3, R^2 = OCH_3, R^3 = H, R^4 = $CONHCH_3$, R^5 = 2-quinoline

Scheme 3.1: Synthesis of potential ABCG2 inhibitors. [a)] Boc_2O, NEt_3, DMF, 99 %. [b)] Ac_2O, HCO_2H, THF, 84 %. [c)] $LiAlH_4$, THF, 73 % [d)] acylchloride, NEt_3, DMAP, DCM, 81 %. [e)] 5 eq $SnCl_2$, EtOH, **8a**: 46 %, **8e**: 73 %. [f)] NHBoc + HCl/ether -> NH_2, 95 %. [g)] 15 mol% HCl/ether, 11 %. [h)] LiOH, DMF, microwave: 80°C, 300 W, 4 min, 10 %.

For comparison of the biological activitiy only the left part of the parent structure **1a-b** was prepared, starting with a palladium-catalysed reduction of the methyl 2-nitrobenzoates **10a-b** to the corresponding amino compounds **11a-c** (Scheme 3.2). The partial structures **12a-d** were derived from the coupling of compounds

11a-c to quinoline-2- or the quinoline-3 carbonyl chloride. The Boc-deprotection of compound **12d** gave compound **12e**.



Scheme 3.2: Partial structure synthesis. [a)] 15 mol% HCl/ether, yield: 95 %.

3.2.2. Biological Activity

The potency of the tariquidar analogues to modulate the ABCG2 transporter was determined in the flow cytometric mitoxantrone assay. The red fluorescent mitoxantrone is not accumulated in MCF7/Topo cells due to its active efflux by overexpressed ABCG2 transporters. Hence, ABCG2 inhibitors can be detected by the change of intramolecular mitoxantrone levels. The relative fluorescence intensity of the cells was measured. Fumitremorgin C and Ko143 were used as positive control compounds, whereas fumitremorgin C was the reference for the maximal inhibitory effect of the test compounds. Additionally, all compounds

were tested on their activity on the ABCB1 transporter in the calcein-AM microtiter assay.[30]

Table 3.1: ABCG2 inhibition by tariquidar analogues determined by the mitoxantrone efflux flow cytometry assay.

No.	IC_{50} [nM]	Max. effect [%] (realtive to Fumitremorgin C)	ABCB1 [nM]
FTC	> 11 000	100	inactive
Ko143	225 ± 33	82 ± 5	inactive
1a	60 ± 10	56 ± 6	> 29000
1b	119 ± 22	41 ± 3	9450 ± 417
5c	inactive [a)]	--	inactive
5f	inactive [a)]	6 ± 3	> 50000
9a	297 ± 75	65 ± 2	> 50000
9b	795 ± 194	39 ± 3	> 50000
9d	664 ± 249	90 ± 5	> 100000
9e	555 ± 48	56 ± 2	> 50000
9f	3414 ± 724	27[b)]	inactive
9g	1900 ± 700	68 ± 6	6000 ± 2000
9h	857 ± 122	26 ± 3	> 50000
9i	622 ± 152	58 ± 3	95308 ± 57364
9j	1470 ± 420	63 ± 7	103417 ± 48104
12a	inactive [a)]	--	> 50000
12b	inactive [a)]	--	> 50000
12c	2353 ± 251	105 ± 4	> 50000
12e	1669 ± 590 [b), c)]	64 ± 3	> 100000

[a)] no effect up to a concentration of 10 μM. [b)] N = 1. [c)] values obtained by Hoechst 33342 assay.

In comparison to the most active compounds **1a** and **1b**, the methyl keto substituted compound **9i** (IC_{50} 622 ± 152 nM) gave a good inhibitory effect, whereas the potency for the propenyl substituted compound **9j** (IC_{50} 1470 ± 420 nM) was reduced, potentially due to sterical more demanding

residue (Table 3.1). The exchange of the methyl ester to methyl amide (**9b** IC_{50} 795 ± 194 nM, **9h** IC_{50} 857 ± 122 nM) decreased the IC_{50} value about one order of magnitude. Compound **9f** possessing the carboxylic acid in position 4 was about 30 times less potent (IC_{50} 3414 ± 724 nM) than the parent ester functionalized analogue **1b** (IC_{50} 119 ± 22). This observation shows that susceptible enzymatic cleavage of the ester would decrease the activity of the compound significantly. Without a substituent in 4-position, compound **9e** still shows acceptable activity (IC_{50} 555 ± 48 nM). The examples indicate that a replacement of the methyl ester by other substituents without decrease in activity is only possible if they are not charged (**9f**) or sterically demanding (**9j**).

The modifications on the tetrahydroisoquinoline part of the molecule, the replacement of the methoxy substituents by hydrogen (**9a**, IC_{50} 297 ± 75 nM) or a primary amine (**9d**, IC_{50} 795 ± 194 nM) lead to derivatives with IC_{50} values only slightly higher than these for Ko143. Additionally, partial structures of the parent compound **1a** and **1b** were investigated. As shown before, all compounds lacking the quinolincarboxamide structure (**5c**, **5f**) are inactive.[23] In the absence of the tetrahydroisoquinoline part as in compound **12c** (IC_{50} 2353 ± 251 nM) and **12e** (IC_{50} 1669 ± 590 nM) the inhibition activity is still low. Compounds **12a** and **12b** are negatively charged at physiological conditions; these compounds show no activity.

Compound **9g**, which was *N*-methylated at the peptide bond connecting the tetrahydroisoquinoline part and the quinolincarboxamide, showed only low inhibition activity for ABCG2. However, this was the only compound also possessing low activity on ABCB1 with a selectivity factor of 3. As this slight structural modification has such strong effect, the phenylbenzamide bond seems to play an essential role in the interaction with the transporter.

In summary, the inhibitory effect of mitoxantrone efflux is more affected by the quinoline carboxamide benzoate substructure than by the tetrahydroisoquinoline substructure. Structural changes on the tetrahydroisoquinoline part are more tolerated in terms of retained inhibition activity. All new derivatives are selective ABCG2 inhibitors.

3.2.3. Stability in Mouse Plasma

Before the intented *in vivo* studies, the biological stability of selected ABCG2 modulators was investigated. Incubation with mouse plasma showed enzymatic degradation of the compounds **1a** and **1b** within 10 to 30 min by at least 50 %.[31] Surprisingly, the ester group remained intact, whereas the benzoic amide was cleaved rapidly. HPLC-MS analysis confirmed the cleavage of the peptide bond into compounds **12a**/**12b** and **5c**. To exclude a hydrolytic cleavage, control experiments in phosphate buffered saline (PBS) and deproteinated plasma revealed the stability of the compounds in the absence of enzymes. The products of the cleavage reaction are inactive in the inhibition of the ABCG2 transporter.

Methylation of amide nitrogen atoms is an established strategy to improve the stability of peptides against enzymatic cleavage. However, compound **9g** with the *N*-methylated amide was again completely degraded within minutes (Figure 3.2).

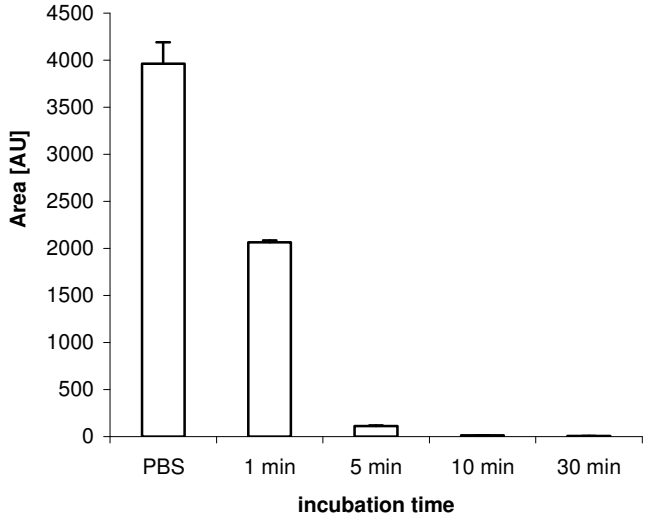


Figure 3.2: Stability of compound **9g** upon incubation with murine plasma; the concentration of **9g** was determined by HPLC, N = 2.

Bio-isosteric replacement of the amide bond e.g. by benzimidazole, by different oxadiazoles or by -CH_2-NH- may improve the stability of derivatives against enzymatic cleavage.[32, 33]

3.3. Conclusion

A series of new tariquidar-derived ABC-transporter inhibitors were synthesised to investigate the effect of structural changes at the tetrahydroisoquinoline substructure and the ester functionality. Furthermore, the effect of *N*-methylation on the enzymatic stabililty was examined to increase enzymatic stability by *N*-methylation. Structural variations on the tetrahydroisoquinoline part (**9a**, **9d**) decreased the inhibitory effect on ABCG2 only little. Exchanging the methyl ester on the central aromatic core by other substituents was tolerated well, as long as no additional charge (**9f**) or a sterical demanding residue (**9j**) was introduced. Partial structures without the tetrahydroisoquinoline part (**12c**, **12e**) showed weak inhibitory activity in the testing. This allows the conclusion that the tetrahydroisoquinoline part plays only a minor role in the interaction between the inhibitor and the transporter protein. *N*-Methylation of the phenylbenzamide moiety as in **9g** decreased the inhibitory activity compared to the parent compound **1a** significantly; the functional group seems to be important for the inhibitory activity on ABCG2.

All new compounds were found to be selective inhibitors for ABCG2 with no significant inhibition activity for ABCB1. Among 15 tested derivatives, only **9g**, the *N*-methylated analogue of **1a**, showed some inhibition activity on ABCB1.

Although it was not possible to obtain an ABCG2 inhibitor that is stable in serum, valuable information on the structure activity-relationship was derived from the study.

3.4. Experimental Section

General. Commercial reagents and starting materials were purchased from Aldrich, Fluka or Acros and used without further purification. Flash chromatography was performed on silica gel (Merck silica gel Si 60 40-63 μm); products were detected by TLC on alumina plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm) and visualized by UV light (λ = 254 nm). Melting points were determined with a Büchi SMP 20 and are uncorrected. NMR spectra were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz; T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz; ^{13}C: 100.6 MHz; T =

300 K) and Bruker Avance 600 (^{1}H: 600.1 MHz; ^{13}C: 150.1 MHz; *T* = 300 K) instruments. Chemical shifts are reported in *δ*/ppm relative to external standards and coupling constants *J* are given in Hz. Abbreviations for the characterization of the signals: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, dd = double doublet. The relative numbers of protons is determined by integration. Error of reported values: chemical shift 0.01 ppm (^{1}H-NMR), 0.1 ppm (^{13}C-NMR), coupling constant 0.1 Hz. The used solvent for each spectrum is reported. Mass spectra were recorded with Finnigan MAT TSQ 7000 (ESI) and Finnigan MAT 90 (HRMS), IR spectra with a Bio-Rad FT-IR-FTS 155 spectrometer and UV/vis spectra with a Cary BIO 50 UV/vis/NIR spectrometer (Varian).

The following compounds were synthesised following literature-known procedures: 1,2,3,4-tetrahydro-7-nitroisoquinoline,[34] 3-(*tert*-butoxycarbonyl-amino)benzoic acid,[35] methyl 4-{4-[2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethylphenyl] aminocarbonyl}-2-nitrobenzoate,[24] methyl 2-amino-4-({4-[2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]phenyl} aminocarbonyl)benzoate,[24] *tert*-butyl 4-aminophenethyl-carbamate,[36] methyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1H]-yl) ethyl] phenylcarbamoyl} -2-(quinoline-2-carboxamido) benzoate (**1a**),[24] methyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl] phenylcarbamoyl}-2-(quinoline-3-carboxamido) benzoate (**1b**),[24] 1,2,3,4-tetrahydroisoquinolin-7-amine (**3b**),[37] 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]ethyl}aniline (**5c**),[38] 3-amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1H]-yl)ethyl]phenyl}benz-amide (**8h**) (see chapter 2), methyl 4-{4-[2-(*tert*-butoxycarbonylamino) ethyl] phenylcarbamoyl}-2-nitrobenzoate (**10b**) (see chapter 2), methyl 2-amino-4-{4-[2-(*tert*-butoxycarbonylamino)ethyl] phenylcarbamoyl} benzoate (**11c**) (see chapter 2), *N*-[4-(2-tert-butoxycarbonylamino-ethyl)-phenyl]-2-[(quinoline-2-carbonyl)-amino]-terephthalamic acid methyl ester (**12d**) (see chapter 2), methyl-4-[4-(2-aminoethyl)phenylcarbamoyl]-2-(isoquinoline-3-carboxamido)-benzoate (**12e**) (see chapter 2), *tert*-butyl-3-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenylcarbamoyl}phenylcarbamate (see chapter 2)

General procedures (GP):

GP 1: Amide coupling over an active ester

The respective carboxylic acid (1.1 eq) was dissolved in CH_2Cl_2. Two equivalents of DIPEA, 1.2 eq of each TBTU and HOBt were added at 0 °C. After 30 minutes the active ester was formed and the respective amine was added. The reaction mixture was stirred for 16 h, taken up in water, diluted with dichloromethane, washed three times with water (3 x 30 mL) and once with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated giving the crude reaction product.

GP 2: Reduction of the nitro function

The respective nitro compound (1 eq) was dissolved in ethyl acetate and methanol followed by the addition of Pd/C (10 % m/m) suspended in ethyl acetate. After stirring the solution under 10 bar of H_2 at room temperature for 15 h, the catalyst was filtered off and the solvent was removed to obtain the solid product.

2-(4-Nitrophenethyl)-1,2,3,4-tetrahydroisoquinoline (4a)

A solution of 4-nitrophenylethyl bromide (1 eq, 4.40 g, 19 mmol), 1,2,3,4-tetrahydro isoquinoline hydrochloride (1.1 eq, 2.45 g, 18 mmol) and potassium carbonate (2.3 eq, 5.53 g, 40 mmol) in 20 mL DMF was heated at 100 °C for 4.5 h. The mixture was filtered and evaporated. The residue was taken up in water and extracted with dichloromethane (3 x 30 mL). The organic layers were dried over sodium sulphate and the solvents were removed under reduced pressure. The resulting solid was recrystallized from ethanol to give 2-(4-nitrophenethyl)-1,2,3,4-tetrahydroisoquinoline as orange solid (3.96 g,14 mmol, 73.4 %), **mp** 108 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.80 (t, *J* = 7.2 Hz, 4 H), 2.92 (t, *J* = 5.7 Hz, 2 H), 3.02 (t, *J* = 8.1 Hz, 2 H), 7.02-7.04 (m, 1 H), 7.09-7.15 (m, 3 H), 7.41 (d, *J* = 8.8 Hz, 2 H), 8.15 (d, *J* = 8.8 Hz, 2 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 29.1 (-, CH_2), 33.8 (-, CH_2), 50.9 (-, CH_2), 56.1 (-, CH_2), 59.2 (-, CH_2), 123.7 (+, 2 x CH_{arom}), 125.7 (+, CH_{arom}), 126.3 (+, CH_{arom}), 126.6 (+, CH_{arom}), 128.7 (+, 2 x CH_{arom}), 129.6 (+, CH_{arom}), 134.2 (C_{quat}), 134.4 (C_{quat}), 146.5 (C_{quat}), 148.4 (C_{quat}) – **MS** (CI, NH_3): m/z (%) = 283 (100) [M-H$^+$] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1339 (N-O), 1519 (N-O) – **MF** $C_{17}H_{18}N_2O_2$ – **MW** 282.34

2-(4-Nitrophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-amine (4b)

A solution of 4-nitrophenylethyl bromide (1 eq, 1.9 g, 8.5 mmol), 1,2,3,4-tetrahydroisoquinolinehydrochloride-7-amine (1.2 eq, 1.4 g, 9.3 mmol) and potassium carbonate (2.3 eq, 2.7 g, 19.5 mmol) in 10 mL DMF was heated at 90 °C for five hours under nitrogen. The mixture was filtered and evaporated. The residue was taken up in water and extracted with dichloromethane (3 x 30 mL). The organic layers were dried over sodium sulphate and the solvents were removed under reduced pressure. The resulting solid was purified by column chromatography (elution with ethyl acetate, R_f: 0.2) to give 2-(4-nitrophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-amine as orange solid (1 g, 3.4 mmol, 45 %), **mp** 137 °C – **^{1}H-NMR:** (300 MHz, $CDCl_3$) δ = 2.75 – 2.80 (m, 6 H), 2.95 – 3.02 (m, 2 H), 3.62 (s, 2 H), 6.37 (d, *J* = 2.3 Hz, 1 H), 6.52 (dd, *J* = 2.4 Hz, 8.1 Hz, 1 H), 6.90 (d, *J* = 8.1 Hz, 1 H), 7.40 (d, *J* = 8.7 Hz, 2 H), 8.14 (d, *J* = 8.7 Hz, 2 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 27.0 (-, CH_2), 32.7 (-, CH_2), 50.1 (-, CH_2), 54.9 (-, CH_2), 58.0 (-, CH_2), 111.7 (+, CH_{arom}), 112.3 (+, CH_{arom}), 122.6 (+, 2 x CH_{arom}), 123.1 (C_{quat}), 128.4 (+, CH_{arom}), 128.6 (+, 2 x CH_{arom}), 134.0 (C_{quat}), 143.2 (C_{quat}), 145.5 (C_{quat}), 147.4 (C_{quat}) – **MS** (CI): m/z (%) = 298 (100) $[M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1339 (N-O), 1514 (N-O), 3350 (N-H), 3453 (N-H) – **MF** $C_{17}H_{19}N_3O_2$ – **MW** 297.35

tert-Butyl 2-(4-nitrophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-yl carbamate (4c)

2-(4-Nitrophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-amine (1.0 g, 3.4 mmol) was dissolved in DMF before 1.5 eq of NEt_3 (0.5 g, 0.7 mL, 5.1 mmol) and 1 eq of Boc_2O (0.7 g, 3.5 mmol), diluted in a few drops of water, were added. After stirring over night at room temperature DMF was evaporated and the residue was taken up with diluted HCl and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated. The reaction yielded 1.35 g (3.3 mmol, 99 %) yellow oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.50 (s, 9 H), 2.75-2.85 (m, 6 H), 2.94-3.00 (m, 2 H), 3.66 (s, 2 H), 6.47 (s, 1H), 6.99 (d, *J* = 3.8 Hz, 1 H), 7.21 (s, 1 H), 7.39 (d, *J* = 8.8 Hz, 2 H), 8.14 (d, *J* = 8.5 Hz, 2 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 28.4 (+, 3 x CH_3,), 29.2 (-, CH_2), 33.7 (-, CH_2), 51.0 (-, CH_2), 56.0 (-, CH_2), 59.2 (-, CH_2), 80.3 (C_{quat}), 117.0 (+, CH_{arom}), 123.6 (+, 2 x CH_{arom}), 128.8 (+, CH_{arom}), 129.0 (+, CH_{arom}), 129.6 (+, 2 x CH_{arom}), 135.1 (C_{quat}), 136.2 (C_{quat}), 146.4 (C_{quat}), 148.5 (C_{quat}), 153.1 (C_{quat}),

162.7 (C_{quat}) – **MS** (CI): m/z (%) = 398 (100) $[M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1341 (N-O), 1511 (N-O), 1664 (C=O) – **MF** $C_{22}H_{27}N_3O_4$ – **MW** 397.47

4-{2-[3,4-Dihydroisoquinolin-2(1*H*)-yl]ethyl}benzenamine (5a)

The preparation followed GP 2 using 3.75 g (13.3 mmol) 2-(4-nitrophenethyl)-1,2,3,4-tetrahydroisoquinoline diluted in ethyl acetate. The reaction yielded 3.02 g (12.0 mmol, 89 %) orange solid, **mp** 88 °C – **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 2.50-2.60 (m, 4 H), 2.68 (t, *J* = 6.0 Hz, 2 H), 2.79 (t, *J* = 5.4 Hz, 2 H), 3.59 (s, 2 H), 6.48 (d, *J* = 8.2 Hz, 2 H), 6.89 (d, *J* = 8.2 Hz, 2 H), 7.02-7.11 (m, 4 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, [D_6]-DMSO): δ = 28.6 (-, CH_2), 32.1 (-, CH_2), 50.4 (-, CH_2), 55.4 (-, CH_2), 60.2 (-, CH_2), 113.9 (+, 2 x CH_{arom}), 125.3 (+, CH_{arom}), 125.7 (+, CH_{arom}), 126.3 (+, CH_{arom}), 127.1 (C_{quat}), 128.3 (+, CH_{arom}), 128.9 (+, 2 x CH_{arom}), 134.1 (C_{quat}), 134.9 (C_{quat}), 146.5 (C_{quat}) – **MS** (CI, NH_3): m/z (%) = 253 (100) $[M\text{-}H^+]$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 3308 (N-H), 3432 (N-H) – **MF** $C_{17}H_{20}N_2$ – **MW** 252.35

***tert*-Butyl 2-(4-aminophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-ylcarbamate (5b)**

The preparation followed GP 2 diluting *tert*-butyl 2-(4-nitrophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-ylcarbamate (0.48 g, 1.2 mmol) in ethyl acetate. The reaction yielded 0.45 g (1.2 mmol, 100 %) yellow solid, **mp** 70 °C decomposition – **^{1}H-NMR** (300 MHz, [D_4]-methanol): δ = 1.05 (s, 3 H), 2.33-2.45 (m, 8 H), 2.89 (s, 2 H), 6.53 (d, *J* = 8.2 Hz, 2 H), 6.71-6.73 (m, 3 H), 6.75-6.79 (m, 2 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, [D_4]-methanol): δ = 28.2 (+, 3 x CH_3), 28.9 (-, CH_2), 32.8 (-, CH_2), 51.9 (-, CH_2), 56.4 (-, CH_2), 61.0 (-, CH_2), 80.9 (C_{quat}), 117.0 (+, 3 x CH_{arom}), 129.9 (+, CH_{arom}), 130.1 (+, CH_{arom}), 130.5 (+, 2 x CH_{arom}), 134.1 (C_{quat}), 138.8 (C_{quat}), 147.1 (C_{quat}), 155.3 (C_{quat}), 164.9 (C_{quat}) – **MS** (CI): m/z (%) = 368 (100) $[M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1515 (C=O), 3222 (N-H), 3300 (N-H) – **MF** $C_{22}H_{29}N_3O_2$ – **MW** 367.48

_N_-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1_H_]-yl)ethyl]phenyl}formamide (5d)

Formic acid (1.5 mL) was added to ice-cold acetic anhydride (3 mL) and heated to 50-60 °C for two hours. After cooling to room temperature dry THF (5 mL) was added. Then a solution of 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl}aniline (0.42 g, 1.3 mmol) in dry THF was added dropwise at -20 °C. After 30 min stirring at low temperature the excess of anhydride and acid was removed under vacuum. The residue was taken up in ethyl acetate and washed twice with 0.1 N NaOH. The organic layer was dried over Na_2SO_4 and evaporated. The reaction resulted in pale yellow solid (0.39 g, 1.1 mmol, 84 %), **mp** 96 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.72-2.82 (m, 8 H), 3.63 (s, 2 H), 3.81 (s, 6 H), 6.52 (s, 1 H), 6.58 (s, 1 H), 6.98 (d, *J* = 8.5 Hz, 1 H), 7.14-7.21 (m, 2 H), 7.44 (d, *J* = 8.2 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 28.65 (-, CH_2), 33.3 (-, CH_2), 51.0 (-, CH_2), 55.7 (+, CH_3), 55.9 (+, CH_3), 56.0 (-, CH_2), 60.0 (-, CH_2), 109.5 (+, CH_{arom}), 111.3 (+, CH_{arom}), 119.2 (+, CH_{arom}), 120.2 (+, CH_{arom}), 126.1 (C_{quat}), 126.4 (C_{quat}), 129.3 (+, CH_{arom}), 130.0 (+, CH_{arom}), 136.9 (C_{quat}), 137.6 (C_{quat}), 147.2 (C_{quat}), 147.6 (C_{quat}), 162.6 (C_{quat}) – **MS** (CI): m/z (%) = 341 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1516 (C=O) – **MF** $C_{20}H_{24}N_2O_3$ – **MW** 340.18

4-{2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1_H_)-yl]ethyl}-_N_-methylaniline (5e)

To a suspension of $LiAlH_4$ (2.6 eq, 30 mg, 0.7 mmol) in dry THF a solution of *N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}formamide (100 mg, 0.3 mmol) was added dropwise at 0 °C. After two hours reaction time the mixture was treated with saturated NH_4Cl solution to destroy the excess of reducing agent. The water layer was then extracted twice with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated. The reaction yielded yellow solid (0.07 g, 0.2 mmol, 73 %), **mp** 86 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.68-2.84 (m, 11 H), 3.64 (s, 2 H), 3.83 (s, 3 H), 3.84 (s, 3 H), 6.54 (s, 1 H), 6.57 (d, *J* = 8.2 Hz, 2 H), 6.59 (s, 1 H), 7.06 (d, *J* = 8.5 Hz, 2 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 27.6 (-, CH_2), 29.9 (+, CH_3), 32.0 (-, CH_2), 50.0 (-, CH_2), 54.7 (-, CH_2), 54.8 (+, CH_3), 54.9 (+, CH_3), 59.7 (-, CH_2), 108.4 (+, CH_{arom}), 110.3 (+, CH_{arom}), 111.5 (+, 2 x CH_{arom}), 125.1 (C_{quat}),

125.5 (C_{quat}), 127.8 (C_{quat}), 128.4 (+, 2 x CH_{arom}), 146.1 (C_{quat}), 146.4 (C_{quat}), 146.7 (C_{quat}) – **MS** (CI): m/z (%) = 327 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 2806 (C-H), 3400 (N-H) – **MF** $C_{20}H_{26}N_2O_2$ – **MW** 326.2

N-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}acetamide (5f)

4-{2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl}aniline (50 mg, 0.16 mmol) was diluted in dichloromethane before 1.5 eq (20 mg, 30 µL, 0.24 mmol) of NEt_3, 0.5 eq (0.01 g, 0.08 mmol) of DMAP and 1.5 eq (20 mg, 0.24 mmol) of acylchloride were added. After stirring three hours at room temperature the mixture was diluted with further dichloromethane and the organic layer was washed with $NaHCO_3$ and water. Drying over Na_2SO_4 and evaporation yielded 0.05 g (0.13 mmol, 81 %) yellow oil, **mp** 105 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.05 (s, 3 H), 2.81-2.86 (m, 8 H), 3.70 (s, 2 H), 3.74 (s, 3 H), 3.75 (s, 3 H), 6.45 (s, 1 H), 6.52 (s, 1 H), 7.05 (d, *J* = 8.5 Hz, 2 H), 7.42 (d, *J* = 8.8 Hz, 2 H), 8.72 (s, 1 H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 24.4 (+, CH_3), 27.8 (-, CH_2), 32.7 (-, CH_2), 50.5 (-, CH_2), 54.9 (-, CH_2), 55.9 (+, CH_3), 56.0 (+, CH_3), 59.3 (-, CH_2), 109.4 (+, CH_{arom}), 111.3 (+, CH_{arom}), 120.2 (+, 2 x CH_{arom}), 125.2 (C_{quat}), 125.5 (C_{quat}), 129.1 (+, 2 x CH_{arom}), 135.3 (C_{quat}), 136.6 (C_{quat}), 144.0 (C_{quat}), 147.4 (C_{quat}), 147.7 (C_{quat}), 168.8 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 355 (100) [$M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1514 (N-H), 1671 (C=O) – **MF** $C_{21}H_{26}N_2O_3$ – **MW** 354.44

*N*4-{4-[2-(3,4-Dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-*N*1-methyl-2-nitroterephthalamide (7a)

The preparation followed GP 1 using 4-(methylcarbamoyl)-3-nitrobenzoic acid (0.27 g, 1.21 mmol), DIPEA (0.28 g, 0.37 mL, 2.2 mmol), HOBt (0.2 g, 1.3 mmol), TBTU (0.5 g, 1.3 mmol) and 4-{2-[3,4-dihydroisoquinolin-2(1H)-yl]ethyl}benzenamine (0.27 g, 1.1 mmol) diluted in dichloromethane with a few drops of DMF. The reaction yielded 0.55 g (1.2 mmol, 75 %) orange solid, **mp** 177 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.73-2.87 (m, 11 H), 3.69 (s, 2 H), 6.95-6.97 (m, 1 H), 7.05-7.08 (m, 3 H), 7.14 (d, *J* = 8.5 Hz, 2 H), 7.37 (d, *J* = 8.0 Hz, 1 H), 7.55 (d, *J* = 8.2 Hz, 2 H), 7.99 (dd, *J* = 1.4 Hz, 8.0 Hz, 1 H), 8.34 (d, *J* = 1.4 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 26.7 (+, CH_3), 28.5

(-, CH_2), 32.8 (-, CH_2), 50.8 (-, CH_2), 55.7 (-, CH_2), 59.8 (-, CH_2), 121.0 (+, 2 x CH_{arom}), 123.4 (+, CH_{arom}), 125.8 (+, CH_{arom}), 126.5 (+, CH_{arom}), 126.6 (+, CH_{arom}), 128.7 (+, CH_{arom}), 129.0 (+, CH_{arom}), 129.2 (+, 2 x CH_{arom}), 132.5 (+, CH_{arom}), 133.7 (2 x C_{quat}), 134.8 (C_{quat}), 135.9 (C_{quat}), 136.6 (C_{quat}), 137.2 (C_{quat}), 146.0 (C_{quat}), 163.7 (C_{quat}), 167.1 (C_{quat}) – **MS** (+ESI): m/z (%) = 459 (100) [M-H^+] - **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1604 (C=O), 1637 (C=O), 3263 (N-H) – **MF** $C_{26}H_{26}N_4O_4$ – **MW** 458.51

Methyl 4-{4-[2-(3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl] phenylcarbamoyl}-2-nitro-benzoate (7b)

The preparation followed GP 1 using 4-(methoxycarbonyl)-3-nitrobenzoic acid (0.6 g, 2.4 mmol), DIPEA (0.56 g, 0.74 mL, 4.4 mmol), HOBt (0.4 g, 2.6 mmol), TBTU (1.0 g, 2.6 mmol) and 4-{2-[3,4-dihydroisoquinolin-2(1H)-yl]ethyl}benzenamine (0.55 g, 2.2 mmol) diluted in dichloromethane with a few drops of DMF. The reaction yielded 0.86 g (1.9 mmol, 85 %) yellow solid, **mp** 79 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.74-2.95 (m, 8 H), 3.72 (s, 1 H), 3.95 (s, 3 H), 7.02-7.04 (m, 1 H), 7.10-7.23 (m, 3 H), 7.24 (d, *J* = 7.1 Hz, 2 H), 7.58 (d, *J* = 8.2 Hz, 2 H), 7.80 (d, *J* = 7.9 Hz, 1 H), 8.21 (dd, *J* = 7.9 Hz, 1.4 Hz, 1 H), 8.43 (d, *J* = 1.4 Hz, 1 H), 8.63 (s, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 29.1 (-, CH_2), 33.4 (-, CH_2), 51.0 (-, CH_2), 53.5 (+, CH_3), 56.1 (-, CH_2), 60.1 (-, CH_2), 121.1 (+, 2 x CH_{arom}), 123.1 (+, CH_{arom}), 125.6 (+, CH_{arom}), 126.2 (+, CH_{arom}), 126.6 (+, CH_{arom}), 128.7 (+, CH_{arom}), 129.3 (+, 2 x CH_{arom}), 129.6 (C_{quat}), 130.0 (+, CH_{arom}), 132.1 (+, CH_{arom}), 134.2 (C_{quat}), 134.6 (C_{quat}), 135.8 (C_{quat}), 137.3 (C_{quat}), 138.7 (C_{quat}), 147.9 (C_{quat}), 165.4 (C_{quat}), 165.7 (C_{quat}) – **MS** (+ESI): m/z (%) = 460 (100) [M-H^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1348 (N-O), 1536 (N-O), 1651 (C=O), 1740 (C=O), 3333 (N-H) – **MF** $C_{26}H_{25}N_3O_5$ – **MW** 459.49

Methyl4-{4-[2-(7-[*tert*-butoxycarbonylamino]-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]-phenyl-carbamoyl}-2-nitrobenzoate (7c)

The preparation followed GP 1 using 4-(methoxycarbonyl)-3-nitrobenzoic acid (0.27 g, 1.21 mmol), DIPEA (0.28 g, 0.37 mL, 2.2 mmol), HOBt (0.2 g, 1.3 mmol), TBTU (0.5 g, 1.3 mmol) and *tert*-butyl 2-(4-aminophenethyl)-1,2,3,4-tetrahydroisoquinolin-7-ylcarbamate (0.4 g, 1.0 mmol) diluted in

dichloromethane with a few drops of DMF. The reaction yielded 0.48 g (0.8 mmol, 40 %) yellow solid, **mp** 92 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.50 (s, 9 H), 2.72-2.86 (m, 8 H), 3.63 (s, 2 H), 3.94 (s, 3 H), 6.49 (s, 1 H), 6.96 (s, 1 H), 7.15 (s, 1 H), 7.21 (d, *J* = 8.5 Hz, 2 H), 7.59 (d, *J* = 8.2 Hz, 2 H), 7.76 (d, *J* = 7.9 Hz, 1 H), 8.19 (d, *J* = 7.9 Hz, 1 H), 8.42 (d, *J* = 1.4 Hz, 1 H), 8.9 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 28.3 (-, CH_2), 28.4 (+, CH_3, 3 x), 33.2 (-, CH_2), 51.0 (-, CH_2), 53.5 (+, CH_3), 55.9 (-, CH_2), 59.9 (-, CH_2), 116.8 (+, CH_{arom}), 117.2 (+, CH_{arom}), 121.1 (+, 2 x CH_{arom}), 123.1 (+, CH_{arom}), 124.9 (C_{quat}), 128.9 (C_{quat}), 129.2 (+, 2 x CH_{arom}), 129.5 (C_{quat}), 129.9 (+, CH_{arom}), 130.0 (+, CH_{arom}), 132.1 (+, CH_{arom}), 135.1 (C_{quat}), 135.9 (C_{quat}), 137.1 (C_{quat}), 138.6 (C_{quat}), 147.9 (C_{quat}), 153.2 (C_{quat}), 162.8 (C_{quat}), 165.4 (C_{quat}), 165.7 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 575 (100) [M-H$^+$]$^-$ - **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] 1513 (N-O), 1601 (C=O), 1673 (C=O), 1732 (C=O) – **MF** $C_{31}H_{34}N_4O_7$ – **MW** 574.62

Methyl 4-{[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl)-phenyl]-(methyl)-carbamoyl}-2-nitrobenzoate (7d)

4-{2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl}-*N*-methylaniline (0.5 g, 1.5 mmol) and 1.5 eq of NEt_3 (0.23 g, 0.32 mL, 2.3 mmol) were dissolved in dichloromethane and methyl 4-(chlorocarbonyl)-2-nitrobenzoate (0.56 g, 2.3 mmol) was added. After stirring the reaction mixture for 15 h at room temperature 10 mL dichloromethane were added and the organic layer was washed with $NaHCO_3$ and water. Then, the organic layer was dried over Na_2SO_4 and evaporated. Further purification was achieved by column chromatography (elution EtOAc > ETOAC/MeOH 6:1, R_f: 0.42). The reaction yielded yellow solid (0.47 g, 0.08 mmol, 44 %), **mp** 57 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.70-2.85 (m, 8 H), 3.48 (s, 3 H), 3.63 (s, 2 H), 3.82 (s, 3 H), 3.83 (s, 3 H), 3.86 (s, 3 H), 6.52 (s, 1 H), 6.58 (s, 1 H), 6.95 (d, *J* = 8.2 Hz, 2 H), 7.15 (d, *J* = 8.2 Hz, 2 H), 7.51 (d, *J* = 7.9 Hz, 1 H), 7.56 (dd, *J* = 1.1 Hz, 7.9 Hz, 1 H), 7.79 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 28.5 (-, CH_2), 33.3 (-, CH_2), 38.6 (+, CH_3), 50.9 (-, CH_2), 53.4 (+, CH_3), 55.6 (-, CH_2), 55.9 (+, CH_3), 56.0 (+, CH_3), 59.5 (-, CH_2), 109.5 (+, CH_{arom}), 111.4 (+, CH_{arom}), 124.4 (+, CH_{arom}), 126.0 (C_{quat}), 126.2 (C_{quat}), 126.9 (+, 2 x CH_{arom}), 128.0 (C_{quat}), 129.4 (+, CH_{arom}), 130.2 (+, 2 x CH_{arom}), 132.9 (+, CH_{arom}), 139.7 (C_{quat}), 140.2 (C_{quat}),

141.5 (C_{quat}), 147.3 (C_{quat}), 147.4 (C_{quat}), 147.6 (C_{quat}), 165.3 (C_{quat}), 166.7 (C_{quat}) – **MS** (CI): m/z (%) = 534 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1514 (C=O), 1733 (C=O) – **MF** $C_{29}H_{31}N_3O_7$ – **MW** 533.57

4-{2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl}phenyl4-(methylcarbamoyl)-3-nitrobenzoate (7e)

The preparation followed GP 1 using 4-(methylcarbamoyl)-3-nitrobenzoic acid (0.3 g, 1.3 mmol), DIPEA (0.31 g, 0.40 mL, 2.4 mmol), HOBt (0.23 g, 1.5 mmol), TBTU (0.56 g, 1.5 mmol) and 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]ethyl}aniline (0.37 g, 1.2 mmol) diluted in dichloromethane with a few drops of DMF. The product was purified with column chromatography (elution with MeOH/EE 1:3, R_f: 0.35). The reaction yielded 0.28 g (0.5 mmol, 42 %) of yellow solid, **mp** 178 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.64-2.80 (m, 8 H), 3.28 (s, 2 H), 3.57 (s, 3 H), 3.72 (s, 3 H), 3.73 (s, 3 H), 6.44 (s, 1 H), 6.51 (s, 1 H), 7.14 (d, *J* = 8.2 Hz, 2 H), 7.47 (d, *J* = 7.7 Hz, 1 H), 7.52 (d, *J* = 8.2 Hz, 2 H), 8.07 (d, *J* = 7.9 Hz, 1 H), 8.46 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, [D_6]-DMSO): δ = 26.0 (+, CH_3), 28.2 (-, CH_2), 32.3 (-, CH_2), 50.5 (-, CH_2), 55.0 (-, CH_2), 55.3 (+, CH_3), 55.4 (+, CH_3), 59.5 (-, CH_2), 109.8 (+, CH_{arom}), 111.6 (+, CH_{arom}), 120.4 (+, 2 x CH_{arom}), 123.1 (+, CH_{arom}), 125.8 (C_{quat}), 126.5 (C_{quat}), 128.8 (+, 2 x CH_{arom}), 128.9 (C_{quat}), 129.2 (+, CH_{arom}), 132.4 (+, CH_{arom}), 134.6 (C_{quat}), 136.4 (C_{quat}), 136.6 (C_{quat}), 146.7 (C_{quat}), 146.8 (C_{quat}), 147.0 (C_{quat}), 162.4 (C_{quat}), 165.1 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 519 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1516 (N-O), 1600 (C=O), 1640 (C=O), 3300 (N-H) – **MF** $C_{28}H_{30}N_4O_6$ – **MW** 518.55

4-Acetyl-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]-phenyl}-3-nitro benzamide (7f)

Preparation following GP 1 using 4-acetyl-3-nitrobenzoic acid (0.74 g, 3.5 mmol), DIPEA (0.82 g, 1.09 mL, 6.4 mmol), HOBt (0.58 g, 3.8 mmol), TBTU (0.38 g, 3.8 mmol) and 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]ethyl}aniline (1.0 g, 3.2 mmol) diluted in dichloromethane. The reaction yielded 1.42 g (88 %) of a yellow solid, **mp** > 190 °C – **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 2.62 (s, 3 H), 2.78-2.86 (m, 8 H), 3.70 (s, 6 H), 5.76 (s, 2 H), 6.66 (s, 1 H), 6.68 (s, 1 H), 7.27 (d, *J* = 8.5 Hz, 2 H), 7.71 (d, *J* = 8.5 Hz, 2 H), 7.94

(d, *J* = 7.9 Hz, 2 H), 8.41 (dd, *J* = 7.6 Hz, *J* = 1.6 Hz, 1 H), 8.64 (d, *J* = 1.6 Hz, 1 H), 10.60 (s, 1 H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 28.4 (-, CH_2), 30.1 (+, CH_3), 30.9 (+, CH_3), 33.2 (-, CH_2), 50.9 (-, CH_2), 55.5 (-, CH_2), 55.9 (+, CH_3), 59.8 (-, CH_2), 109.4 (+, CH_{arom}), 111.3 (+, CH_{arom}), 120.9 (+, CH_{arom}), 123.2 (+, CH_{arom}), 124.5 (+, CH_{arom}), 125.9 (C_{quat}), 126.1 (C_{quat}), 127.7 (+, CH_{arom}), 129.3 (+, 2 x CH_{arom}), 133.2 (+, CH_{arom}), 135.6 (C_{quat}), 137.2 (C_{quat}), 137.5 (C_{quat}), 139.9 (C_{quat}), 145.6 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 199.3 (C_{quat}), 208.2 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 504 (100) [MH^+] – **UV/Vis** ($CHCl_3$) λ_{max} [nm] (lg ε): 276 (4.9732) – **IR** (KBr) $\tilde{\nu}$ [cm^{-1}] = 3307 (N-H), 2933 (C-H), 1707 (C=O), 1647 (C=O; amide), 1599 (N-H), 1518 (N-H) – **MF** $C_{28}H_{29}N_3O_6$ – **MW** 503.55 g/mol

N-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-4-(1-hydroxypropyl)-3-nitrobenzamide (7g)

Preparation according to GP 1 using 4-(1-hydroxypropyl)-3-nitrobenzoic acid (0.25 g, 1.1 mmol), DIPEA (0.26 g, 0.33 mL, 2.0 mmol), HOBt (0.14 g, 1.2 mmol), TBTU (0.45 g, 1.2 mmol) and 4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)aniline (0.31 g, 1.0 mmol) diluted in dichloromethane and a few drops of DMF. After further purification with column chromatography (elution with EtOAc/MeOH, 4:1, R_f: 0.72) and evaporation of the solvent the reaction yielded 0.46 g (88 %) of a yellow oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.03 (t, *J* = 7.4 Hz, 3 H), 1.59-1.86 (m, 2 H), 2.86-2.94 (m, 8 H), 3.61 (s, 1 H), 3.79 (s, 3 H), 3.80 (s, 3 H), 5.13-5.19 (m, 1 H), 6.46 (s, 1 H), 6.54 (s, 1 H), 7.22 (d, *J* = 8.5 Hz, 2 H), 7.59 (d, *J* = 8.5 Hz, 2 H), 7.84 (d, *J* = 8.2 Hz, 1 H), 8.03 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1 H), 8.32 (d, *J* = 1.6 Hz, 1 H), 8.55 (s, 1 H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 10.5 (+, CH_3), 28.5 (-, CH_2), 31.6 (-, CH_2), 33.2 (-, CH_2), 51.0 (-, CH_2), 55.7 (-, CH_2), 55.9 (+, 2 x CH_3), 60.0 (-, CH_2), 70.1 (+, CH), 109.4 (+, CH_{arom}), 111.3 (+, CH_{arom}), 120.8 (+, 2 x CH_{arom}), 123.0 (+, CH_{arom}), 126.0 (C_{quat}), 126.1 (C_{quat}), 128.8 (+, CH_{arom}), 129.3 (+, 2 x CH_{arom}), 131.6 (+, CH_{arom}), 134.8 (C_{quat}), 135.8 (C_{quat}), 136.9 (C_{quat}), 144.2 (C_{quat}), 147.1 (C_{quat}), 147.3 (C_{quat}), 147.5 (C_{quat}), 165.6 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 520.2 (100) [$M\text{-}H^+$] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 3559 (O-H), 1664 (C=O, amide), 1604 (N-H, amide), 1514 (N-O), 1324 (N-O) – **MF** $C_{29}H_{33}N_3O_6$ – **MW** 519.59 g/mol

2-Amino-*N*4-{4-[2-(3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-*N*1-methylterephthalamide (8a)

*N*4-{4-[2-(3,4-Dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-*N*1-methyl-2-nitroterephthal-amide (0.5 g, 1.2 mmol) was dissolved in ethanol (p.a.) and treated with 5 eq $SnCl_2$ (1.14 g, 6.0 mmol) and then heated under nitrogen to 70 °C for 30 min. The reaction mixture was poured on ice and the pH was adjusted to 9 and extracted with ethyl acetate. Then the organic layer was dried over Na_2SO_4 and evaporated. The reaction yielded 0.38 g (0.88 mmol, 73 %) of yellow solid, **mp** 147 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.79 - 2.88 (m, 8 H), 2.91 (s, 3 H), 3.70 (s, 2 H), 6.96-7.00 (m, 2 H), 7.07 – 7.09 (m, 3 H), 7.15 (d, *J* = 8.5 Hz, 2 H), 7.30 (d, *J* = 8.1 Hz, 1 H), 7.54 (d, *J* = 8.4 Hz, 2 H), 7.92 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 26.3 (+, CH_3), 28.5 (-, CH_2), 32.9 (-, CH_2), 50.8 (-, CH_2), 55.8 (-, CH_2), 59.9 (-, CH_2), 115.0 (+, CH_{arom}), 115.8 (+, CH_{arom}), 118.9 (C_{quart}), 120.4 (+, CH_{arom}), 120.8 (+, CH_{arom}), 120.9 (C_{quart}), 125.8 (+, CH_{arom}), 126.4 (+, CH_{arom}), 126.6 (+, 2 x CH_{arom}), 127.9 (+, CH_{arom}), 128.6 (+, CH_{arom}), 129.1 (+, 2 x CH_{arom}), 133.8 (2 x C_{quart}), 136.2 (C_{quart}), 138.1 (C_{quart}), 148.3 (C_{quart}), 162.9 (C_{quart}), 169.7 (C_{quart}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 429 (100) $[M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1599 (C=O), 1635 (C=O), 3318 (N-H) – **MF** $C_{26}H_{28}N_4O_2$ – **MW** 428.53

Methyl 2-amino-4-{4-[2-(3,4-dihydroisoquinolin-[1*H*]-yl)ethyl]phenyl-carbamoyl}benzoate (8b)

The preparation followed GP 2 using methyl 4-{4-[2-(3,4-dihydroisoquinolin-2 [1*H*]-yl)ethyl]phenylcarbamoyl}-2-nitro-benzoate (1.1 g, 2.4 mmol) diluted in ethyl acetate. The reaction yielded 1.03 g (2.4 mmol, 100 %) yellow solid, **mp** 89 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.55-2.94 (m, 8 H), 3.71 (s, 2 H), 3.87 (s, 3 H), 5.90 (s, 2 H, NH_2), 6.98-7.03 (m, 2 H), 7.09-7.13 (m, 3 H), 7.20-7.23 (m, 3 H), 7.55 (d, *J* = 8.2 Hz, 2 H), 7.90 (d, *J* = 8.2 Hz, 1 H), 8.08 (s, 1H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 29.0 (-, CH_2), 33.3 (-, CH_2), 50.9 (-, CH_2), 51.8 (+, CH_3), 56.0 (-, CH_2), 60.2 (-, CH_2), 112.7 (C_{quat}), 113.8 (+, CH_{arom}), 115.9 (+, CH_{arom}), 120.6 (+, 2 x CH_{arom}), 125.6 (+, CH_{arom}), 126.2 (+, CH_{arom}), 126.6 (+, CH_{arom}), 128.7 (+, CH_{arom}), 129.2 (+, 2 x CH_{arom}), 131.8 (+, CH_{arom}), 134.2 (C_{quat}), 134.6 (C_{quat}), 136.0 (C_{quat}), 136.7 (C_{quat}), 140.0 (C_{quat}), 150.5 (C_{quat}), 165.8 (C_{quat}), 168.0 (C_{quat}) – **MS** (+ESI): m/z (%) = 430 (100) $[M\text{-}H^+]$ – **IR**

(ATR) $\tilde{\nu}$ [cm^{-1}] = 1620 (C=O), 1695 (C=O), 3330 (N-H), 3490 (N-H) – **MF** $C_{26}H_{27}N_3O_3$ – **MW** 429.51

Methyl 2-amino-4-{4-[2-(7-[tert-butoxycarbonylamino]-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenylcarbamoyl}benzoate (8c)

The preparation followed GP 2 diluting methyl4-{4-[2-(7-[tert-butoxycarbonylamino]-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]-phenyl-carbamoyl}-2-nitrobenzoate (0.3 g, 0.52 mmol) in ethanol. The reaction yielded 0.1 g (0.2 mmol, 35 %) yellow solid, **mp** 172 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.49 (s, 9 H), 2.71-2.85 (m, 8 H), 3.66 (s, 2 H), 3.86 (s, 3 H), 6,58 (s, 1H), 6.96-7.01 (m, 2 H), 7.13-7.19 (m, 3 H), 7.53 (d, *J* = 8.5 Hz, 2 H), 7.86 (d, *J* = 8.2 Hz, 1 H), 8.16 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 28.0 (-, CH_2), 28.8 (+, CH_3, 3 x), 32.9 (-, CH_2), 51.9 (-, CH_2),52.2 (+, CH_3), 56.2 (-, CH_2), 60.3 (-, CH_2), 112.2 (C_{quat}), 113.2 (C_{quat}), 114.7 (+, CH_{arom}), 117.0 (+, CH_{arom}), 117.6 (C_{quat}), 122.5 (+, 2 x CH_{arom}), 122.8 (+, CH_{arom}), 124.4 (+, CH_{arom}), 126.4 (C_{quat}), 128.3 (C_{quat}), 130.1 (+, CH_{arom}), 130.2 (+, 2 x CH_{arom}), 132.6 (+, CH_{arom}), 133.7 (C_{quat}), 136.5 (C_{quat}), 138.9 (C_{quat}), 141.4 (C_{quat}), 152.6 (C_{quat}), 155.3 (C_{quat}), 169.4 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 545 (100) $[M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1514 (C=O), 1599 (C=O), 1685 (C=O) – **MF** $C_{31}H_{36}N_4O_5$ – **MW** 544.64

Methyl 2-amino-4-{[4-(2-[6,7-dimethoxy-3,4- dihydroisoquinolin-2(1*H*)-yl] ethyl) phenyl] [methyl] carbamoyl} benzoate (8d)

The preparation followed GP 2 dissolving methyl 4-{[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]ethyl)phenyl](methyl)carbamoyl}-2-nitrobenzoate (0.5 g, 0.9 mmol) in methanol and yielded 0.5 g (0.9 mmol, 100 %) yellow solid, **mp** 114 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.27-3.30 (m, 8 H), 3.40 (s, 3 H), 3.46 (s, 2 H), 3.79 (s, 3 H), 3.81 (s, 3 H), 3.84 (s, 3 H), 6.48 (d, *J* = 8.23 Hz, 1 H), 6.52 (s, 1 H), 6.61 (s, 1 H), 6.71 (s, 1 H), 6.99 (d, *J* = 6.86 Hz, 2 H), 7.13 (d, *J* = 7.68 Hz, 2 H), 7.62 (d, *J* = 8.2 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 23.8 (-, CH_2), 30.0 (-, CH_2), 49.2 (-, CH_2), 50.4 (+, CH_3), 51.7 (+, CH_3), 52.2 (-, CH_2), 55.3 (-, CH_2), 56.0 (+, CH_3), 56.1 (+, CH_3), 109.3 (+, CH_{arom}), 111.1 (+, CH_{arom}), 111.7 (C_{quat}), 117.2 (+, CH_{arom}), 118.1 (C_{quat}), 122.4 (C_{quat}), 127.1 (+, 2 x CH_{arom}), 127.3(+, CH_{arom}), 129.7 (+, 2 x CH_{arom}), 130.9 (+,

CH_{arom}), 134.7 (C_{quat}), 141.2 (C_{quat}), 143.2 (C_{quat}), 148.5 (C_{quat}), 149.0 (C_{quat}), 149.1 (C_{quat}), 167.8 (C_{quat}), 169.9 (C_{quat}) – **MS** (CI): m/z (%) = 504 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1518 (C=O), 1690 (C=O), 3445 (N-H) – **MF** $C_{29}H_{33}N_3O_5$ – **MW** 503.24

2-Amino-*N*4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]-phenyl}-*N*1-methylterephthalamide (8e)

4-{2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2 (1*H*)-yl]ethyl] phenyl4- (methyl-carba-moyl)-3-nitrobenzoate (0.16 g, 0.3 mmol) was dissolved in ethanol (p.a.) and treated with 5 eq $SnCl_2$ (0.29 g, 1.5 mmol) and then heated under nitrogen to 70 °C for 30 min. The reaction mixture was poured on ice and the pH was adjusted to 9 and extracted with ethyl acetate. Then the organic layer was dried over Na_2SO_4 and evaporated. The reaction yielded 0.07 g (0.14 mmol, 46 %) of yellow solid, **mp** 174 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.73 - 2.92 (m, 8 H), 2.95 (d, 4.7 Hz, 3 H), 3.67 (s, 2 H), 3.80 (s, 3 H), 3.81 (s, 3 H), 6.49 (s, 1 H), 6.56 (s, 1 H), 6.99 (d, 8.5 Hz, 1 H), 7.13-7.18 (m, 2 H), 7.33 (d, 8.2 Hz, 1 H), 7.51 (d, 7.9 Hz, 2 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 26.4 (-, CH_2), 27.7 (+, CH_3), 32.7 (-, CH_2), 50.7 (-, CH_2), 55.1 (-, CH_2), 55.9 (+, CH_3), 55.9 (+, CH_3), 59.5 (-, CH_2), 109.5 (+, CH_{arom}), 111.3 (+, CH_{arom}), 114.5 (C_{quat}), 115.0 (+, CH_{arom}), 115.6 (+, CH_{arom}), 115.9 (+, CH_{arom}), 116.0 (C_{quat}), 118.9 (C_{quat}), 120.8 (+, CH_{arom}), 125.1 (C_{quat}), 125.5 (C_{quat}), 127.9 (+, CH_{arom}), 129.2 (+, CH_{arom}), 129.5 (+, CH_{arom}), 135.8 (C_{quat}), 136.3 (C_{quat}), 138.1 (C_{quat}), 147.4 (C_{quat}), 147.8 (C_{quat}), 148.4 (C_{quat}) – **MS** (+ESI): m/z (%) = 489 (100) [$M-H^+$] - **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1531 (C=O), 1637 (C=O), 3197 (N-H), 3263 (N-H) – **MF** $C_{28}H_{32}N_4O_4$ – **MW** 488.58

4-Acetyl-3-amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)-ethyl]phenyl} benzamide (8f)

Preparation according to GP 2 with 4-acetyl-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroiso-quinolin-2[1*H*]-yl)ethyl]phenyl}-3-nitro benzamide (0.4 g, 0.8 mmol). Evaporation of the solvents yielded 0.3 g of a yellow solid. The substance was purified by column chromatography on silica gel (elution with MeOH/ETOAC, 1:2, R_f: 0.55). The yield was 0.2 g (0.4 mmol, 50 %), **mp** > 190 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.57 (s, 3 H), 2.93-3.10 (m, 8 H), 3.79 (s, 3 H), 3.81 (s, 3

H), 4.00 (s, 2 H), 6.45 (s, 1 H), 6.54 (s, 1 H), 7.02 (d, *J* = 8.5 Hz, 2 H), 7.17 (d, *J* = 8.5 Hz, 2 H), 7.57 (d, *J* = 7.4 Hz, 1 H), 7.73 (d, *J* = 8.5 Hz, 1 H), 8.39 (s, 1 H) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 474 (100) [MH^+] – **MF** $C_{28}H_{31}N_3O_4$ – **MW** 473.56 g/mol

3-Amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]-phenyl}-4-(1-hydroxypropyl)benzamide (8g)

Preparation following GP 2 with *N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-4-(1-hydroxypropyl)-3-nitrobenzamide (30 mg, 0.06 mmol) diluted in ethyl acetate/methanol 1:1. Evaporation of the solvents yielded 20 mg (0.04 mmol, 56 %) of a yellow solid, **mp** 132 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.9 (t, *J* = 7.3 Hz, 3 H), 1.75-1.88 (m, 2 H), 2.70-2.90 (m, 8 H), 3.82 (s, 3 H), 3.83 (s, 3 H), 4.58 (t, *J* = 7.0 Hz, 1 H), 6.5 (s, 1H), 6.6 (s, 1 H), 7.06 (d, *J* = 5.7 Hz, 2), 7.12 (s, 1 H), 7.17 (s, 1 H), 7.19 (s, 1 H), 7.55 (d, *J* = 8.5 Hz, 2 H), 7.98 (s, 1 H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 3.1 (+, CH_3), 10.7 (+, 2 x CH_3,), 19.4 (-, CH_2), 27.0 (-, CH_2), 27.9 (-, CH_2), 31.8 (-, CH_2), 32.5 (-, CH_2), 42.8 (-, CH_2), 43.7 (+, CH), 89.4 (+, CH_{arom}), 109.3 (+, CH_{arom}), 111.2 (+, CH_{arom}), 113.0 (C_{quat}), 116.1 (C_{quat}), 120.8 (+, 2 x CH_{arom}), 127.8 (+, CH_{arom}), 129.1 (+, 2 x CH_{arom}), 131.2 (+, CH_{arom}), 134.3 (C_{quat}), 136.7 (C_{quat}), 136.9 (C_{quat}), 137.4 (C_{quat}), 145.5 (C_{quat}), 147.6 (C_{quat}), 148.0 (C_{quat}), 166.3 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 490 (100) [M-H^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 3310 (O-H), 3057 (N-H, amine), 1602 (N-H, amine), 1522 (C=O, amide) – **MF** $C_{29}H_{35}N_3O_4$ – **MW** 489.61 g/mol

Methyl 4-{4-[2-(3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenylcarbamoyl}-2-(quinoline-2-carboxamido)benzoate (9a)

Methyl 2-amino-4-{4-[2-(3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenylcarbamoyl} benzoate (0.28 g, 0.65 mmol) was dissolved in dry DMF before 1.5 eq of NEt_3 (0.1 g, 0.14 mL, 1.0 mmol) and 0.5 eq of DMAP (40 mg, 0.32 mmol) were added. Under nitrogen atmosphere the acid chloride (0.18 g, 1.0 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. Then the DMF was removed, the residue was taken up with water and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. Parts of the product were purified with

HPLC to get 1 mg pale yellow solid for pharmacological studies. Due to the small scale, purity was checked with HPLC, **mp** 145 °C (decomposition) – **MS** (+ ESI): m/z (%): 585.2 (100 %) [M-H$^+$] – **MF** $C_{36}H_{32}N_4O_4$ – **MW** 584.66

_N_4-{4-[2-(3,4-Dihydroisoquinolin-2[1_H_]-yl)ethyl]phenyl}-_N_1-methyl-2-(quinoline-2-carboxamido)terephthalamide (9b)

2-Amino-_N_4-{4-[2-(3,4-dihydroisoquinolin-2[1_H_]-yl)ethyl]phenyl}-_N_1-methylterephthal-amide (0.36 g, 0.85 mmol) was dissolved in dry DMF before 1.5 eq of NEt_3 (0.13 g, 0.18 mL, 1.3 mmol) and 0.5 eq of DMAP (50 mg, 0.43 mmol) were added. Under nitrogen atmosphere the acid chloride (0.24 g, 1.3 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. Then the DMF was removed, the residue was taken up with water and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. Parts of the product were purified with HPLC to get 1 mg pale yellow solid for pharmacological studies. Due to the small scale, purity was checked with HPLC, **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 584 (100) [M-H$^+$]$^-$ – **MF** $C_{36}H_{33}N_5O_3$ – **MW** 583.68

Methyl 4-{4-[2-(7-amino-3,4-dihydroisoquinolin-2[1_H_]-yl)ethyl]phenylcarbamoyl}-2-(quinoline-2-carboxamido)benzoate (9d)

Methyl 2-amino-4-{4-[2-(7-[tert-butoxycarbonylamino]-3,4-dihydroisoquinolin-2[1_H_]-yl)ethyl]phenylcarbamoyl}benzoate (100 mg, 0.36 mmol) was dissolved in dry DMF, 1.5 eq NEt_3 (54 mg, 77 µL, 0.54 mmol), 0.5 eq DMAP (21 mg, 0.18 mmol) and 1.5 eq quinoline-2 carboyl chloride (103 mg, 0.54 mmol) were added. The reaction mixture was stirred for two hours at 80 °C and for further 15 h at room temperature. Then, the solution was diluted with dichloromethane and washed twice with saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. Without further purification methyl 4-{4-[2-(7-[tert-butoxycarbonylamino]-3,4-dihydroisoquinolin-2[1H]-yl)ethyl]phenylcarbamoyl}-2-(quinoline-2-carboxamido)benzoate (0.01 g, 0.01 mmol, 1 eq) was dissolved in dichloromethane and HCl-Ether (15 % /mmol, 0.1 mL). After 15 h stirring at room temperature the solvent was removed. The product was then purified with preparative HPLC to get 1 mg pale

yellow solid for pharmacological studies. Due to the small scale, purity was analyzed with HPLC, **MS** (ESI): m/z (%) = 600 (100) [MH^+] – **MF** $C_{36}H_{33}N_5O_4$ – **MW** 599.68

N-{3-[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl)-phenylcarbamoyl]phenyl}quinoline-2-carboxamide (9e)

3-Amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}benzamide (100 mg, 0.2 mmol) was dissolved in dry DMF before 1.5 eq of NEt_3 (30 mg, 0.04 mL, 6.7 mmol) and 0.5 eq of DMAP (12 mg, 0.1 mmol) were added. Under nitrogen atmosphere the acid chloride (60 mg, 0.34 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. Then the DMF was removed, the residue was taken up with water and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The product was purified with column chromatography (elution with MeOH/ETOAC 1:9, R_f: 0.48). The reaction yielded 20 mg (0.03 mmol, 17 %) of a pale yellow solid, **mp** 180 °C (decomposition) – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.78-2.93 (m, 8 H), 3.69 (s, 2 H), 3.82 (s, 6 H), 6.52 (s, 1 H), 6.58 (s, 1 H), 7.23 (d, *J* = 8.5 Hz, 2 H), 7.51 (t, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 7.7 Hz, 2 H), 7.68 (s, 1 H), 7.74 (d, *J* = 7.7 Hz, 1 H), 7.82 (t, *J* = 6.7 Hz, 2 H), 7.91 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* = 8.5 Hz, 1 H), 8.35-8.40 (m, 3 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 32.1 (-, CH_2), 37.0 (-, CH_2), 54.8 (-, CH_2), 59.4 (-, CH_2), 59.7 (+, CH_3), 59.8 (+, CH_3), 63.9 (-, CH_2), 95.1 (C_{quat}), 113.3 (+, CH_{arom}), 115.2 (+, CH_{arom}), 122.4 (+, CH_{arom}), 122.5 (+, CH_{arom}), 124.7 (+, CH_{arom}), 127.0 (C_{quat}), 148.0 (+, CH_{arom}), 129.8 (C_{quat}), 129.9 (C_{quat}), 131.8 (+, CH_{arom}), 132.4 (+, CH_{arom}), 133.0 (+, 2 x CH_{arom}), 133.2 (C_{quat}), 133.4 (+, CH_{arom}), 133.5 (+, 2 x CH_{arom}), 134.5 (+, CH_{arom}), 140.0 (C_{quat}), 140.4 (C_{quat}), 141.3 (+, CH_{arom}), 142.0 (+, CH_{arom}), 150.2 (C_{quat}), 151.1 (C_{quat}), 151.5 (C_{quat}), 152.8 (C_{quat}), 160.7 (C_{quat}), 169.9 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 587 (100) [M^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1651 (C=O), 1668 (C=O), 2342 (N-$(C)_3$), 2360 (N-$(C)_3$), 3344 (N-H) – **MF** $C_{36}H_{34}N_4O_4$ – **MW** 586.68

4-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenylcarbamoyl}-2-(quinoline-3-carboxamido)benzoic acid (9f)

Compound methyl 4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenylcarbamoyl}-2-(quinoline-3-carboxamido)benzoate (50 mg, 0.077 mmol) was dissolved in 5 mL of DMF and 3 eq of LiOH dissolved in a small amount of water were added. The reaction was carried out in the microwave (80 °C, 300 W, 4 min). To work-up the product, the solution was acidified with diluted HCl and extracted with dichloromethane. The crude product was purified by column chromatography on silica gel (elution with chloroform/methanol 10:7, R_f: 0.38) and preparative HPLC (acetonitrile/water (TFA 0.0059 %)) and yielded 5 mg (0.007 mmol, 10 %) of a yellow solid, **mp** 185 °C – **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 2.65-3.38 (m, 8 H), 3.76 (s, 6 H), 3.85-4.05 (m, 2 H), 6.79 (d, *J* = 4.9 Hz, 1 H), 7.30 (d, *J* = 8.5 Hz, 2 H), 7.68-7.78 (m, 4 H), 7.91 (t, *J* = 2.27, 1 H), 8.11-8.20 (m, 3 H), 8.96 (s, 1 H), 9.19 (s, 1 H), 9.46 (s, 1 H), 10.47 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, [D_6]-DMSO): δ = 25.4 (-, CH_2), 33.0 (-, CH_2), 34.2 (-, CH_2), 39.8 (-, CH_2), 42.8 (-, CH_2), 56.4 (+, CH_3), 56.5 (+, CH_3), 109.9 (+, CH_{arom}), 114.3 (C_{quat}), 117.8 (+, CH_{arom}), 118.2 (+, CH_{arom}), 120.6 (+, CH_{arom}), 122.0 (+, CH_{arom}), 122.8 (+, CH_{arom}), 125.6 (C_{quat}), 129.2 (+, CH_{arom}), 134.2 (+, CH_{arom}), 134.7 (+, CH_{arom}), 135.1 (+, CH_{arom}), 137.7 (+, CH_{arom}), 138.6 (+, CH_{arom}), 138.7 (C_{quat}), 139.3 (C_{quat}), 139.5 (C_{quat}), 141.8 (+, CH_{arom}), 142.3 (C_{quat}), 142.4 (+, CH_{arom}), 143.0 (C_{quat}), 145.0 (C_{quat}), 146.9 (C_{quat}), 147.8 (C_{quat}), 148.6 (C_{quat}), 150.8 (+, CH_{arom}), 151.2 (C_{quat}), 164.0 (C_{quat}), 174.1 (C_{quat}), 177.1 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 631 (100) [MH^+] – **UV/Vis** (DMSO) λ_{max} [nm]: 245 – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 3359 (O-H, acid), 1631 (C=O, amide) – **MF** $C_{37}H_{34}N_4O_6$ – **MW** 630.69 g/mol

Methyl 4-{[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl)-phenyl][methyl] carbamoyl}-2-(quinoline-2-carboxamido)benzoate (9g)

Methyl 2-amino-4-{[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl] ethyl)-phenyl][methyl]carbamoyl} benzoate (0.5 g, 0.99 mmol) was dissolved in dichloromethane before 1.5 eq of NEt_3 (0.15 g, 0.2 mL, 1.5 mmol) were added. Under nitrogen atmosphere the acid chloride (0.28 g, 1.5 mmol) was added. After two hours at 40 °C the reaction mixture was stirred over night at room temperature. The residue was taken up with water and extracted with

dichloromethane and washed with saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and evaporated. Further purification was achieved with column chromatography (elution EtOAc > MeOH, R_f: 0.35). The reaction yielded 0.45 g (0.7 mmol, 69 %) yellow solid, **mp** 65 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.60-2.77 (m, 8 H), 3.39 (s, 2 H), 3.75 (s, 3 H), 3.76 (s, 3 H), 3.79 (d, *J* = 1.6 Hz, 3 H), 3.96 (s, 3 H), 6.39 (s, 1 H), 6.48 (s, 1 H), 6.55 (s, 1 H), 6.66 (d, *J* = 1.4 Hz, 1 H), 6.92 (d, *J* = 7.9 Hz, 1 H), 7.02-7.15 (m, 5 H), 7.58 (t, *J* = 6.9 Hz, 1 H), 7.74 (t, *J* = 6.9 Hz, 1 H), 7.83 (d, *J* = 8.2 Hz, 1 H), 7.92 (d, *J* = 8.2 Hz, 1 H), 8.23 (d, *J* = 8.5 Hz, 1 H), 8.93 (s, 1H, NH), 13.09 (s, 1H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 21.0 (+, CH_3), 28.4 (-, CH_2), 33.3 (-, CH_2), 50.8 (-, CH_2), 52.5 (+, CH_3), 55.5 (-, CH_2), 55.8 (+, CH_3), 55.9 (+, CH_3), 59.6 (-, CH_2), 109.4 (+, CH_{arom}), 111.2 (+, CH_{arom}), 111.3 (+, CH_{arom}), 118.9 (+, CH_{arom}), 122.4 (+, CH_{arom}), 125.9 (C_{quat}), 126.0 (C_{quat}), 126.2 (C_{quat}), 126.6 (+, CH_{arom}), 127.0 (+, CH_{arom}), 127.6 (+, CH_{arom}), 128.3 (+, CH_{arom}), 129.3 (C_{quat}), 129.5 (+, CH_{arom}), 129.6 (+, CH_{arom}), 130.1 (+, CH_{arom}), 130.2 (+, CH_{arom}), 130.9 (+, CH_{arom}), 137.6 (+, CH_{arom}), 139.0 (C_{quat}), 139.2 (C_{quat}), 140.3 (C_{quat}), 142.0 (C_{quat}), 146.5 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 149.9 (C_{quat}), 163.2 (C_{quat}), 167.4 (C_{quat}), 169.6 (C_{quat}) – **MS** (CI): m/z (%) = 659 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1568 (C=O), 1643 (C=O), 1687 (C=O) – **MF** $C_{39}H_{38}N_4O_6$ – **MW** 658.74

*N*4-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-*N*1-methyl-2-(quinoline-2-carboxamido)terephthalamide (9h)

2-Amino-*N*4-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-*N*1-methylterephthalamide (70 mg, 0.14 mmol) was dissolved in dry DMF before 1.5 eq of NEt_3 (20 mg, 0.03 mL, 0.2 mmol) and 0.5 eq of DMAP (8 mg, 0.07 mmol) were added. Under nitrogen atmosphere the acid chloride (40 mg, 0.2 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. Then the DMF was removed under reduced pressure, the residue was taken up with water and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and evaporated. Parts of the product were purified with HPLC to get 1 mg pale yellow solid for pharmacological studies. Due to the small scale, purity was checked with HPLC, **MS** (+ESI): m/z (%) = 644 (100) [$M\text{-}H^+$] – **MF** $C_{38}H_{37}N_5O_5$ – **MW** 643.73

_N_-{2-Acetyl-5-[4-(2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1_H_)-yl]ethyl)-phenylcarbamoyl] phenyl} quinoline-3-carboxamide (9i)

4-Acetyl-3-amino-_N_-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1_H_]-yl)ethyl]-phenyl} benzamide (0.12 g, 0.25 mmol), Et_3N (1.5 eq, 0.14 g, 0.5 mmol, 0.2 mL) and DMAP (0.5 eq, 15 mg, 0.12 mmol) were dissolved in dry DMF and under nitrogen atmosphere quinoline-3-carbonyl chloride (0.1 g, 0.5 mmol) was added. The reaction mixture was heated for two hours at 90 °C. After stirring another 16 h at room temperature it was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The reaction yielded 70 mg (0.12 mmol, 50 %) of an orange solid, **mp** > 190 °C (decomposition) – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.51 (s, 3 H), 3.06-3.26 (m, 6 H), 3.36-3.43 (m, 2 H), 3.77 (s, 3 H), 3.78 (d, 3 H), 4.11 (s, 2H), 6.48 (s, 1 H), 6.56 (s, 1 H), 7.02 (dd, _J_ = 1.3 Hz, _J_ = 8.2 Hz, 1 H), 7.16 (d, _J_ = 8.5 Hz, 2 H), 7.55 (t, _J_ = 7.5 Hz, 1 H), 7.58-7.63 (m, 3 H), 7.76 (dt, _J_ = 1.3 Hz, _J_ = 7.0 Hz, 1 H), 7.87 (d, _J_ = 7.9 Hz, 1 H), 8.13 (d, _J_ = 8.5 Hz, 1 H), 8.71 (s, 1 H), 8.82 (d, _J_ = 1.6 Hz, 1 H), 9.49 (d, _J_ = 1.9 Hz, 1 H) – **^{13}C-NMR**, HSQC (75 MHz, $CDCl_3$): δ = 27.9 (+, CH_3), 31.5 (-, CH_2), 44.9 (-, CH_2), 49.5 (-, CH_2), 53.4 (-, CH_2), 55.8 (+, CH_3), 55.9 (+, CH_3), 57.2 (-, CH_2), 100.1 (+, CH_{arom}), 109.3 (+, CH_{arom}), 111.2 (+, CH_{arom}), 112.9 (C_{quat}), 113.3 (+, CH_{arom}), 113.9 (C_{quat}), 116.3 (+, CH_{arom}), 120.6 (+, CH_{arom}), 120.7 (+, CH_{arom}), 121.0 (C_{quat}), 122.2 (+, CH_{arom}), 125.2 (C_{quat}), 126.9 (+, CH_{arom}), 127.3 (C_{quat}), 128.8 (+, CH_{arom}), 129.0 (+, CH_{arom}), 129.3 (+, CH_{arom}), 130.9 (+, CH_{arom}), 132.5 (+, CH_{arom}), 136.8 (+, CH_{arom}), 138.3 (C_{quat}), 148.0 (C_{quat}), 148.4 (C_{quat}), 148.7 (C_{quat}), 151.1 (+, CH_{arom}), 157.5 (C_{quat}), 162.2 (C_{quat}), 170.1 (C_{quat}), 170.3 (C_{quat}), 171.2 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 629 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1651 (C=O, ketone), 1602 (C=O, amide), 1573 (N-H, amide), 1520 (N-H, amide) –**MF** $C_{38}H_{36}N_4O_5$ – **MW** 628.72 g/mol

(_E_)-_N_-{5-[4-(2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1_H_)-yl]ethyl)phenyl carbamoyl]-2-[prop-1-enyl]phenyl}quinoline-3-carboxamide (9j)

3-Amino-_N_-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1_H_]-yl)ethyl]phenyl}-4-(1-hydroxypropyl)benzamide (20 mg, 0.03 mmol) and 15 eq Et_3N (40 mg, 0.05 mL, 0.5 mmol) were dissolved in a few mL dry DMF. The acid chloride **15** was added (10 eq, 60 mg, 0.3 mmol) before the solution was heated by microwave

irradiation for 5 min at 160 °C. DMF was removed under reduced pressure and the substance was extracted with dichloromethane and water. The organic layer was dried with Na_2SO_4 and the solvent was evaporated. For further purification the substance was separated by preparative TLC. The reaction yielded 8 mg (0.012 mmol, 42 %) of an orange solid, **mp** > 190 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.14-2.24 (m, 3 H), 3.20-3.22 (m, 8 H), 3.72 (s, 6 H), 3.55-3.59 (m, 2 H), 4.25-4.30 (m, 1 H), 5.47 (d, *J* = 5.2 Hz, 1 H), 6.50 (s, 1 H), 6.55 (s, 1 H), 7.07 (d, *J* = 8.2 Hz, 2 H), 7.12-7.22 (m, 3 H), 7.30 (s, 1 H), 7.56 (d, *J* = 5.7 Hz, 2 H), 7.70 (s, 1 H), 7.88 (d, *J* = 7.4 Hz, 1 H), 8.03 (d, *J* = 7.4 Hz, 1 H), 8.79 (s, 1 H), 9.46 (s, 1 H) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 627 (100) [M-H$^+$] – **MF** $C_{39}H_{38}N_4O_4$ – **MW** 626.74 g/mol

Methyl 4-(methylcarbamoyl)-2-nitrobenzoate (10a)

4-(Methoxycarbonyl)-3-nitrobenzoic acid (0.6 g, 2.6 mmol) was dissolved in dichloromethane and a few drops of DMF. Subsequently, 1.2 eq (0.14 g, 0.1 mL, 1.1 mmol) oxalyl dichloride were added dropwise. After two hours stirring at room temperature methylamine (0.1 mL of 40 % solution in water) was added over 5 min to the clear solution. The resulting white precipitate was collected and washed with water and finally re-dissolved in dichloromethane. Remaining starting material was removed by washing with aqueous $NaHCO_3$. The reaction yielded 0.32 g (1.3 mmol, 50 %) white solid, **mp** 140 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.02 (d, *J* = 4.6 Hz, 3 H), 3.92 (s, 3 H), 7.74 (d, *J* = 7.9 Hz, 1 H), 8.07 (dd, *J* = 1.6 Hz, 7.9 Hz, 1 H), 8.28 (d, *J* = 1.4 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 26.1 (+, CH_3), 52.5 (+, CH_3), 121.5 (+, CH_{arom}), 128.6 (C_{quat}), 129.2 (+, CH_{arom}), 130.4 (+, CH_{arom}), 137.0 (C_{quat}), 146.9 (C_{quat}), 164.0 (C_{quat}), 164.3 (C_{quat}) – **MS** (EI): m/z (%) = 238 (100) [M$^{+\bullet}$] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1639 (C=O), 1728 (C=O), 3337 (N-H) – **MF** $C_{10}H_{10}N_2O_5$ – **MW** 238.20

Methyl 2-amino-4-(methylcarbamoyl)benzoate (11a)

The preparation followed GP 2 diluting methyl 4-(methylcarbamoyl)-2-nitrobenzoate (0.3 g, 1.2 mmol) in ethanol. The reaction yielded 0.23 g (1.1 mmol, 92 %) pale yellow solid, **mp** 134 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.93 (d, *J* = 4.6 Hz, 3 H), 3.84 (s, 3 H), 7.64 (d, *J* = 7.9 Hz, 1 H), 8.00 (dd, *J* =

1.4 Hz, 7.9 Hz, 1 H), 8.24 (d, *J* = 1.4 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 27.1 (+, CH_3), 53.5 (+, CH_3), 122.7 (+, CH_{arom}), 129.5 (C_{quat}), 130.1 (+, CH_{arom}), 131.6 (+, CH_{arom}), 138.0 (C_{quat}), 147.9 (C_{quat}), 165.2 (C_{quat}), 165.4 (C_{quat}) – **MS** (CI): m/z (%) = 209 (100) [MH^+] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1638 (C=O), 1731 (C=O), 3334 (N-H) – **MF** $C_{10}H_{12}N_2O_3$ – **MW** 208.21

4-(Methoxycarbonyl)-3-(quinoline-2-carboxamido)benzoic acid (12a)

3-Amino-4-(methoxycarbonyl)benzoic acid (100 mg, 0.5 mmol) was dissolved in dry DMF before 1.5 eq of NEt_3 (80 mg, 0.11 mL, 0.8 mmol) and 0.5 eq of DMAP (30 mg, 0.25 mmol) were added. Under nitrogen atmosphere the acid chloride (0.14 g, 0.75 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. Then the DMF was removed, the residue was taken up with dichloromethane. A pale yellow solid precipitated which was collected. The reaction yielded 0.01 g (0.03 mmol, 6 %), **mp** 255 °C – **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 2.08 (s, 1 H, NH), 4.03 (s, 3 H), 7.74-7.77 (m, 2 H), 7.91-7.96 (m, 1 H), 8.12-8.19 (m, 3 H), 8.28 (d, 8.5 Hz, 1 H), 8.66 (d, 8.5 Hz, 1 H), 9.47 (d, 1.4 Hz, 1 H), 13.06 (s, 1 H, COOH) – **^{13}C-NMR**, DEPT 135 (75 MHz, [D_6]-DMSO): δ = 52.8 (+, CH_3), 118.5 (+, CH_{arom}), 119.4 (C_{quat}), 120.7 (+, CH_{arom}), 123.5 (+, CH_{arom}), 128.1 (+, CH_{arom}), 128.6 (+, CH_{arom}), 129.0 (C_{quat}), 129.2 (+, CH_{arom}), 130.9 (+, CH_{arom}), 131.3 (+, CH_{arom}), 135.8 (C_{quat}), 138.5 (+, CH_{arom}), 139.7 (C_{quat}), 145.6 (C_{quat}), 149.0 (C_{quat}), 162.7 (C_{quat}), 166.4 (C_{quat}), 166.6 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 351 (100) [$M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1523 (C=O), 1574 (C=O), 1698 (C=O) – **MF** $C_{19}H_{14}N_2O_5$ – **MW** 350.32

4-(Methoxycarbonyl)-3-(quinoline-3-carboxamido) benzoic acid (12b)

3-Amino-4-(methoxycarbonyl)benzoic acid (150 mg, 0.8 mmol) was dissolved in dry DMF, then 1.5 eq of NEt_3 (120 mg, 0.15 mL, 1.2 mmol) and 0.5 eq of DMAP (40 mg, 0.4 mmol) were added. Under nitrogen atmosphere the acid chloride (230 mg, 1.2 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. DMF was removed under reduced pressure and the residue was taken up with dichloromethane. A pale yellow solid precipitated which was collected. The reaction yielded 0.09 g (0.3 mmol, 37 %), **mp** 241 °C – **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 3.89 (s, 3 H), 7.75 (t,

J = 7.27 Hz, 1 H), 7.82 (dd, *J* = 1.6 Hz, 8.2 Hz, 1 H), 7.94 (t, *J* = 8.4 Hz, 1 H), 8.06 (d, *J* = 8.2 Hz, 1 H), 8.14 (d, *J* = 8.2 Hz, 1 H), 8.20 (d, *J* = 7.9 Hz, 1 H), 8.85 (d, *J* = 1.4 Hz, 1 H), 8.97 (d, *J* = 2.2 Hz, 1 H), 9.38 (d, *J* = 2.2 Hz, 1 H), 11.53 (s, 1 H, COOH) – **^{13}C-NMR**, DEPT 135 (75 MHz, [D_6]-DMSO): δ = 52.8 (+, CH_3), 123.0 (+, CH_{arom}), 123.3 (C_{quat}), 124.6 (+, CH_{arom}), 126.5 (C_{quat}), 126.9 (C_{quat}), 127.7 (+, CH_{arom}), 128.8 (+, CH_{arom}), 129.4 (+, CH_{arom}), 130.8 (+, CH_{arom}), 131.8 (+, CH_{arom}), 135.2 (C_{quat}), 136.1 (+, CH_{arom}), 138.8 (C_{quat}), 148.4 (+, CH_{arom}), 148.7 (C_{quat}), 163.7 (C_{quat}), 166.3 (C_{quat}), 167.1 (C_{quat}) – **MS** (+ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 351 (100) $[M\text{-}H^+]^-$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1527 (C=O), 1547 (C=O), 1691 (C=O) – **MF** $C_{19}H_{14}N_2O_5$ - **MW** 350.32

Methyl 2-(2-naphthamido)-4-(methylcarbamoyl)benzoate (12c)

The amine (0.2 g, 0.9 mmol) was dissolved in dry DMF, then 1.5 eq of NEt_3 (0.13 g, 0.18 mL, 1.3 mmol) and 0.5 eq of DMAP (0.08 g, 0.5 mmol) were added. Under nitrogen atmosphere the acid chloride (0.23 g, 1.2 mmol) was added. After two hours at 80 °C the reaction mixture was stirred over night at room temperature. DMF was removed under reduced pressure, the residue was taken up with dichloromethane and washed with $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and evaporated. The reaction yielded 0.09 g (0.3 mmol, 33 %), **mp** 202 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.96 (d, *J* = 4.6 Hz, 3 H), 4.00 (s, 3 H), 7.61 (t, *J* = 8.1 Hz, 1 H), 7.66 (dd, *J* = 1.4 Hz, 8.2 Hz, 1 H), 7.77 (t, *J* = 7.1 Hz, 1 H), 7.85 (d, *J* = 8.2 Hz, 1 H), 8.12 (d, *J* = 8.5 Hz, 1 H), 8.22-8.33 (m, 3 H), 9.06 (s, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 26.9 (+, CH_3), 52.7 (+, CH_3), 117.8 (+, CH_{arom}), 118.7 (+, CH_{arom}), 118.8 (C_{quat}), 122.9 (+, CH_{arom}), 127.7 (+, CH_{arom}), 128.5 (+, CH_{arom}), 129.5 (+, CH_{arom}), 130.2, 130.4 (+, CH_{arom}), 131.8 (+, CH_{arom}), 137.9 (+, CH_{arom}), 139.7 (C_{quat}), 140.0 (C_{quat}), 146.6 (C_{quat}), 149.4 (C_{quat}), 163.9 (C_{quat}), 167.4 (C_{quat}), 167.5 (C_{quat}) – **MS** (CI): m/z (%) = 364 (100) $[MH^+]$ – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 1569 (C=O), 1633 (C=O), 1686 (C=O) – **MF** $C_{21}H_{18}N_2O_4$ – **MW** 363.37

4-(1-Hydroxypropyl)-3-nitrobenzoic acid

Ethyl 3-nitro-4-propionylbenzoate (0.30 g, 1.2 mmol) was dissolved in methanol. A portion of 3 eq LiOH (80 mg, 3.5 mmol) was added in a suspension of water. The mixture was stirred at room temperature for two hours. Then it was acidified

with diluted HCl and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The reaction yielded brown oil (0.26 g, 1.1 mmol, 96 %), **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.06 (t, *J* = 7.4 Hz, 3 H), 1.80 (m, 2 H), 5.26 (dd, *J* = 3.8 Hz, *J* = 8.2 Hz, 1 H), 7.9 (d, *J* = 8.2 Hz, 1 H), 8.32 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1 H), 8.59 (d, *J* = 1.6 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 10.2 (+, CH_3), 31.3 (-, CH_2), 70.6 (+, CH), 126.0 (+, CH_{arom}), 128.7 (+, CH_{arom}), 134.2 (+, CH_{arom}), 145.5 (C_{quat}), 160.9 (C_{quat}), 168.4 (C_{quat}), 177.5 (C_{quat}) – **MS** (CI -MS, NH_3): m/z (%) = 226.0 (100) [M-H$^+$] – **IR** (ATR) $\tilde{\nu}$ [cm^{-1}] = 3334 (O-H, acid), 3090 (O-H), 1686 (C=O, acid), 1529 (N-O), 1345 (N-O), 1251 (C-O, acid) – **MF** $C_{10}H_{11}NO_5$ – **MW** 225.2 g/mol

Modulation of ABCB1 and ABCG2 was performed as described.[30], [24]

Stability investigations in mouse plasma. Preparation of mouse plasma and determination of esterase activity: Blood of BL6 mice was collected by heart puncture in deep anesthesia using heparin-coated syringes. Samples were immediately centrifuged for 7 min at 7000 rpm (Eppendorf centrifuge 5415R, Eppendorf, Hamburg, Germany) and the supernatant was carefully removed. After pooling, the plasma was fractioned into small aliquots for long-term storage at -80 °C. The enzymatic activity of esterases in the plasma samples was measured spectrophotometrically. The coloured product *o*-nitrophenol, formed by enzymatic cleavage of the chromogenic substrate nitrophenyl butyrate was determined as a function of time. Absorbance was plotted against time, the linear slope at the beginning of the reaction was determined and used for the calculation of volume activity:

$$V_A = \frac{\frac{\Delta A \cdot V}{t}}{\varepsilon \cdot d \cdot v}$$

The abbreviations of the present equation define the following parameters:
V_A = volume activity [U · mL^{-1}] multiplied with 1000 for [U · L^{-1}], Δ*A* = absorbance of *o*-nitrophenol at 414 nm, *t* = time [min], *V* = total volume [mL], $\varepsilon_{o\text{-nitrophenol}}$ = 3190 [L · mol^{-1} · cm^{-1}], d = path length [cm], v = plasma volume [mL]

The test compounds were dissolved in DMSO at a concentration of 1.5 mM. Subsequently, test compound stocks, mouse plasma, deproteinated plasma, culture medium and phosphate buffered saline were equilibrated on a Wealtec heat plate (Wealtec, Sparks, USA) to 37 °C for approximately 15 min. Thereafter, a 1:50 dilution of the substances with the corresponding medium was prepared in 1.5-mL polypropylene reaction vessels (Eppendorf, Hamburg, Germany). The samples were shortly vortexed and immediately incubated at 37 °C. After increasing periods of time, aliquots were taken, and the reaction was stopped by the addition of two parts of ice-cold acetonitrile. For quantitative precipitation of the denatured proteins, the samples were efficiently vortexed and stored at 4 °C for 30 min. Finally, samples were centrifuged for 5 min at 17000 g, using an Eppendorf MiniSpin plus centrifuge, and the supernatants were transferred into new plastic cups. For HPLC analysis the samples were further diluted (1:2) with acetonitrile and stored at -80 °C until the measurement. For HPLC analysis samples were unfrozen at room temperature and injected into the HPLC system (Waters, Eschborn, Germany). Analysis was accomplished by gradient elution with water containing TFA (0.05 %) and acetonitrile (0 min, 15 %; 19 min, 60 %; 20 min, 95 %; 24.5 min, 95 %; 25 min, 15 %; 38 min, 15 %), at a constant flow rate of 0.8 mL/min. The HPLC system was equipped with a Luna RP-18 (2), 3 µm, 150 mm x 4.6 mm column. Analysis was carried out via UV-detection at a wavelength of 210 nm.

3.5. References

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4. Photochromic Tariquidar Derivatives as Potential ABCG2 Modulators*

Despite the wide spectrum of substrates and inhibitors known for the multi-drug-resistance causing ABCG2 transporter mechanistic details are not yet clear. Photochromic tariquidar-derived compounds as potential ABCG2 modulators may be useful as tool in mechanistic investigation. The synthetic approach was focused on the replacement of characteristic structural moieties of a potent and selective ABCG2 inhibitor by photochromic dithienylethenes. Photochemical properties, thermostability, cycle performance, photostationary state and photostability at assay conditions are suitable for all four compounds for pharmacological *in vitro* investigations.

* All synthesis and spectroscopical investigations were done by Carolin Fischer, compounds **3** and **8** were synthsisized by Natascha Kuzmanović, inhibition assay for ABCB1 and ABCG2 were performed by Maria Beer-Krön.

4.1. Introduction

The ABCG2 transporter (BCRP, breast cancer resistance protein) has been shown to play a protective role due to blocking absorption of xenobiotics. As it is a member of the ABC (ATP-binding cassette) transporter family, they can be found in increased concentrations in different biological barriers like the blood-brain-barrier[1], the blood-testis-barrier[2], the maternal-fetal-barrier[3] and the apical membrane of the intestine.[4] Also high levels of ABCG2 are found in cancer cells of acute myeloid leukemia and breast cancer causing multi-drug-resistance of cytostatic there.[5] Since its discovery in 1998, the list of substrates and inhibitors continuously grew. Most of the substrates are chemotherapeutic agents such as mitoxantrone, topotecan, methotrexate, flavopiridol and others.[6] Also fluorescent dyes like Hoechst 33342, rhodamine 123, bodipy-prazosin have been found to be substrates.[7-8] Fumitremorgin C[9] was the first selective ABCG2 inhibitor described and the class of selective inhibitors was supplemented by the highly potent analog Ko143[10], novobiocin[11], tariquidar derivatives[12] and 4-(2-hydroxyethyl)phenyl 2-acylaminobenzoates[13] in the last years. Beside the specific inhibitors, the class of broad spectrum inhibitors like elacridar (GF120918) and cyclosporine a, which are also modulating ABCB1 (p-gp, p-glycoprotein) or ABCC1 (MRP1), the family of natural occurring flavonoids and tyrosine kinase inhibitors were identified to inhibit ABCG2.[14-16] Despite the remarkable number of substrates and inhibitors, no clear structure-function-relationship has been identified yet. Mechanistic knowledge is still lacking. Although there is no 3-dimensional structure reported, this broad range of substrates and inhibitors indicates that the transporter contains more than one distinct binding pocket. Matsson *et al*. also predict a large binding surface where several compounds can bind simultaneously instead of docking into defined binding sites.[14]

In this study, four new ABCG2 modulators containing a photoswitchable unit were synthesised. Upon irradiation with 312 nm, an electrocyclization of the photochromic unit occurs turning the flexible molecule into a more rigid structure (Figure 4.1). This reversible interconversion of two isomers can be monitored by different absorption spectra. Diarylethens have been intensively studied concerning their properties and their use as optical memory media.[15] Also, successful applications for molecular light switches in medicinal chemistry have

already been shown.[16] In particular, dithienylethenes (DTE) were applied for the inhibition of human carbonic anhydrase.[17-18] Targeting the structure-activity-relationship between the membrane-located ABCG2 transporter and its ligands, different shapes may result in different biological activity and therefore provide information about essential interactions between the modulator and the transporter.

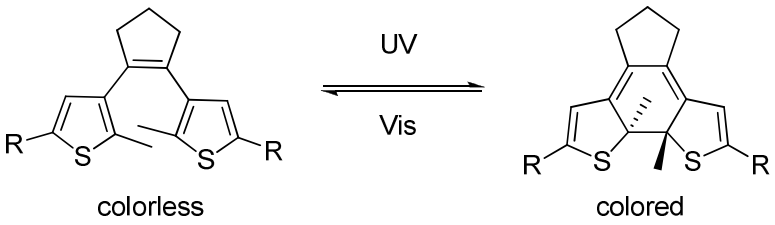
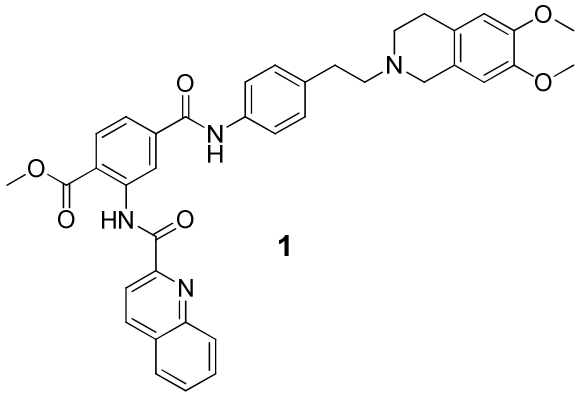


Figure 4.1: Photochromism of dithienylethenes.

The synthetic strategy towards new photoswitchable ABCG2 inhibitors was the exchange of molecular parts of the parent compound **1** to the photochromic unit (Figure 4.2). With this approach three different aims should be achieved: retention of the potency, determination of the essential molecular structures and similarity of the size and the molecular weight to the parent ABCG2 inhibitor. To obtain photochromic ABCG2 inhibitors the quinoline moiety, the tetrahydroisoquinoline moiety and the central aromatic core were replaced by dithienylethenes.



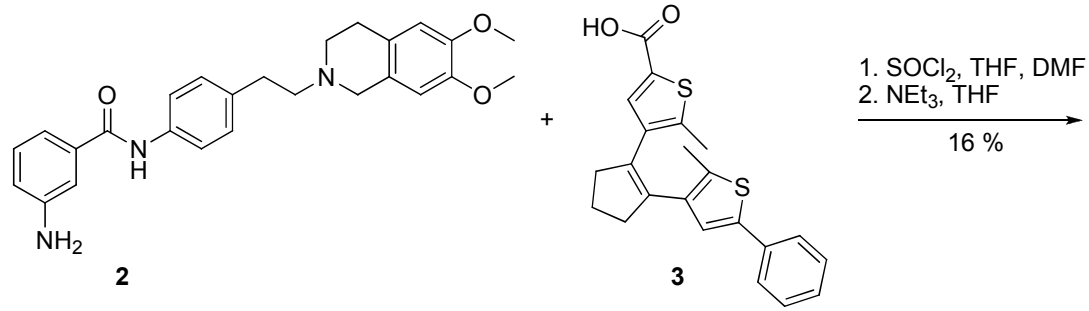
1

Figure 4.2: Potent and selective ABCG2 inhibitor, parent compound.

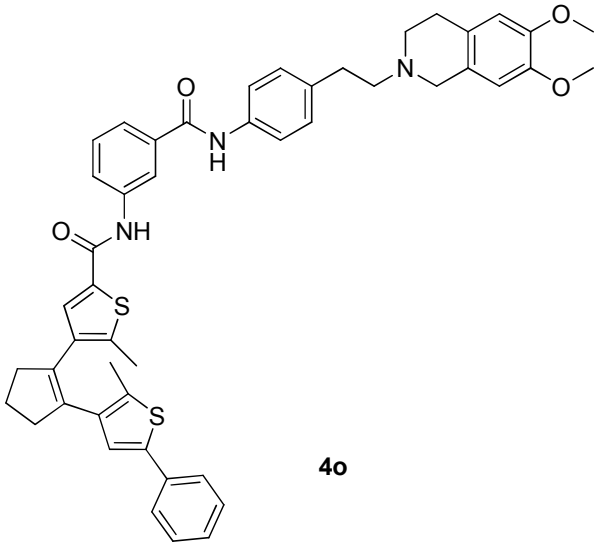
4.2. Results and Discussion

4.2.1. Synthesis

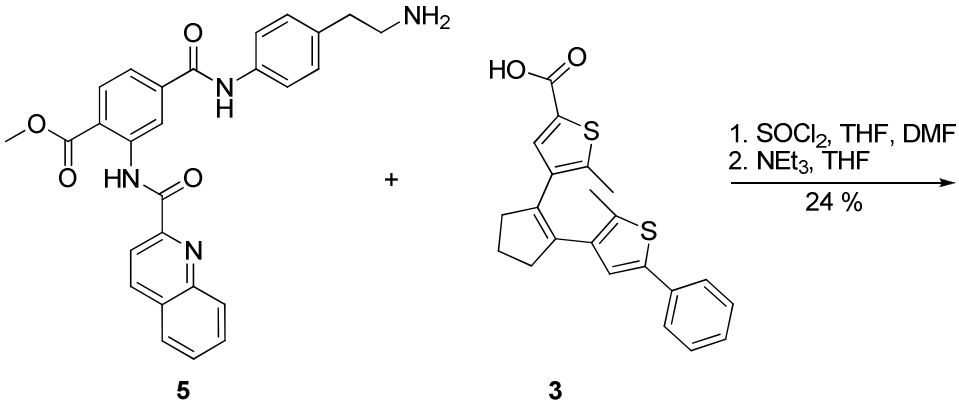
Compound **4o** was accessible replacing the quinoline moiety by the photoswitchable DTE unit. The amine **2** was reacted with phenyl-substituted dithienylethene **3** by first generating the acid chloride. In the same way compound **6o** was synthesised in one step from compound **3** and **5**. For the preparation of the symmetrically substituted compound **9o** two equivalents of quinoline-2-amine **7** were coupled with the diacid dithienylethene **8**. The synthesis of the non-symmetrically substituted compound **11o** was achieved using 0.3 equivalents of compound **10** and 1 equivalent of compound **7** (Scheme 4.1). All photoswitchable ABCG2 modulators were purified by preparative HPLC and obtained in moderate isolated yields.



2 **3**



4o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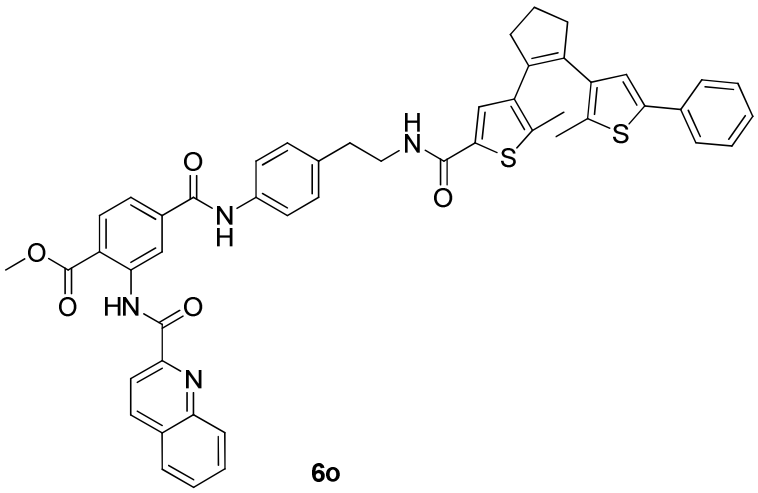
1. SOCl2, THF, DMF
2. NEt3, THF
24 %

5 **3**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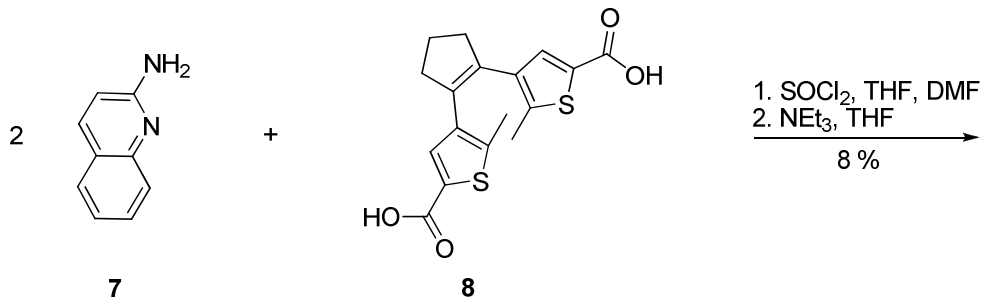
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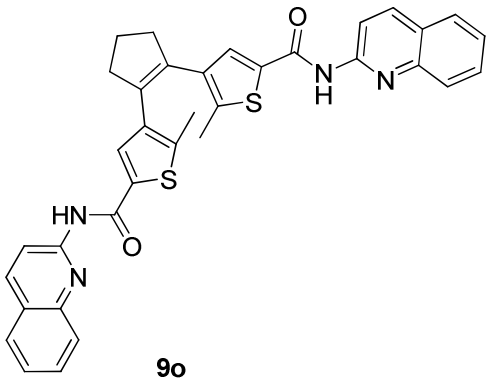
1. SOCl2, THF, DMF
2. NEt3, THF
8 %

7 **8**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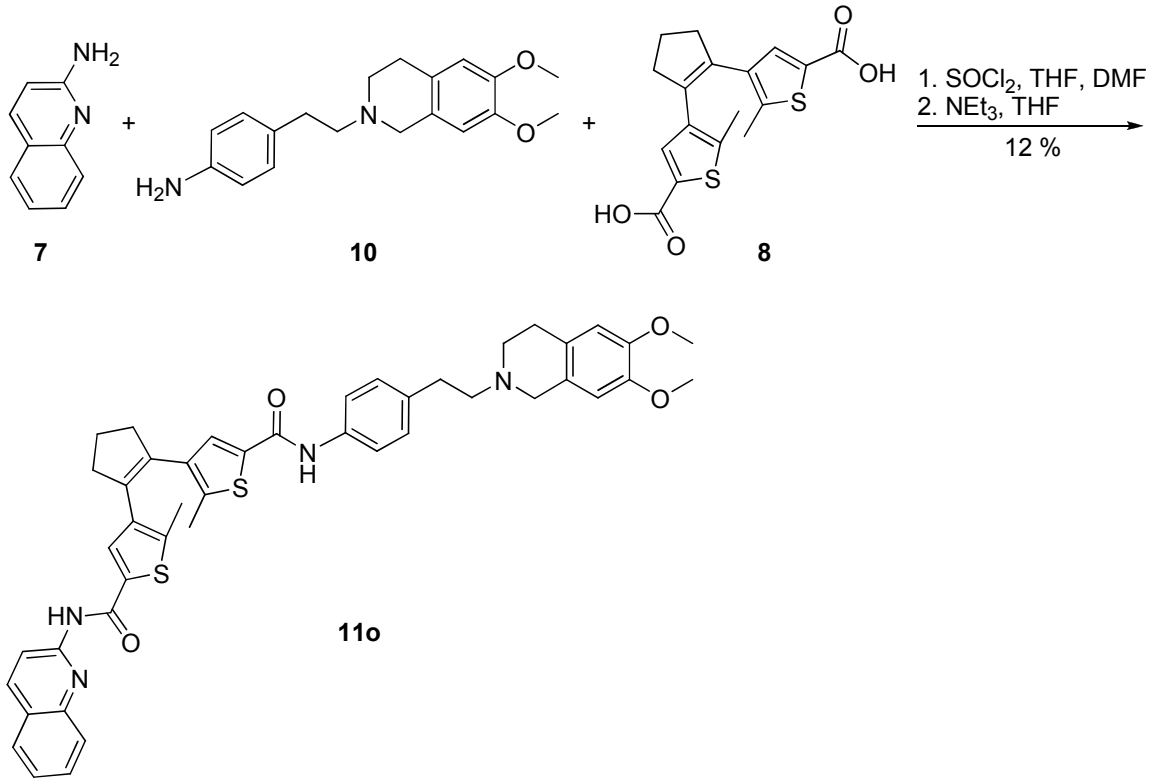
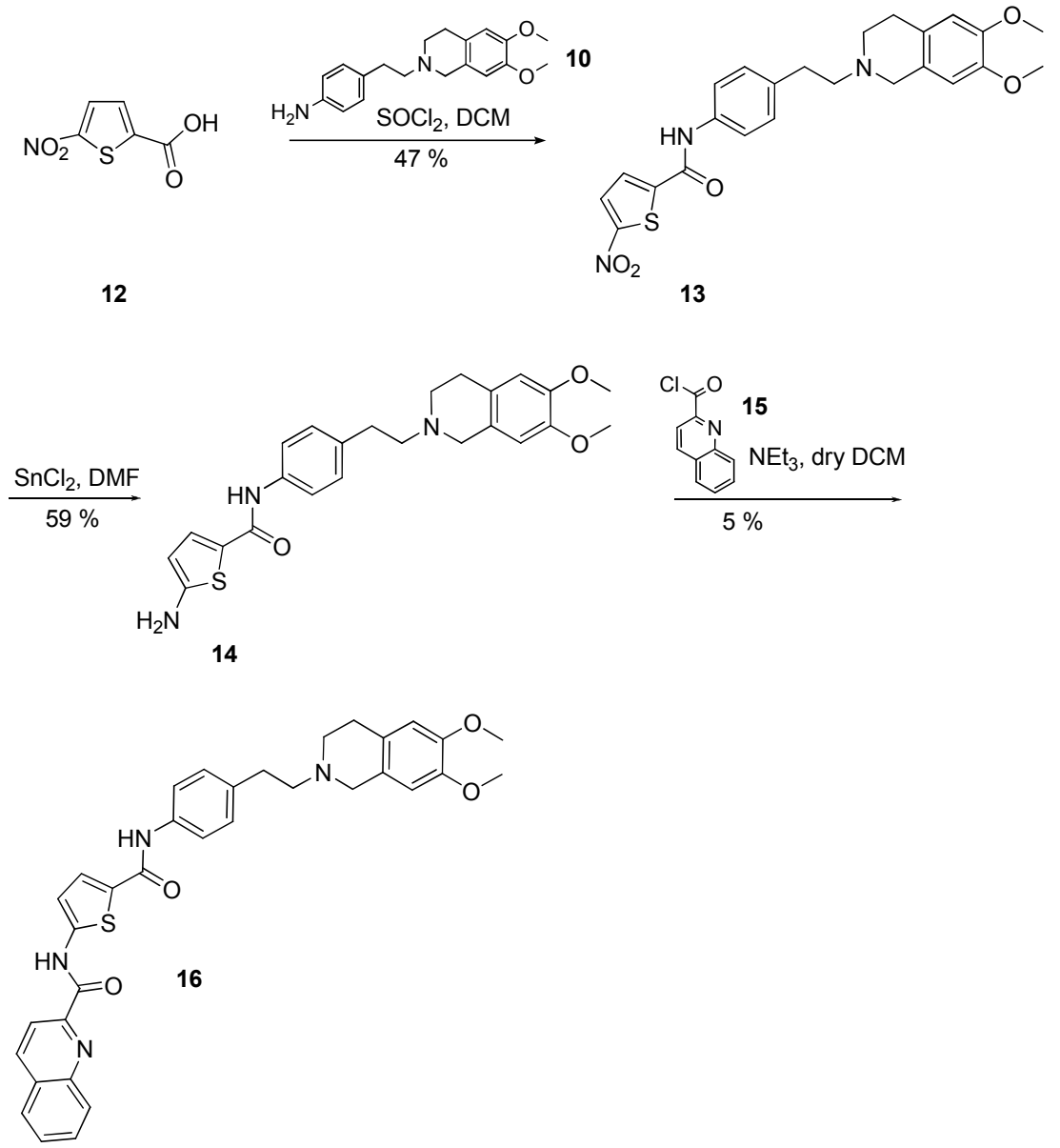


Scheme 4.1: Synthesis of potential photoswitchable ABCG2 modulators.

With the intention to prepare an ABCG2 modulator containing a heteroaromatic moiety instead of the central aromatic core, the synthetic sequence was started with the coupling of 5-nitrothiophene-2-carboxylic acid **12** and the amine **10**. The reduction of the nitrofunction of compound **13** to the corresponding amine was performed with $SnCl_2$ in good yield. The synthesis of the thiophene compound **16** was completed by the second amid bond formation (Scheme 4.2).



Scheme 4.2: Synthesis of thiophene containing ABCG2 modulator.

4.2.2. Photochemical Properties

Irradiating solutions of **4o**, **6o**, **9o** and **11o** in methanol (c = 10^{-5} mol/L) with 312 nm resulted in immediate changes of the UV/VIS absorption spectra that are typical for photo-responsive dithienylethenes.[17] Depending on the different compounds, the high energy band around λ_{max} = 270 nm decreased in intensity and a new absorption band in the visible spectral region appeared (λ_{max} = 530-560 nm). For compounds **4c**, **9c** and **11c** additional absorption bands appeared at λ_{max} = 378 nm (**4c**), λ_{max} = 370 nm (**9c**) and λ_{max1} = 239 nm, λ_{max2} = 358 nm (**11c**). The solution changed its colour from colourless to purple upon ring-

closure. These spectral changes were complete after several seconds of irradiation (compound **4**: 40 s; compound **6**: 20 s; compound **9**: 10 s; compound **11**: 14 s). Longer irradiation led, in some cases, to destruction of the molecules (see Figure 4.3).

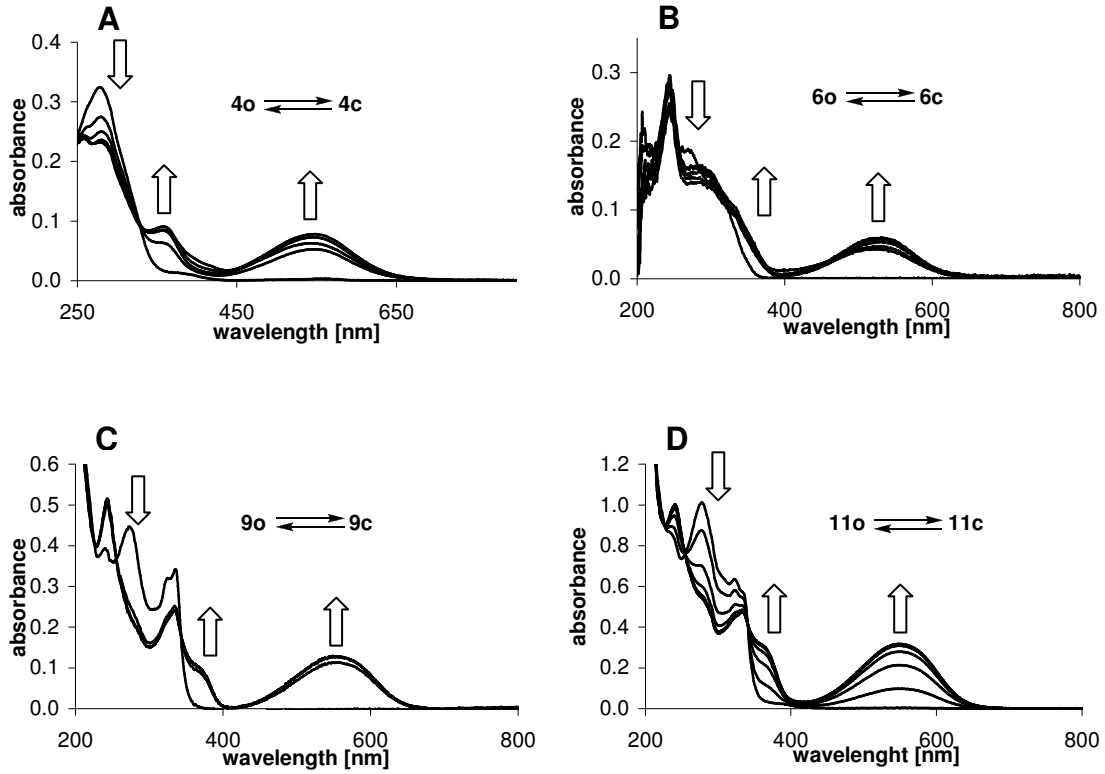


Figure 4.3: Electrocyclization upon irradiation with 312 nm, concentration 10^{-5} mol/L in methanol, irradiation periods: **A**: **4o**: 0, 10, 22, 33, 53 s; **B**: **6o**: 0, 10, 22, 40, 60, 120 s; **C**: **9o**: 0, 5, 10, 15, 20 s; **D**: **11o**: 0, 2, 4, 6, 8, 10, 12, 14 s.

Thermal stability of the ring-closed derivatives could be proven for all compounds at least for 10 h in the dark (Figure 4.4).

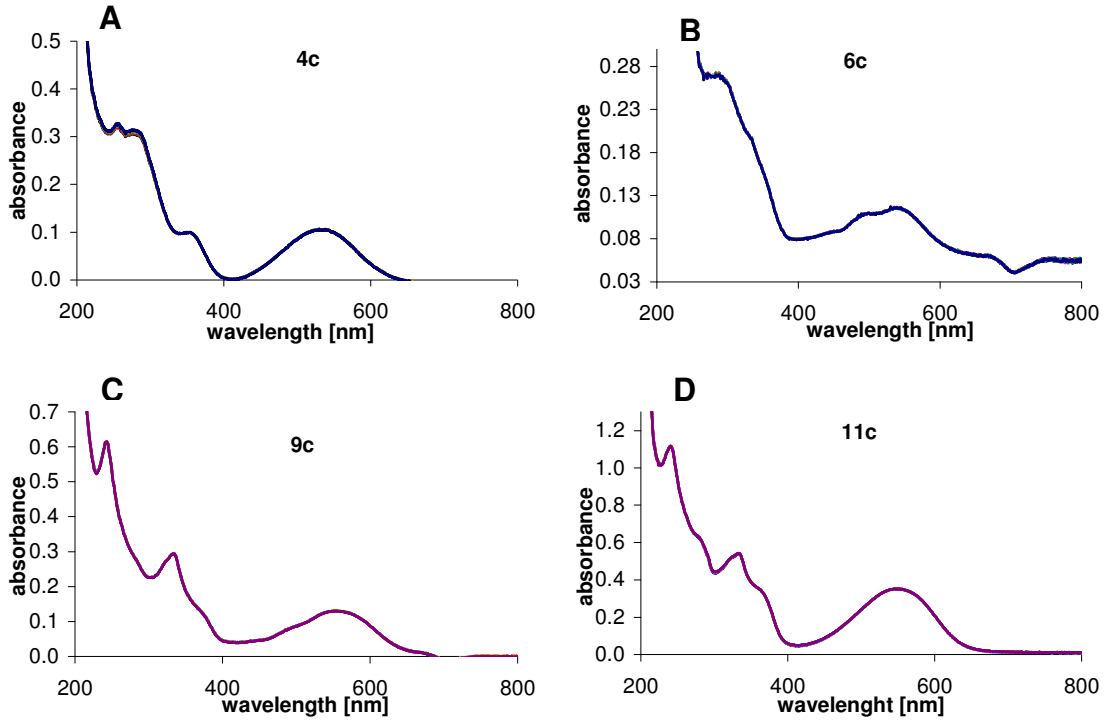


Figure 4.4: Thermal stability in the dark for 10 h, spectra taken each hour, **A**: compound **4c**, **B**: compound **6c**, **C**: compound **9c**, **D**: compound **11c**.

In order to study the durability, the photoswitchable compounds **4**, **6**, **9** and **11** were alternately irradiated with 312 nm and with visible light > 420 nm. The absorption band as well as the visible light band were followed and plotted against the cycle number. Compound **4** showed a fast loss of performance and therefore a high tendency of photo-degradation. After nine cycles the absorption intensity decreased about 50 %. The performance was improved for compounds **6** and **9**. The intensity of the visible band drops by 32 % for compound **6** and only 10-12 % for compounds **9** and **11** after ten cycles (Figure 4.5).

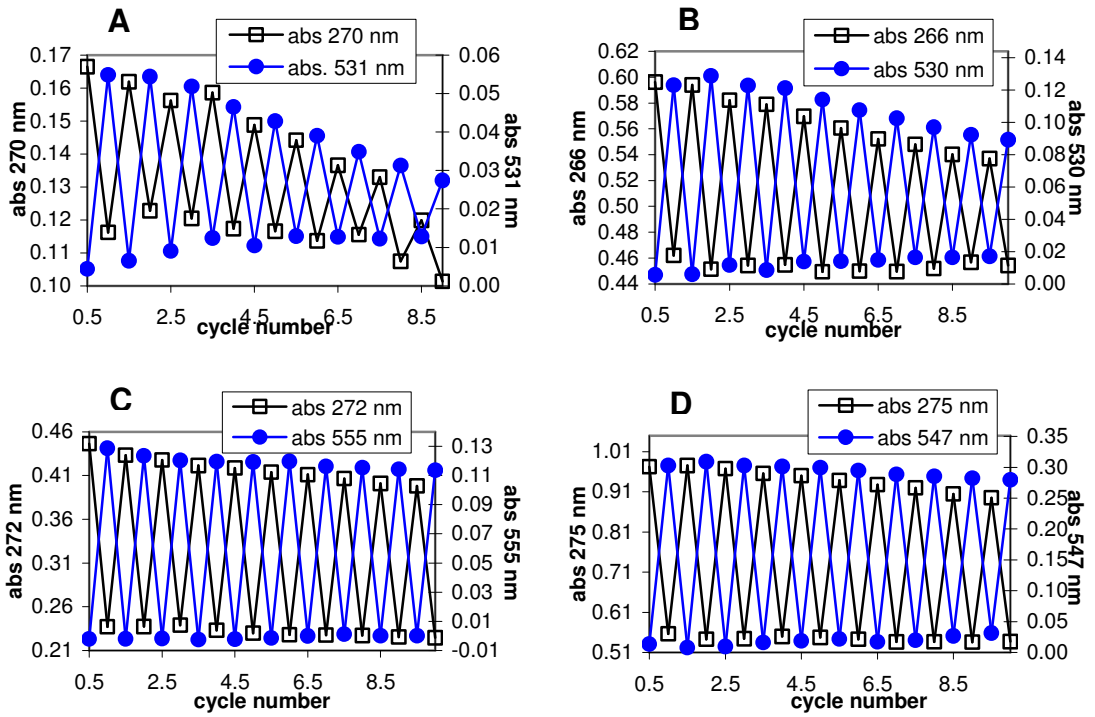


Figure 4.5: A :Cycle performance of **4**, changes in absorption at 270 nm (open scares) and 531 nm (blue circles) during an alternated irradiation of a solution in methanol (10^{-5} mol/L) with 312 nm light for 40 s and greater than 420 nm light for 7 min., **B**: Cycle performance of **6**, changes in absorption at 266 nm (open scares) and 530 nm (blue circles) during an alternated irradiation of a solution in methanol (10^{-5} mol/L) with 312 nm light for 120 s and greater than 420 nm light for 5 min; **C**: Cycle performance of **9**, changes in absorption at 272 nm (open scares) and 555 nm (blue circles) during an alternated irradiation of a solution in methanol (10^{-5} mol/L) with 312 nm light for 10 s and greater than 420 nm light for 2 min; **D**: Cycle performance of **11**, changes in absorption at 275 nm (open scares) and 547 nm (blue circles) during an alternated irradiation of a solution in methanol (10^{-5} mol/L) with 312 nm light for 30 s and greater than 420 nm light for 5 min.

The photostationary state was investigated by ^{1}H-NMR exemplarily for compound **9c**. Irradiating a 35 μM solution of **9o** in $CDCl_3$ 60 min with light of a 312 nm lamp resulted in a photostationary state containing 86 % of the ring-closed isomer **9c**.

In preparation for biological activity investigations, the photostability of the photoswitchable modulators was tested in the opened and in the closed form upon irradiation with 340 nm. This irradiation wavelength is applied for assay using the Hoechst 33342 dye. The ring-opened and ring-closed isomers of all

four compounds, dissolved in methanol (c = 10^{-5} mol/L) turned out to be stable to irradiation for several minutes (Figure 4.6 and Figure 4.7).

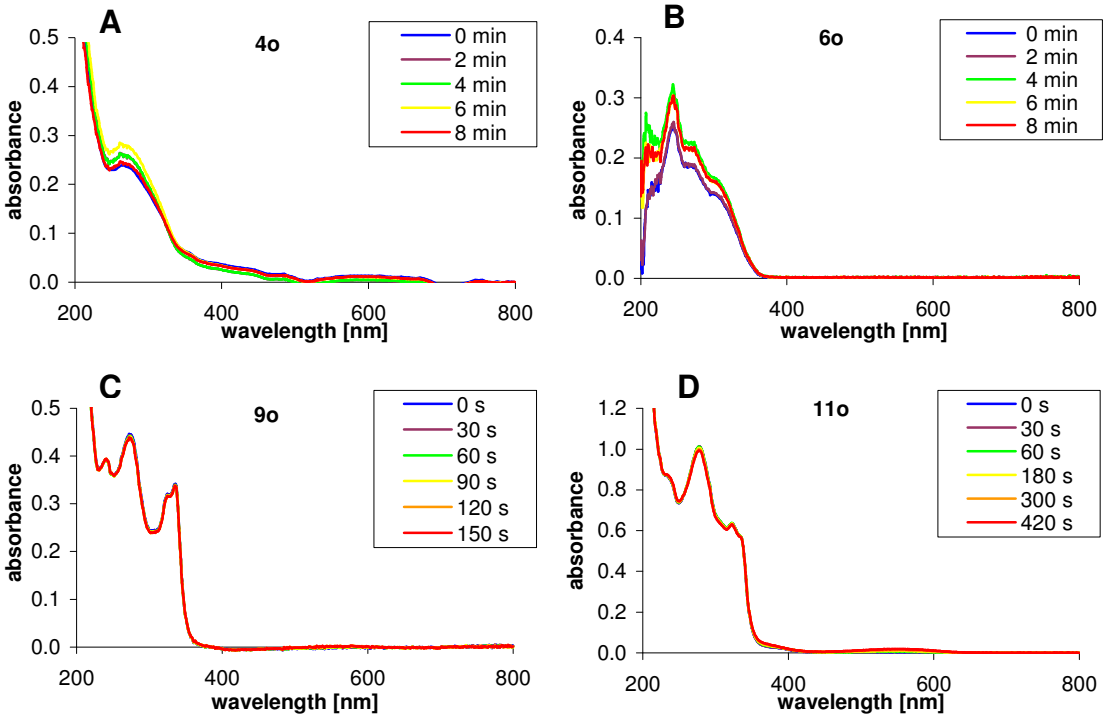


Figure 4.6: Irradiation of the open derivatives with 340 nm, **A**: compound **4o**, **B**: compound **6o**, **C**: compound **9o**, **D**: compound **11o**.

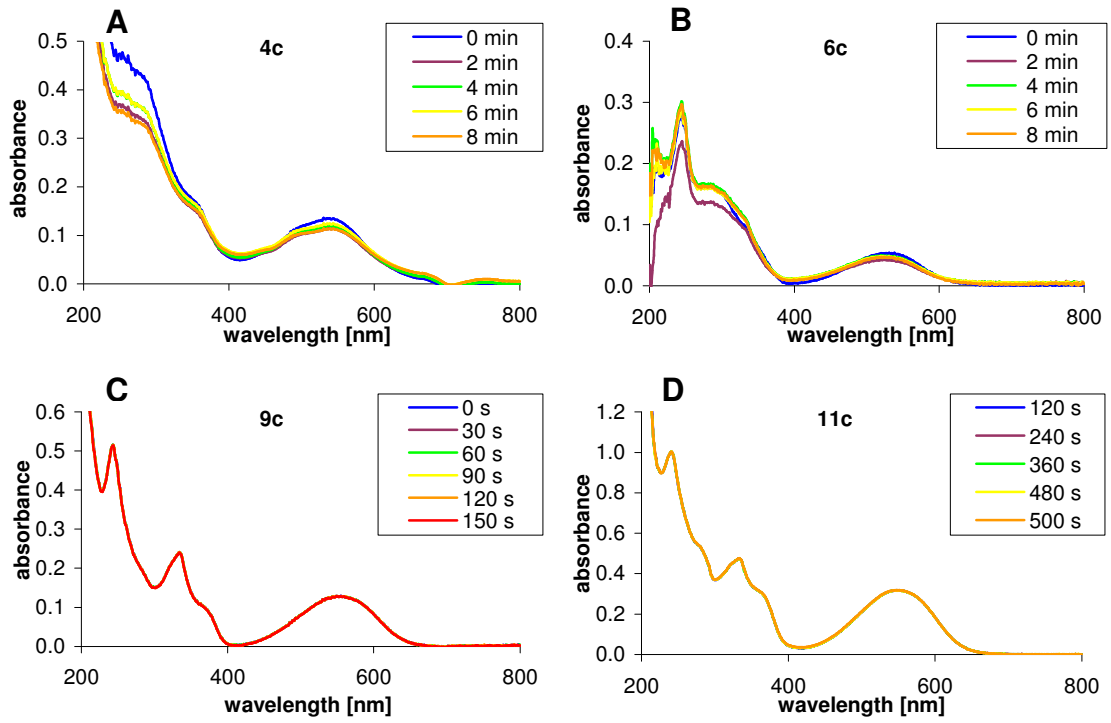


Figure 4.7: Irradiation of the closed derivatives with 340 nm, **A**: compound **4c**, **B**: compound **6c**, **C**: compound **9c**, **D**: compound **11c**.

Daylight exposure resulted in immediate conversion into the ring-opened isomer. Within 10 min the long wavelength absorption intensity already dropped about 50 % (Figure 4.8).

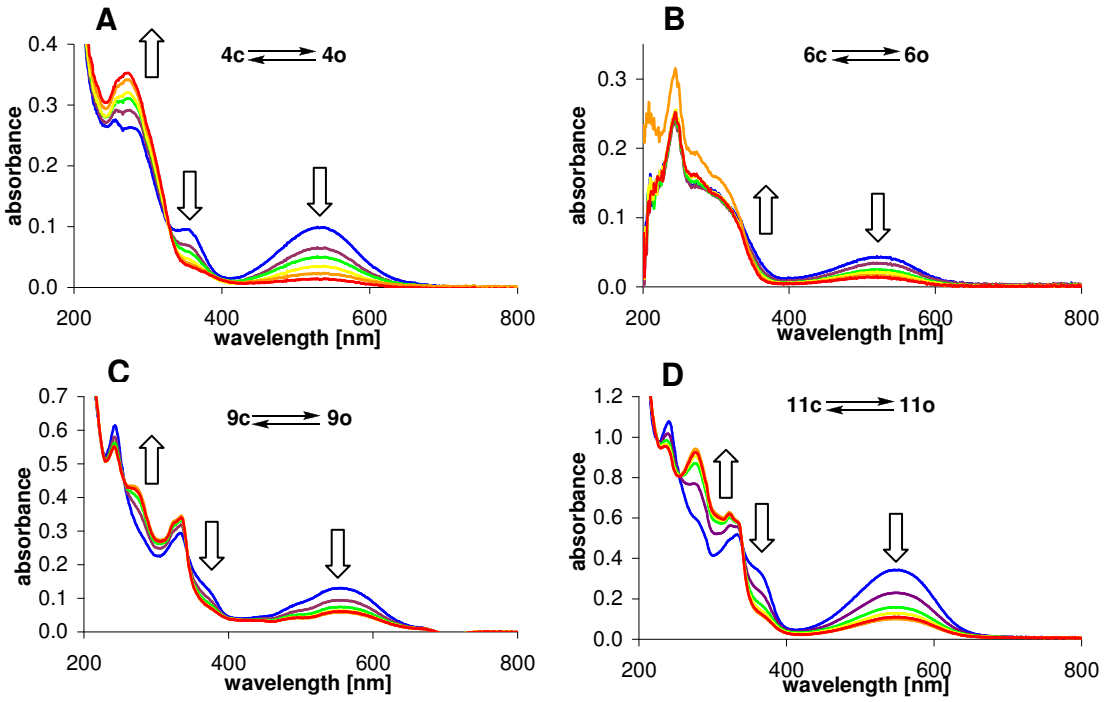


Figure 4.8: Daylight exposure for 0 min (blue), 10 min (purple), 20 min (green), 30 min (yellow), 60 min (orange), 120 min (red) of the ring-closed derivatives, **A**: compound **4c**, **B**: compound **6c**, **C**: compound **9c**, **D**: compound **11c**.

Switching of the photochromic compounds in aqueous PBS-buffer containing 1 % of BSA and EMEM + 5 % FCS was hampered by a very small extinction coefficients for compounds **6** and **11**. It was not possible to monitor the conversion between the open and the closed isomer after irradiation with 312 nm (data no shown). However, the ring-closed **11c** isomer was detectable by UV-spectroscopy in aqueous buffer after switching the molecule in the DMSO stock solution (Figure 4.9).

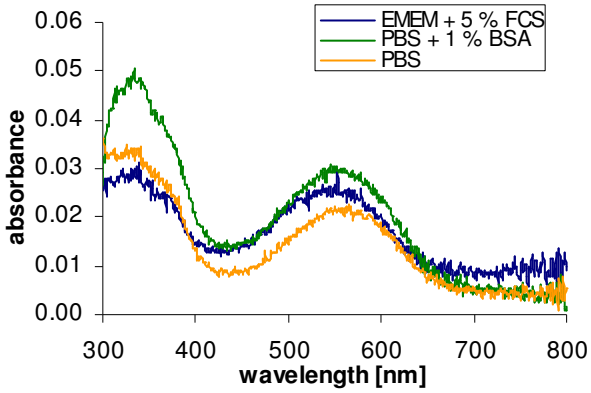


Figure 4.9: UV-absorbance of compound **11c** ($c = 10^{-5}$ M) in different aqueous media, all samples prepared in 1 : 100 ratio (DMSO/aqueous media).

4.2.3. Biological Activity

The potency of the photochromic compounds to modulate the ABCG2 transporter was determined in the fluorescent based Hoechst 33342 (H33342) microtiter plate assay. The blue fluorescent Hoechst 33342 is not accumulated in MCF7/Topo cells due to its active efflux by overexpressed ABCG2 transporters. Hence, ABCG2 inhibitors can be detected by the change of intramolecular H33342 levels. The relative fluorescence intensity of the cells was measured. Fumitremorgin C and Ko143 were used as positive control compounds, whereas fumitremorgin C was the reference for the maximal inhibitory effect of the test compounds. This assay was suitable since there was no interference between the spectral properties of the analyte H33342 and the test compound. As shown above, all photochromic compounds remain as open or closed isomer upon exiting H33342 at 340 nm.

Table 4.1: Inhibitory effect of photoswitchable compounds on the transport of ABCG2.

Compound	IC_{50} [μM]	Max. inhibition [%][b)]
4o	0.433 ± 0.034	42 ± 2 (10 μM)
6o	-[a)]	14 ± 2 (50 μM)
9o	-[c)]	31 ± 2 (30 μM)
11o	-[c)]	25 ± 1 (30 μM)
16	0.625 ± 0.051	34 ± 2 (7 μM)

[a)] no significant inhibitory effect, [b)] N = 3, [c)] plateau not reached.

Until now only the ring-opened isomers were tested on their biological activity and resulted in weak or no inhibitory effect (Table 4.1). Compound **16** was additionally tested on its inhibitory effect on ABCB1, was found to be inactive.

4.3. Conclusion

Potential modulators of the ABCG2 transporter containing a photoswitchable dithienylethene unit were prepared. The replacement of parts of the parent inhibitor **1** to DTE led to four photochromic compounds. The investigation of the photochemical properties resulted in a fast conversion into the ring-closed isomer after 10-40 s of irradiation with 312 nm light, monitored by changes in the UV/Vis spectra. All ring-closed isomers remained stable in the dark for at least 10 h. Cycle performance revealed high stabilities for compounds **9** and **11**, even after 10 cycles. The photostationary state was determined to be 86 % for compound **9c**.

The photostability at the pharmacological assay conditions using irradiation of 340 nm to detect the analyte Hoechst 33342, was shown for all compounds, either in the ring-opened or the ring-closed isomer. Daylight exposure led to immediate ring-opening and must be avoided during assays. The photoconversion into ring-closed isomers was not possible in aqueous media, but in the DMSO stock solution before.

Preliminary pharmacological tests showed low activity of the ring-opened isomers.

4.4. Experimental Section

General. Commercial reagents and starting materials were purchased from Aldrich, Fluka or Acros and used without further purification. Flash chromatography was performed on silica gel (Merck silica gel Si 60 40-63 *μ*m); products were detected by TLC on alumina plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm) and visualized by UV light (λ = 254 nm). Melting points were determined with a Optimelt MPA 100 and are uncorrected. NMR spectra were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz; *T* = 300 K), Bruker Avance 400 (1H: 400.1 MHz; 13C: 100.6 MHz; *T* = 300 K), and Bruker Avance 600 (^{1}H: 600.1 MHz; ^{13}C: 150.1 MHz; *T* = 300 K) instruments. Chemical shifts are reported in *δ*/ppm relative to external standards and coupling constants *J* are given in Hz. Abbreviations for the characterization of the signals: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, dd = double doublet. The relative numbers of protons is determined by integration. Error of reported values: chemical shift 0.01 ppm (^{1}H-NMR), 0.1 ppm (^{13}C-NMR), coupling constant 0.1 Hz. The used solvent for each spectrum is reported. Mass spectra were recorded with Finnigan MAT TSQ 7000 (ESI) and Finnigan MAT 90 (HRMS), IR spectra with a Bio-Rad FT-IR-FTS 155 spectrometer and UV/vis spectra with a Cary BIO 50 UV/vis/NIR spectrometer (Varian).

Photochemistry. Standard hand-held lamps used for visualizing TLC plates (Herolab, 6 W) were used to carry out the ring-closing reactions at 312 nm. The ring-opening reactions were carried out using the light of a 200 W tungsten source that was passed through a 420 nm cut-off filter to eliminate higher energy light. The power of the light source is given based on the specifications supplied by the company where the lamps were purchased. A light detector was not used to measure the intensity during the irradiation experiments.

The following compounds were prepared following known procedures: 3-amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}benzamide (**2**) (see chapter 2), 5-methyl-4-[2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-enyl]thiophene-2-carboxylic acid (**3**),[23] methyl 4-[4-(2-

aminoethyl)phenylcarbamoyl]-2-(quinoline-2-carboxamido)benzoate (**5**) (see chapter 2), 4,4'-(cyclopentene-1,2-diyl)bis(5-methylthiophene-2-carboxylic acid) (**8**),[20-21] 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl}aniline (**10**),[19] 5-nitrothiophene-2-carboxylic acid (**12**)[24], quinoline-2-carbonyl chloride (**15**)[22]

_N_-{3-[4-(2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl)phenylcarbamoyl] phenyl}-5-methyl-4-[2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-enyl]thiophene-2-carboxamide (4o)

5-Methyl-4-[2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-enyl]thiophene-2-carboxylic acid (1.5 eq, 100 mg, 0.24 mmol) was dissolved in 5 mL dry THF and 4 trops of DMF were added followed by $SOCl_2$ (2 eq, 60 mg, 0.04 mL, 0.5 mmol) and stirred at room temperature for one hour. Then, the solvent was removed under reduced pressure; the residue was dried in high *vacuo* for two hours and was taken up with dry THF. Triethylamine (1.5 eq, 40 mg, 0.05 mL; 0.36 mmol) was added in one portion followed by solid 3-amino-N-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1H]-yl)ethyl]phenyl}benz-amide (1 eq, 70 mg, 0.16 mmol) and the resulting mixture was stirred at room temperature for 20 h. The solvent was evaporated in *vacuo* and the residue was purified by preparative HPLC affording compound **4o** (20 mg, 0.03 mmol, 16 %) as pale yellow solid, **mp** 189 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.91 (s, 3 H), 1.94 (s, 3 H), 1.98-2.02 (m, 2 H), 2.75-2.77 (m, 6 H), 2.88- 2.93 (m, 4 H), 3.06-3.14 (m, 4 H), 3.73 (s, 3 H), 3.75 (s, 3 H), 6.37 (s, 1 H), 6.48 (s, 1 H), 6.93-6.96 (m, 2 H), 7.14 (t, *J* = 6.9 Hz, 2 H), 7.23-7.26 (m, 3 H), 7.41 (d, *J* = 7.3 Hz, 2 H), 7.48-7.52 (m, 3 H), 7.56 (s, 1 H), 7.74 (d, *J* = 8.0 Hz, 1 H), 8.01 (s, 1 H), 8.66 (s, 1 H, N-H), 8.86 (s, 1 H, N-H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 14.4 (+, CH_3), 14.8 (+, CH_3), 22.9 (-, CH_2), 24.1 (-, CH_2), 29.7 (-, CH_2), 30.0 (-, CH_2), 38.4 (-, CH_2), 38.5 (-, CH_2), 49.3 (-, CH_2), 52.2 (-, CH_2), 55.9 (+, CH_3), 56.0 (+, CH_3), 109.0 (+, CH_{arom}), 111.0 (+, CH_{arom}), 118.2 (+, CH_{arom}), 118.3 (+, CH_{arom}), 118.7 (+, CH_{arom}), 121.4 (+, 2 x CH_{arom}), 122.3 (+, CH_{arom}), 123.1 (+, CH_{arom}), 123.5 (C_{quat}), 123.8 (+, CH_{arom}), 125.2 (+, 2 x CH_{arom}), 127.1 (+, CH_{arom}), 128.8 (+, 2 x CH_{arom}), 129.1 (2 x C_{quat}), 129.2 (C_{quat}), 130.4 (C_{quat}), 131.7 (C_{quat}), 133.8 (+, CH_{arom}), 134.2 (C_{quat}), 134.3 (C_{quat}), 134.4 (C_{quat}), 135.1 (C_{quat}), 135.8 (C_{quat}), 136.4 (+, CH_{arom}), 137.3 (C_{quat}), 138.4 (C_{quat}), 140.0 (C_{quat}), 141.7 (C_{quat}), 148.5

(C_{quat}), 149.2 (C_{quat}), 160.5 (C_{quat}), 165.7 (C_{quat}) – **MS** (+ESI): m/z (%) = 794 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1600 (C=O), 1656 (C=O), 3466 (N-H) – **MF** $C_{48}H_{47}N_3O_4S_2$ – **MW** 794.03

Methyl 4-{4-[2-(5-methyl-4-[2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-enyl]thiophene-2-carboxamido)ethyl]phenylcarbamoyl}-2-(quinoline-2-carboxamido)benzoate (6o)

5-Methyl-4[2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-enyl]thiophene-2-carboxylic acid (1.3 eq, 32 mg, 0.08 mmol) was dissolved in dry THF and one drop of DMF. Thionylchloride (2 eq, 18 mg, 11 µL, 0.16 mmol) was added; the mixture was stirred for one hour at room temperature and evaporated to dryness. Then the residue was taken up with dry THF, triethylamine (1.5 eq, 13 mg, 20 µL, 0.13 mmol) and methyl 4-[4-(2-aminoethyl)phenylcarbamoyl]-2-(quinoline-2-carboxamido)benzoate (1.0 eq, 30 mg, 0.06 mmol) were added and the solution was stirred for 15 h at room temperature. The solvent was evaporated under reduced pressure and the residue was further purified by preparative HPLC to yield compound **6o** (12 mg, 0.014 mmol, 24 %) as colourless solid, **mp** 180 °C (decomposition) – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.93 (s, 3 H), 1.99 (s, 3 H), 2.05 (t, *J* = 7.4 Hz, 2 H), 2.76-2.80 (m, 4 H), 2.86 (t, *J* = 6.9 Hz, 2 H), 3.59-3.65 (m, 2 H), 4.08 (s, 3 H), 5.94-5.97 (m, 1 H, NH), 6.99 (s, 1 H), 7.17-7.22 (m, 4 H), 7.31 (t, *J* = 7.8 Hz, 2 H), 7.47 (d, *J* = 7.4 Hz, 2 H), 7.61 (d, *J* = 8.2 Hz, 2 H), 7.66-7.72 (m, 2 H), 7.79-7.85 M, 1 H), 7.90 (d, *J* = 8.2 Hz, 1 H), 8.20 (d, *J* = 8.5 Hz, 1 H), 8.30 (s, 1 H), 8.33-8.35 (m, 3 H), 9.43 (s, 1 H, NH), 13.3 (s, 1 H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 14.4 (+, CH_3), 14.7 (+, CH_3), 22.9 (-, CH_2), 35.3 (-, CH_2). 38.4 (-, CH_2), 38.5 (-, CH_2), 41.1 (-, CH_2), 52.7 (+, CH_3), 117.9 (+, CH_{arom}), 118.7 (+, CH_{arom}), 118.9 (C_{quat}), 120.9 (+, CH_{arom}), 122.6 (+, CH_{arom}), 123.8 (+, CH_{arom}), 125.3 (+, 2 x CH_{arom}), 127.0 (+, CH_{arom}), 127.7 (+, CH_{arom}), 128.4 (+, CH_{arom}), 128.8 (+, 2 x CH_{arom}), 129.3 (+, 3 x CH_{arom}), 129.4 (+, CH_{arom}), 129.5 (C_{quat}), 130.2 (+, CH_{arom}), 130.3 (+, CH_{arom}), 131.9 (+, CH_{arom}), 133.8 (C_{quat}), 134.0 (C_{quat}), 134.3 (C_{quat}), 134.5 (C_{quat}), 135.4 (C_{quat}), 135.5 (C_{quat}), 136.2(C_{quat}), 136.3(C_{quat}), 136.7 (C_{quat}), 137.8 (+, CH_{arom}), 139.9 (C_{quat}), 140.0 (C_{quat}), 140.1 (C_{quat}), 140.8 (C_{quat}), 146.6 (C_{quat}), 149.5 (C_{quat}), 161.9 (C_{quat}), 163.9 (C_{quat}), 164.7 (C_{quat}), 167.3 (C_{quat}) –

MS (+ESI): m/z (%) = 832 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1568 (C=O), 1621 (C=O), 1654 (C=O), 1696 (C=O) – **MF** $C_{49}H_{42}N_4O_5S_2$ – **MW** 831.01

4,4'-(Cyclopentene-1,2-diyl)bis[5-methyl-*N*-(quinolin-2-yl)thiophene-2-carboxamide] (9o)

4,4'-(Cyclopentene-1,2-diyl)bis(5-methylthiophene-2-carboxylic acid) (0.5 g, 1.4 mmol) was dissolved in dry THF (10 mL) and treated with 4 drops of DMF followed by $SOCl_2$ (0.67 g, 0.41 mL, 5.7 mmol). The solution was stirred at room temperature for 15 h and then evaporated to dryness. The residue was taken up in dry THF and under nitrogen atmosphere NEt_3 (3.3 eq, 0.46 g, 0.66 mL, 4.62 mmol) was added in one portion, followed by solid quinolin-2-amine (0.40 g, 2.8 mmol). After stirring at room temperature for additional 50 h, insoluble materials were filtered off, washed with THF and the filtrate was evaporated to dryness. Further purification was achieved by column chromatography (elution with EtOAc/PE 3:7, R_f: 0.8) and yielded compound **9o** (70 mg, 0.1 mmol, 8.3 %) as purple solid, – **mp** 130 °C (decomposition) – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.85 (s, 6 H), 1.91 (t, *J* = 7.2 Hz, 2 H), 2.55 (t, *J* = 7.4 Hz, 4 H), 7.36 (t, *J* = 7.9 Hz, 2 H), 7.40 (s, 2 H), 7.54 (t, *J* = 8.4 Hz, 2 H), 7.72 (d, *J* = 8.8 Hz, 4 H), 8.12 (d, *J* = 9.0 Hz, 2 H), 8.46 (d, *J* = 8.8 Hz, 2 H), 9.38 (s, 2 H, NH) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 14.6 (+, CH_3), 14.8 (+, CH_3), 22.7 (-, CH_2), 38.4 (-, 2 x CH_2), 125.2 (+, 4 x CH_{arom}), 126.2 (2 x C_{quat}), 127.6 (+, 4 x CH_{arom}), 128.0 (2 x C_{quat}), 130.0 (+, 2 x CH_{arom}), 130.4 (+, 2 x CH_{arom}), 134.7 (2 x C_{quat}), 136.3 (2 x C_{quat}), 136.7 (2 x C_{quat}), 138.7 (+, 2 x CH_{arom}), 146.3 (2 x C_{quat}), 151.3 (2 x C_{quat}), 160.5 (2 x C_{quat}) – **MS** (+ESI): m/z (%) = 601 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1597 (C=O), 1655 (C=O), 3505 (N-H) – **MF** $C_{35}H_{28}N_4O_2S_2$ – **MW** 600.75

***N*-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-5-methyl-4-{2-[2-methyl-5-(quinolin-2-ylcarbamoyl)thiophen-3-yl]cyclopent-1-enyl}thiophene-2-carboxamide (11o)**

4,4'-(Cyclopentene-1,2-diyl)bis(5-methylthiophene-2-carboxylic acid) (0.5 g, 1.4 mmol) was dissolved in dry THF (10 mL) and treated with 4 drops of DMF followed by $SOCl_2$ (0.67 g, 0.41 mL, 5.7 mmol). The solution was stirred at room temperature for 15 h and then evaporated to dryness. The residue was taken up

in dry THF and under nitrogen atmosphere NEt_3 (3.3 eq, 0.46 g, 0.66 mL, 4.62 mmol) was added in one portion, followed by a solution of 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]ethyl}aniline (0.14 g, 0.45 mmol) in dry THF dropwise over 4 h. After stirring at room temperature for 1.5 h, solid quinolin-2-amine (0.40 g, 2.8 mmol) was added in one portion. After stirring at room temperature for additional 50 h, insoluble materials were filtered off, washed with THF and the filtrate was evaporated to dryness. Parts of the residue (80 mg) were further purified with preparative HPLC (R_t 13.39 min) and yielded compound **11o** (9 mg, 0.01 mmol, 12 %) as yellow solid.

mp 179 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$, HSQC, HMBC): δ = 2.10-2.13 (m, 5 H), 2.25 (s, 3 H), 2.76 (t, *J* = 7.0 Hz, 2 H), 2.82 (t, *J* = 7.0 Hz, 2 H), 2.91-2.94 (m, 1 H), 3.11-3.16 (m, 2 H), 3.24-3.32 (m, 4 H), 3.67-3.72 (m, 1 H), 3.82 (s, 3 H), 3.85 (s, 3 H), 3.98 (d, *J* = 14.7 Hz, 1 H), 4.57 (d, *J* = 14.7 Hz, 1 H), 6.50 (s, 1 H), 6.62 (s, 1 H), 7.10 (d, *J* = 8.2 Hz, 2 H), 7.69-7-75 (m, 4 H), 7.93-7.98 (m, 3 H), 8.22 (s, 1 H), 8.55 (d, *J* = 9.3 Hz, 1 H), 8.78 (d, *J* = 9.3 Hz, 1H), 9.23 (s, 1H, NH) – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 14.2 (+, CH_3), 14.9 (+, CH_3), 23.0 (-, CH_2), 23.9 (-, CH_2), 30.2 (-, CH_2), 37.6 (-, CH_2), 38.2 (-, CH_2), 49.2 (-, CH_2), 52.3 (-, CH_2), 55.9 (-, CH_2), 56.0 (+, CH_3), 56.1 (+, CH_3), 109.2 (+, CH_{arom}), 111.1 (+, CH_{arom}), 114.7 (+, CH_{arom}), 117.3 (C_{quat}), 118.1 (C_{quat}), 119.5 (+, CH_{arom}), 120.9 (+, 2 x CH_{arom}), 122.4 (C_{quat}), 124.2 (C_{quat}), 128.2 (+, CH_{arom}), 128.6 (+, CH_{arom}), 128.9 (+, 2 x CH_{arom}), 130.0 (C_{quat}), 130.5 (+, CH_{arom}), 131.0 (C_{quat}), 134.5 (+, CH_{arom}), 135.3 (+, CH_{arom}), 135.4 (C_{quat}), 135.8 (C_{quat}), 135.9 (C_{quat}), 136.3 (C_{quat}), 137.0 (C_{quat}), 138.0 (C_{quat}), 140.9 (C_{quat}), 145.9 (+, CH_{arom}), 146.3 (C_{quat}), 148.6 (C_{quat}), 149.2 (C_{quat}), 150.5 (C_{quat}), 160.8 (C_{quat}), 162.6 (C_{quat}) – **HR-MS** calcd for $C_{45}H_{44}N_4O_4S_2$ 768.2804 [M^{+}], found: 768.2788 – **IR** $\tilde{\nu}$ [cm^{-1}] = 1598 (C=O), 1644 (C=O) – **MF** $C_{45}H_{44}N_4O_4S_2$ – **MW** 768.99

N-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-5-nitrothio-phene-2-carboxamide (13)

5-Nitrothiophene-2-carboxylic acid (0.5 g, 2.9 mmol) was refluxed for 30 min in 5 mL $SOCl_2$. The solvent was evaporated to complete dryness, then the residue was taken up with dichloromethane. Triethylamine (1.5 eq, 0.29 g, 0.41 mL; 2.9 mmol) was added in one portion followed by solid 4-{2-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]ethyl}aniline (0.56 g, 1.9 mmol) and the resulting

mixture was stirred at room temperature for 15 h. The yellow precipitate was filtered off and washed with DCM, yielding 0.42 g (0.90 mmol, 47 %) yellow solid, **mp** 220 °C (decomposition) – **^{1}H-NMR** (300 MHz, d-6 DMSO): δ = 3.04-3.51 (m, 6 H), 3.34-3.37 (m, 2 H), 3.73 (s, 3 H), 3.74 (s, 3 H), 4.31 (s, 2 H), 6.77 (s, 1 H), 6.80 (s, 1 H), 7.31 (d, *J* = 8.2 Hz, 2 H), 7.77 (d, *J* = 8.5 Hz, 2 H), 8.19 (d, *J* = 4.4 Hz, 1 H), 8.27 (d, *J* = 4.4 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, d-6 DMSO: δ = 24.7 (-, CH_2), 29.2 (-, CH_2), 48.9 (-, CH_2), 51.5 (-, CH_2), 55.4 (+, CH_3), 55.5 (+, CH_3), 55.9 (-, CH_2), 109.5 (+, CH_{arom}), 111.4 (+, CH_{arom}), 120.3 (C_{quat}), 120.8 (+, 2 x CH_{arom}), 123.4 (C_{quat}), 128.5 (+, CH_{arom}), 128.9 (+, 2 x CH_{arom}), 130.0 (+, CH_{arom}), 133.3 (C_{quat}), 136.6 (C_{quat}), 146.4 (C_{quat}), 147.5 (C_{quat}), 148.1 (C_{quat}), 153.2 (C_{quat}), 158.1(C_{quat}) – **MS** (+ESI): m/z (%) = 468 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1317 (N-O), 1509 (N-O), 1601 (C-O) – **MF** $C_{24}H_{25}N_3O_5S$ – **MW** 467.54

5-Amino-*N*-{4-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}thio-phene-2-carboxamide (14)

N-{4-[2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2[1*H*]-yl)ethyl]phenyl}-5-nitrothio-phene-2-carboxamide (0.4 g, 0.85 mmol) was dissolved in 5 mL DMF and 5 eq of $SnCl_2$ (0.8 g, 4.2 mmol) were added. The reaction mixture was heated to 70 °C for 30 min and then poured on ice. After neutralisation with diluted NaOH the solution was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The reaction yielded compound **14** (0.15 g, 0.34 mmol, 59 %) as red solid, **mp** 200 °C (decomposition) – **^{1}H-NMR** (300 MHz, d-6 DMSO): δ = 2.78-2.87 (m, 8 H), 3.68 (s, 2 H), 3.80 (s, 3 H), 3.82 (s, 3 H), 6.52 (s, 1 H), 6.58 (s, 1 H), 7.11 (d, *J* = 6.0 Hz, 2 H), 7.35 (d, *J* = 3.6 Hz, 1 H), 7.50-7.55 (m, 3 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, d-6 DMSO: δ = 27.9 (-, CH_2), 32.7 (-, CH_2), 50.6 (-, CH_2), 55.1 (-, CH_2), 55.7 (+, CH_3), 55.8 (+, CH_3), 59.5 (-, CH_2), 109.4 (+, CH_{arom}), 111.3 (+, CH_{arom}), 115.3 (+, CH_{arom}), 120.5 (+, 2 x CH_{arom}), 122.6 (C_{quat}), 125.6 (C_{quat}), 129.0 (+, 2 x CH_{arom}), 129.4 (+, CH_{arom}), 135.0 (C_{quat}), 136.5 (C_{quat}), 136.6 (C_{quat}), 150.9 (C_{quat}), 158.8 (C_{quat}), 160.2 (C_{quat}), 161.0 (C_{quat}) – **MS** (+ESI): m/z (%) = 438 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1517 (N-H), 3320 (N-H) – **MF** $C_{24}H_{27}N_3O_3S$ – **MW** 437.55

***N*-{5-[4-(2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl)phenylcarbamoyl] thiophen-2-yl}quinoline-2-carboxamide (16)**

4-{2-[6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]ethyl}aniline (150 mg, 0.34 mmol) was dissolved in 2 mL dry DCM. Triethylamine (1.5 eq, 50 mg, 0.07 mL, 0.5 mmol) and quinoline-2-carbonyl chloride (1.5 eq, 98 mg, 0.5 mmol) were added. The reaction mixture was stirred for 15 h at room temperature and then washed with $NaHCO_3$. The aqueous phase was extracted with dichloromethane (3 x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. Further purification was achieved with preparative HPLC (R_t 8.87 min) affording compound **16** (5 mg, 0.008 mmol, 2 %) as yellow solid, **mp** 48 °C – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.82-2.98 (m, 2 H), 3.02-3.15 (m, 2 H), 3.20-3.38 (m, 4 H), 3.81 (s, 3 H), 3.83 (s, 3 H), 3.98-4.02 (m, 2 H), 6.49 (s, 1 H), 6.60 (s, 1 H), 6.92 (s, 1 H), 7.13 (d, *J* = 6.8 Hz, 2 H), 7.57 (d, *J* = 6.8 Hz, 2 H), 7.64-7.68 (m, 2 H), 7.81 (t, *J* = 7.8 Hz, 1 H), 7.91 (d, *J* = 8.5 Hz, 1 H), 8.18 (d, *J* = 7.7 Hz, 2 H), 8.35 (q, *J* = 7.6 Hz, 2 H), 10.91 (s, 1 H) – **IR** $\tilde{\nu}$ [cm^{-1}] = 1519 (C=O), 1669 (C=O) – **HR-MS** calcd for $C_{34}H_{32}N_4O_4S$ 592.2144 [M^{+}], found: 592.2130– **MF** $C_{34}H_{32}N_4O_4S$ – **MW** 592.71

Photochemical synthesis of the ring-closed isomer 9c. A solution of compound **9o** (15 mg, mmol) in $CDCl_3$ (0.7 mL) was irradiated for 60 min with 312 nm lamp in a silica glass NMR tube, yielding a purple solution of a photostationary state containing 86 % of the ring closed isomer according to the ^{1}H-NMR spectra. The remaining 36 % were assigned to the ring-open isomer **9o**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.92 (t, *J* = 7.1 Hz, 2 H), 2.08 (s, 6 H), 2.48 (t, *J* = 7.4 Hz, 4 H), 6.85 (s, 2 H), 7.47 (t, *J* = 6.9 Hz, 2), 7.68 (t, *J* = 7.0 Hz, 2 H), 7.78-7.82 (m, 4 H), 8.02 (d, *J* = 9.0 Hz, 2 H)

Modulation of ABCB1 and ABCG2 was performed as described.[25]

4.5. References

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5. Stereoselective Synthesis of alpha-disubstituted Secondary Amines as Potential ACE Inhibitors*

Secondary amines containing two carbon stereocentres in both α-positions are a typical substructure of ACE inhibitors and other biological active compounds. Their stereoselective synthesis was aspired in different approaches, but industrial applications mostly still apply the separation of diastereomers. Here, two synthetic routes for the conversion of natural amino acids into the target structures using an S_N2 reaction or an oxazolidine mediated reaction sequence as key steps were compared concerning their diastereoselectivity. Synthesis via the oxazolidine gave only one entantiomer for the substituted secondary amine. The synthesised compounds showed weak or no cytotoxicity. Selected compounds were tested as inhibitors of ACE-2, but revealed no biological activity.

* All synthesis were done by Carolin Fischer, the MTT-assay was done by Gabriele Brunner and the ACE-2 assay was done by the company CEREP (France).

5.1. Introduction

The angiotensin I converting enzyme (ACE) plays a key role in the control of the renin-angiotensin-aldosterone system (RAAS). The biologically inactive angiotensin I is converted into the active vasoconstrictor angiotensin II and the vasodilatory bradykinin is inactivated. The membrane-bound zinc metalloprotease, a dipeptidyl carbopeptidase cleaves the C-terminal dipeptide His-Leu from the decapeptide angiotensin I and the dipeptide Phe-Arg from the nonapeptide bradykinin.[1] The recently discovered related homolog angiotensin-converting enzyme 2 (ACE-2) converts angiotensin I into angiotensin 1-9, which has no effect on blood vessels, but is then converted by ACE to the blood vessel dilator angiotensin 1-7.[2-3] These activities explain the predominant role of ACE and ACE-2 in the regulation of cardic function and blood pressure and justify the use of ACE inhibitors in antihypertensive therapy.[4-5] Based on isolated potent peptides of snake venom a huge number of peptidomimetic ACE inhibitors were developed and released to the market in the 1980s and 1990s. Since the crystal structure of the human angiotensin converting enzyme was published in 2003 by Natesh *et al.*, ACE has become a popular target for structure based drug design.[6] Most of the known inhibitors contain an iminodiaceticacid structure substituted in both alpha positions to the carboxylic acids. The stereocenters are either (*S*)/(*S*) or (*S*)/(*R*) configured. One of the carboxylic acids is in most cases linked to proline or an alternative rigidified ring structure (see Figure 5.1).[7]

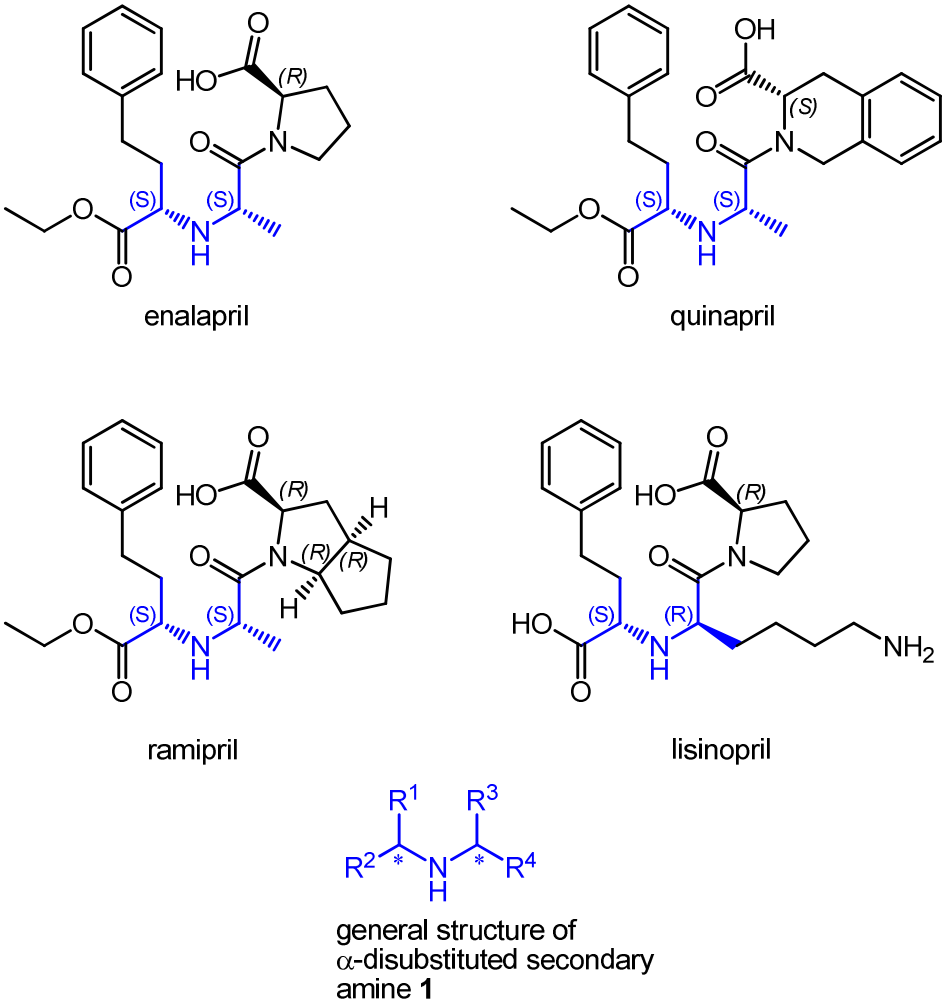


Figure 5.1: Common clinically used ACE inhibitors.

For the synthesis of the secondary amine substructures owning two defined stereocentres in both α-positions, several different approaches were reported resulting in no or low excess for one of the two possible diastereomers: reductive amination using Raney nickel or $NaCNBH_3$,[8-11] aza-Michael reaction,[12] S_N2 reaction applying bromide, mesylate, tosylate or triflate as leaving groups [9, 13-15] and the palladium-catalysed asymmetric reductive alkylation.[19] Only one diastereomer was detected for the synthesis via a β-lactam intermediate,[16] stereoselective alkylation[20] and for microbial reduction.[9, 21] Only few of these reactions resulted in high stereoselectivity. As the defined stereochemistry is important for the biological activity, HPLC separation of the two diastereomers was necessary in most cases.

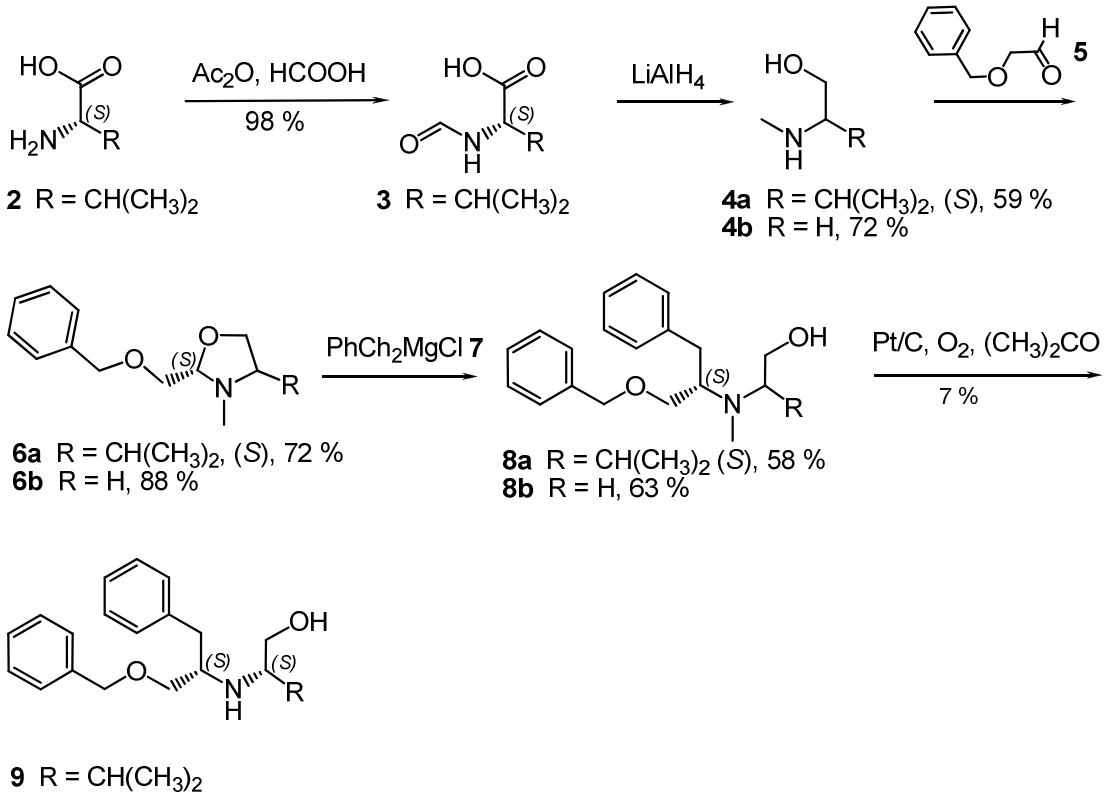
Improved diastereoselective synthesis of α-disubstituted secondary amines would be of advantage for the commercial production of ACE inhibitors, but also interesting for other fields of medicinal chemistry where e.g. acyclic amine inhibitors are used as phosphorylase or nucleosidase inhibitors[22] or secondary amine derivatives are found in naturally occurring opines. These α-substituted 2,2'-azanediyldiacetic acids are formed by the condensation of an amino acid and a keto acid by parasitic bacteria of the genus *Agrobacterium*.[23]

We have investigated two methods for the synthesis of chiral secondary amines of the general structure **1** starting from natural amino acids and compare their diastereoselectivity. S_N2 reactions using triflate as leaving group are often described to give high diastereoselectivity.[13, 24] The second approach proceeds via an oxazolidine intermediate and was not used before for the synthesis of ACE inhibitors. The cytotoxic properties and the inhibitory effect on human ACE-2 of the synthesised derivatives were tested.

5.2. Results and Discussion

5.2.1. Synthesis

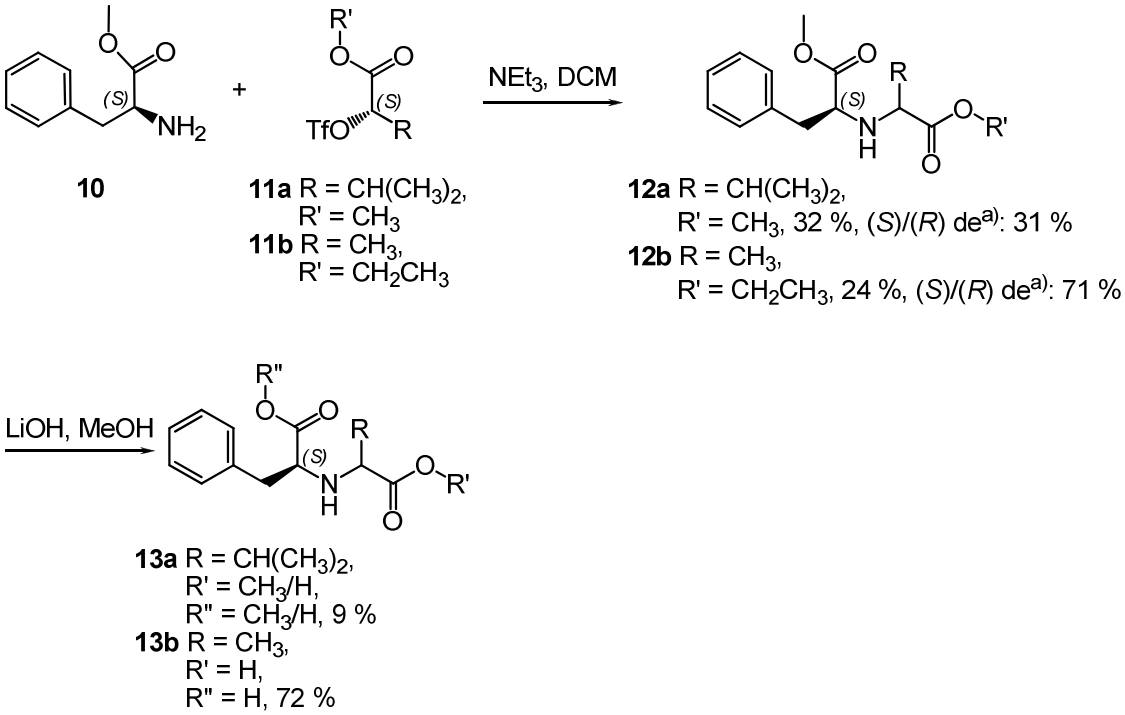
The natural amino acid valine **2** was converted by formylation and reduction into the corresponding *N*-methyl alcohol **4a**. Additionally, the *N*-methylated alcohol of glycine (**4b**) was used (Scheme 5.1).



Scheme 5.1: Oxazolidine-mediated diastereoselective synthesis of amines **8**.

Conversion with 2-(benzyloxy)acetaldehyde **5** gave oxazolidines **6a** and **6b**. The attack of the benzylmagnesium chloride (**7**) was now only possible from the convex side of the molecule and gave one diastereomer of compounds **8a** and **8b** as confirmed by HPLC analyses. The synthetic strategy was used previously for the diastereoselective synthesis of analgesic compounds[25] and the antidepressant cericlamine.[26] Heyns oxidation condition (Pt/C, O_2) with the intention to oxidise the hydroxy function to the corresponding acid was not successful and gave only a small yield of the demethylated compound **9**.

The second synthetic strategy used an S_N2 substitution of a triflate group. Two different triflate compounds were prepared possessing methyl or isopropyl substituents in α-position. The diastereomeric excess for the (*S*,*S*) isomer was 31 % de (**12a**) and 71 % de (**12b**) (Scheme 5.2), respectively, as determined by HPLC. The basic cleavage of the ester groups was incomplete for the isopropyl-substituted compound **13a**.



Scheme 5.2: Synthesis of amines **12** via S_N2 reaction; [a)] de [%] derived by HPLC analysis.

5.2.2. Cytotoxicity and ACE-2 Inhibition

The cytotoxicity of compounds **8**, **9**, **12** and **13** was tested using the colourimetric MTT assay. The assay takes advantage of the cleavage of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolin bromide) into the blue coloured formazan by the mitochondrial enzyme succinate-dehydrogenase.[27-29] The conversion takes only place in living cells and the amount of the red formazan is proportional to the number of cells alive.

Table 5.1: Cytotoxicity determined by the colourimetric MTT-assay.

Compound	IC_{50} [mM]	logP[a)]
8a	51.89 ± 4.24	4.53
8b	173.48 ± 3.80	3.30
9	78.56 ± 1.68	4.40
12a	114.91 ± 6.39	3.58
12b	-[b)]	3.24
13a	-[b)]	3.07
13b	-[b)]	0.90
N-methylimino-diaceticacid	-[b)]	-1.22

[a)] logP values calculated by ACD/labs 12.0, [b)] no cytotoxic effect determined

The tested compounds showed weak or even no toxicity. Their toxicity correlates with the calculated logP values: more lipophilic compounds of this series are more toxic (Table 5.1).

Compound **13b** was previously considered as a potential ACE-2 inhibitor.[30] Therefore selected compounds covering different structural elements were tested on their inhibitory effect on human ACE-2.[1] The assay reference compound was Ac-GG-26-NH_2 with an IC_{50} value of 610 nM and n_H = 1.5. All compounds were investigated at 10 μM final concentration. However, the tested compounds did not show significant inhibition of the human ACE-2 function.

5.3. Conclusion

Di-substituted secondary amines **1** are potential ACE inhibitors and were prepared from natural amino acids via an oxazolidine-intermediate **6** or via a S_N2 reaction. While the first route gave the target compounds in high diastereoselectivity, S_N2 conditions showed only moderate selectivity of 71 %

[1] The ACE-2 inhibition assay was performed by the company CEREP (Celle l'Evescault, France).

de or 31 % de depending on the triflate reagent. Compounds **8**, **9**, **12** and **13** are not or very weakly cytotoxic in an MTT assay, but have no inhibitory effect on human ACE-2.

5.4. Experimental Section

General. Commercial reagents and starting materials were purchased from Aldrich, Fluka or Acros and used without further purification. Flash chromatography was performed on silica gel (Merck silica gel Si 60 40-63 *μ*m); products were detected by TLC on alumina plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm) and visualized by UV light (λ = 254 nm). Melting points were determined with Optimelt MPA 100 and are uncorrected. NMR spectra were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz; T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz; ^{13}C: 100.6 MHz; T = 300 K) and Bruker Avance 600 (^{1}H: 600.1 MHz; ^{13}C: 150.1 MHz; T = 300 K) instruments. Chemical shifts are reported in δ/ppm relative to external standards and coupling constants J are given in Hz. Abbreviations for the characterization of the signals: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, dd = double doublet. The relative numbers of protons is determined by integration. Error of reported values: chemical shift 0.01 ppm (^{1}H NMR), 0.1 ppm (^{13}C NMR), coupling constant 0.1 Hz. The used solvent for each spectrum is reported. Mass spectra were recorded with Finnigan MAT TSQ 7000 (ESI) and Finnigan MAT 90 (HRMS), IR spectra with a Bio-Rad FT-IR-FTS 155 spectrometer and UV/vis spectra with a Cary BIO 50 UV/vis/NIR spectrometer (Varian), optical rotation was determined with Krüss P8000.

Compounds prepared after literature known procedures: (*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)formamide (**3**),[31] (*S*)-3-methyl-2-(methylamino)butan-1-ol (**4a**),[31] (*S*)-2-(methylamino)-3-phenylpropan-1-ol (**4b**),[32] (*S*)-ethyl 2-(trifluoromethylsulfonyloxy)propanoate (**11b**),[33] (*S*)-methyl 2-[1-ethoxy-1-oxopropan-2-ylamino]-3-phenylpropanoate (**12b**) [13] and (*S*)-2-[1-carboxyethylamino]-3-phenylpropanoic acid (**13b**)[13]

(2*S*,4*S*)-2-(Benzyloxymethyl)-4-isopropyl-3-methyloxazolidine (6a)

N-Methylvalinol was dissolved under nitrogen atmosphere in DMSO. Freshly distilled 2-(benzyloxy)acetaldehyde (0.1 g, 0.68 mmol) was added. The reaction was stirred over night at room temperature. The mixture was then diluted with dichloromethane and washed with water. After evaporating the solvent, the reaction yielded (2S,4S)-2-(benzyloxymethyl)-4-isopropyl-3-methyloxazolidine (0.12 g, 0.48 mmol, 72 %) as yellow oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.87 (d, 6.9 Hz, 3 H), 0.91 (d, 6.9 Hz, 3 H), 1.70-1.77 (m, 1 H), 2.49-2.55 (m, 1 H), 2.58 (s, 3 H), 3.72-3.75 (m, 1 H), 3.84-3.90 (m,1 H), 4.21-4.24 (m, 1 H), 4.53-4.68 (m, 4 H), 7.29-7.36 (m, 5 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 17.2 (+, CH_3), 19.7 (+, CH_3), 30.4 (+, CH), 39.8 (+, CH_3), 67.4 (-, CH_2), 70.5 (+, CH), 72.4 (-, CH_2), 73.5 (-, CH_2), 97.3 (+, CH), 127.6 (+, CH_{arom}), 127.7 (+, 2 x CH_{arom}), 128.4 (+, 2 x CH_{arom}), 138.3 (C_{quat}) – **MS** (CI): m/z (%) = 250 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1023 (C-O), 1064 (C-O), 1449 (C-N) – **MF** $C_{15}H_{23}NO_2$ – **MW** 249.35

2-(Benzyloxymethyl)-3-methyloxazolidine (6b)

Freshly distilled 2-(methylamino)ethanol (0.75 g, 10 mmol) was dissolved at 0 °C in DMSO under nitrogen atmosphere. Molecular sieves (3 Å) and freshly distilled 2-(benzyloxy)acetaldehyde (1.5 g, 10 mmol) were added. The reaction was stirred over night at room temperature. The mixture was then diluted with dichloromethane and washed with water. After evaporating the solvent, the reaction yielded 2-(benzyloxymethyl)-3-methyloxazolidine (1.82 g, 8.8 mmol, 88 %) as pale oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.32 (s, 3 H), 3.03-3.10 (m, 1 H), 3.35-3.54 (m, 3 H), 3.68-3.77 (m, 2 H), 4.05 (t, *J* = 4.6 Hz, 1 H), 4.53 (s, 2 H), 7.27-7.34 (m, 5 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 43.9 (+, CH_3), 59.2 (-, CH_2), 69.2 (-, CH_2), 76.9 (-, CH_2), 77.6 (-, CH_2), 100.8 (+, CH), 132.5 (+, 2 x CH_{arom}), 132.6 (+, CH_{arom}), 133.4 (+, 2 x CH_{arom}), 143.6 (C_{quat}) – **MS** (CI): m/z (%) = 208 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1027 (C-O), 1059 (C-O), 1454 (C-N) – **$[\alpha]_D^{20}$** -13.9 ° (c: 1.0, $CHCl_3$) – **MF** $C_{12}H_{17}NO_2$ – **MW** 207.27

(*S*)-2-{[(*S*)-1-(Benzyloxy)-3-phenylpropan-2-yl](methyl)amino}-3-methyl-butan-1-ol (8a)

(4*S*)-2-(Benzyloxymethyl)-4-isopropyl-3-methyloxazolidine (0.25 g, 1 mmol) was dissolved in dry THF and benzylmagnesium chloride (5 eq, 2.5 mL of a 2 M solution in THF; 5.00 mmol) was added dropwise. After stirring the mixture 3-4 h at room temperature the reaction was treated with diethyl ether and washed with water. The organic layer was dried over sodium sulphate and evaporated. The reaction yielded 0.2 g (0.58 mmol, 58 %) yellow oil. LC-MS analysis showed only one diastereomer, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.97 (d, 6.6 Hz, 3 H), 1.05 (d, 6.8 Hz, 3 H), 1.90-1.97 (m, 1 H), 2.55 (s, 3 H), 2.62-2.67 (m, 1 H), 2.90-3.02 (m, 3 H), 3.28-3.35 (m, 4 H), 4.56 (s, 2 H), 7.15-7.24 (m, 10 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 17.2 (+, CH_3), 19.8 (+, CH_3), 34.7 (-, CH_2), 38.0 (-, CH_2), 38.2 (-, CH_2), 39.4 (-, CH_2), 39.9 (+, CH_3), 56.2 (+, CH), 65.9 (+, CH), 76.3 (+, CH), 126.0 (+, CH_{arom}), 126.1 (+, CH_{arom}), 126.2 (+, CH_{arom}), 127.1 (+, CH_{arom}), 128.4 (+, CH_{arom}), 128.5 (+, CH_{arom}), 128.6 (+, CH_{arom}), 128.7 (+, CH_{arom}), 129.3 (+, CH_{arom}), 131.1 (+, CH_{arom}), 136.7 (C_{quat}), 139.1 (C_{quat}) – **MS** (+ESI): m/z (%) = 342 (100) [MH^+] – **$[\alpha]_D^{20}$** -15.3 ° (c: 1.0, $CHCl_3$) – **IR** $\tilde{\nu}$ [cm^{-1}] = 1454 (C-N), 3375 (O-H) – **MF** $C_{22}H_{31}NO_2$ – **MW** 341.49

(*S*)-2-{[1-(Benzyloxy)-3-phenylpropan-2-yl][methyl]amino}ethanol (8b)

(4*S*)-2-(Benzyloxymethyl)-4-isopropyl-3-methyloxazolidine (1 eq, 2.1 g, 10 mmol) was dissolved in dry THF and benzylmagnesium chloride (5 eq, 25 mL of a 2 M solution in THF; 50.0 mmol) was added dropwise. After stirring the mixture 3-4 h at room temperature the reaction was treated with diethyl ether and washed with water. The organic layer was dried over sodium sulphate and evaporated. The reaction yielded (*S*)-2-{[1-(benzyloxy)-3-phenylpropan-2-yl][methyl]amino}ethanol (1.8 g, 6 mmol, 63 %) as yellow oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.36 (s, 3 H), 2.64-2.74 (m, 4 H), 3.06-3.15 (m, 1 H), 3.38-3.61 (m, 4 H), 4.48 (d, *J* = 2.19 Hz, 2 H), 7.15-7.32 (m, 10 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 34.5 (-, CH_2), 36.1 (+, CH_3), 56.0 (-, CH_2), 58.1 (-, CH_2), 64.9 (+, CH), 69.8 (-, CH_2), 73.2 (-, CH_2), 126.2 (+, CH_{arom}), 127.6 (+, CH_{arom}), 127.7 (+, 2 x CH_{arom}), 128.4 (+, 2 x CH_{arom}), 128.5 (+, 2 x CH_{arom}), 129.0 (+, 2 x CH_{arom}), 138.2 (C_{quat}), 139.8 (C_{quat}) – **MS** (+ESI): m/z (%) = 300

(100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1028 (C-O), 1453 (C-N), 3440 (O-H) – **[α]$_D^{20}$** -58.5 ° (c: 1.0, $CHCl_3$) – **MF** $C_{19}H_{25}NO_2$ – **MW** 299.41

(*S*)-2-[(*S*)-1-(Benzyloxy)-3-phenylpropan-2-ylamino]-3-methylbutan-1-ol (9)

(*S*)-2-{[(*S*)-1-(Benzyloxy)-3-phenylpropan-2-yl][methyl]amino}-3-methylbutan-1-ol (50 mg, 0.14 mmol) was dissolved in acetone. $NaHCO_3$ (60 mg, 0.7 mmol) and Pt/C (14 mg of a 100 g/mol batch) were added. The flask was closed with an oxygen balloon and the reaction mixture was stirred for 15 h at 60 °C. The solid Pt/C was filtered off over celite and washed with acetone. After evaporation the residue was taken up in ethyl acetate and washed with water. Lyophilisation of the aqueous layer resulted in 7 mg (0.02 mmol, 7.3 %) of (*S*)-2-((*S*)-1-(benzyloxy)-3-phenylpropan-2-ylamino)-3-methylbutan-1-ol as yellow oil. Due to small amount, purity was checked by HPLC-MS (R_t: 5.63 min), **MS** (+ESI): m/z (%) = 328 (100) [MH^+] – **MF** $C_{21}H_{29}NO_2$ – **MW** 327.46

(*S*)-Methyl 3-methyl-2-(trifluoromethylsulfonyloxy)butanoate (11a)

Methyl 2-hydroxy-3-methylbutanoate (0.55 g, 4.1 mmol) was added to a solution of trifluoromethanesulfonic anhydride (1 eq, 1.17 g, 41. mmol) and pyridine (1 eq, 0.33 g, 0.33 mL, 4.1 mmol) in dichloromethane at 0 °C. The solution was warmed up to room temperature and evaporated. The remaining residue was taken up with pentane and washed with water. Then the organic layer was dried over sodium sulphate and the solvent was removed under reduced pressure. The reaction yielded methyl 3-methyl-2-(trifluoromethylsulfonyloxy)butanoate (0.9 g, 3.4 mmol, 83 %) as purple liquid, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.91 (d, *J* = 6.9 Hz, 3 H), 1.00 (d, *J* = 6.9 Hz, 3 H), 2.28-2.34 (m, 1 H), 3.74 (s, 3 H), 4.91 (d, *J* = 3.84 Hz, 1 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 15.9 (+, CH_3), 17.8 (+, CH_3), 30.9 (+, CH), 52.5 (+, CH_3), 88.0 (+, CH), 116.3 (C_{quat}), 167.02 (C_{quat}) – **MS** (CI): m/z (%) = 282 (100) [MNH_4^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1145 (S=O), 1213 (C-F), 1736 (C=O), 2966 (C-H) – **[α]$_D^{20}$** -13.5 ° (c: 1.0, $CHCl_3$) – **MF** $C_7H_{11}F_3O_5S$ – **MW** 264.22

Methyl 2-[(*S*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino]-3-methyl-butanoate (12a)

Phenylalaninmethylester (0.34 g, 1.8 mmol, 1 eq) was dissolved in a mixture of dichloromethane and 1.1 eq of Et_3N (0.2 g, 0.3 mL, 2 mmol) and a solution of (*S*)-ethyl 3-methyl-2-(trifluoromethylsulfonyloxy)butanoate (0.50 g, 1.8 mmol, 1 eq) in dichloromethane was added at 0 °C. The reaction was stirred for 15 h at room temperature and then washed with water. The organic layers were dried over sodium sulphate and the solvents were removed under reduced pressure. Further purification was achieved by column chromatography (elution with ethyl acetate, R_f = 0.75). The reaction yielded methyl 2-[(R)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino]-3-methylbutanoate (0.17 g, 0.58 mmol, 32 %) as yellow oil, **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.90 (d, *J* = 6.86 Hz, 3 H), 0.99 (d, *J* = 7.1 Hz, 3 H), 1.70-1.78 (m, 1 H), 2.73-2.90 (m, 2 H), 3.30 (t, *J* = 6.7 Hz, 1 H), 3.52 (s, 3 H), 3.55 (s, 3 H), 7.08-7.15 (m, 5 H) – **^{13}C-NMR**, DEPT 135 (75 MHz, $CDCl_3$): δ = 18.2 (+, CH_3), 19.1 (+, CH_3), 31.9 (+, CH), 39.9 (-, CH_2), 51.5 (+, CH_3), 51.7 (+, CH_3), 63.0 (+, CH), 66.6 (+, CH), 126.5 (+, CH_{arom}), 128.2 (+, 2 x CH_{arom}), 129.4 (+, 2 x CH_{arom}), 137.7 (C_{quat}), 174.5 (C_{quat}), 175.0 (C_{quat}) – **MS** (CI): m/z (%) = 294 (100) [MH^+] – **IR** $\tilde{\nu}$ [cm^{-1}] = 1734 (C=O), 2955 (C-H) – **MF** $C_{16}H_{23}NO_4$ – **MW** 293.36

(2*S*)-2-(1-Methoxy-3-methyl-1-oxobutan-2-ylamino)-3-phenylpropanoic acid (13a)

Methyl 2-[(*R*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino]-3-methylbutanoate (50 mg, 0.17 mmol) was dissolved in methanol. After the addition of LiOH (6 eq, 24 mg, 1.0 mmol) the reaction mixture was stirred for 15 h at room temperature. To work-up the reaction the pH was adjusted to 2 with diluted HCl and extracted with ethyl acetate. The organic layer was dried over sodium sulphate and the solvent was evaporated. The reaction yielded the mono-ester (5 mg, 0.02 mmol, 9 %) as yellow oil. Due to small amount, purity was checked by HPLC-MS (R_t: 5.66 min), **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.68 (d, 6.6 Hz, 3 H), 0.75 (d, 6.9 Hz, 3 H), 1.88-1.92 (m, 1 H), 2.89-2.96 (m, 2 H), 3.26-3.32 (m, 1 H), 3.40-3.44 (m, 1 H), 3.70 (s, 3 H), 7.23-7.33 (m, 5 H) – **MS** (+ESI): m/z (%) = 280 (100) [MH^+] – **MF** $C_{14}H_{19}NO_4$ – **MW** 265.30

Cell Lines and Cell Culture Conditions.

The human epithelial cervical cancer cell line HeLa [CCL-2, American Type Culture Collection (ATCC)] was cultured in MEM Earle's medium. An amount of 500 mL MEM was supplemented with 0.8 mL amphotericin B (250 μg/mL), 5 mL penicillin/streptomycin (10,000 U/mL/ 10,000 μg/mL), 5 mL L-glutamine (200 mM), 5 mL NEA (100×), and 50 mL fetal bovine serum (all from Biochrom AG, Berlin, Germany). Cells were split in 1:6 ratio twice a week. All cells were cultured in a humidified incubator at 37 °C in a 5 % CO_2 atmosphere.

Determination of Cytotoxicity via MTT assay.

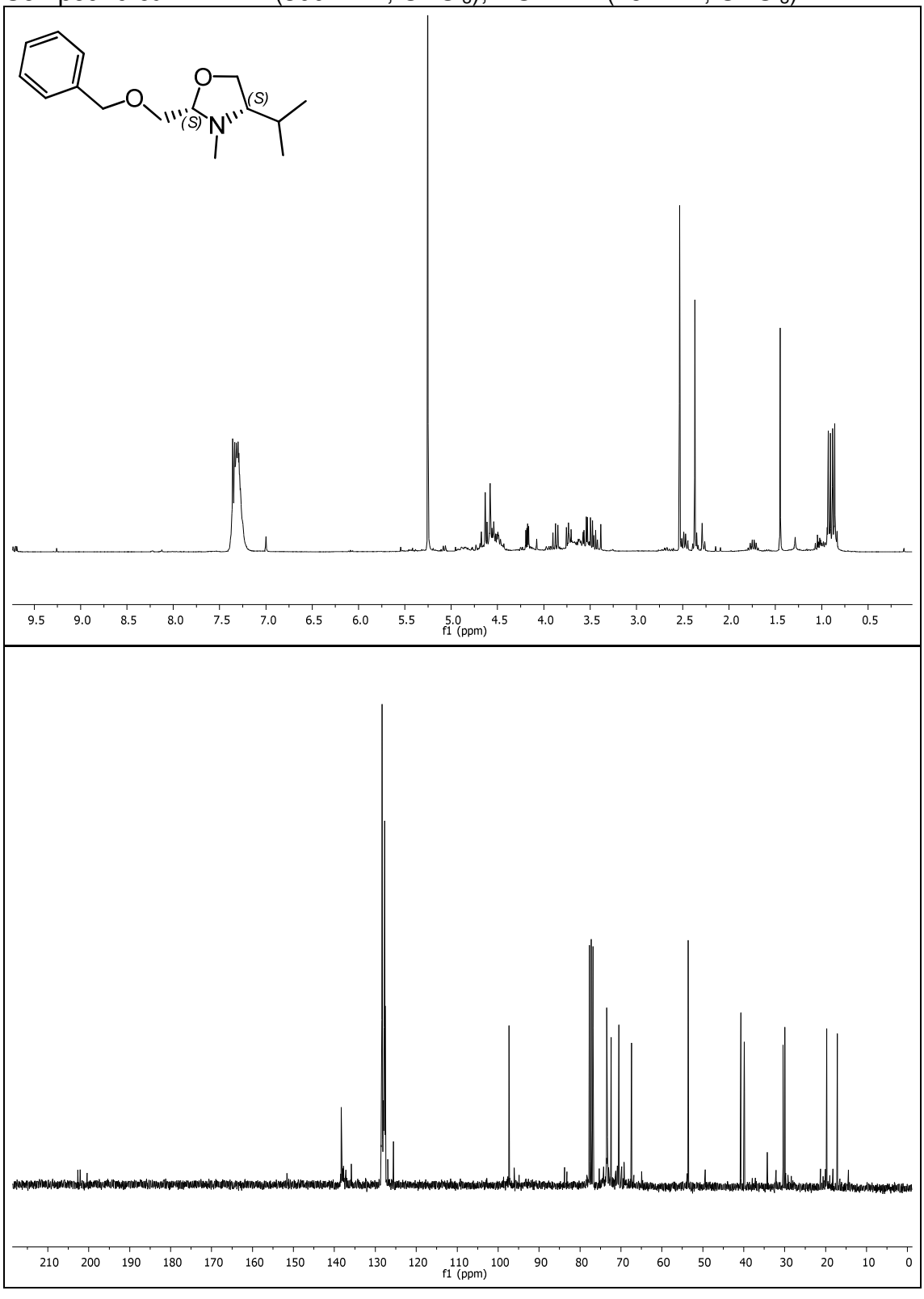
The cytotoxic effect of **8a**, **8b**, **9**, **12a**, **12b**, **13a** and **13b**, respectively, was determined using the non-metabolizing HeLa (ATCC CCL-2) cell line. For evaluation of cytotoxicity on HeLa cells, the colourimetric MTT (tetrazolium) assay was conducted as previously described by Mosmann[27] in a modified manner according to Heilmann[29] *et al*. In brief, following trypsinization, HeLa cells were counted and 11250 cells/well were seeded into a 96 well plate (Techno Plastic Products AG, Trasadingen, Switzerland). Afterward, the cells were exposed to the compounds (final concentrations) **8a** (12.5 μM, 25 μM, 50 μM, 75 μM, 100 μM), **8b** (25 μM, 50 μM, 75 μM, 100 μM, 150 μM, 200 μM, 300 μM), **9** (25 μM, 50 μM, 75 μM, 100 μM, 150 μM, 200 μM), **12a** (25 μM, 50 μM, 75 μM, 100 μM, 150 μM, 200 μM, 300 μM), **12b** (12.5 μM, 25 μM, 50 μM, 75 μM, 100 μM), **13a** (12.5 μM, 25 μM, 50 μM, 75 μM, 100 μM) and **13b** (12.5 μM, 25 μM, 50 μM, 75 μM, 100 μM) dissolved in 30 % of EtOH for 72 h at 37 °C and 5 % CO_2. For quantification of viability, cells were incubated with 15 μL/well MTT (tetrazolium) solution (4 mg/mL) for four hours, which was then converted into insoluble, violet formazan. After removal of the supernatant, 150 μL SDS solution (10 %) was added to dissolve the formazan crystals. The next day, absorption was measured at 560 nm using a SpectraFluorPlus microplate reader (Tecan GmbH, Crailsheim/Germany). All tests were performed in sextuplicates. Solvent control was set to 100 %.

Human ACE-2 inhibition. Assay was done by the company CEREP (Le Bois l'Evêque BP 30001 - 86600 Celle l'Evescault, France). The assay reference compound was Ac-GG-26-NH_2 with IC_{50} = 610 nM and n_H = 1.5. All compounds were tested at 10 μM final concentration.

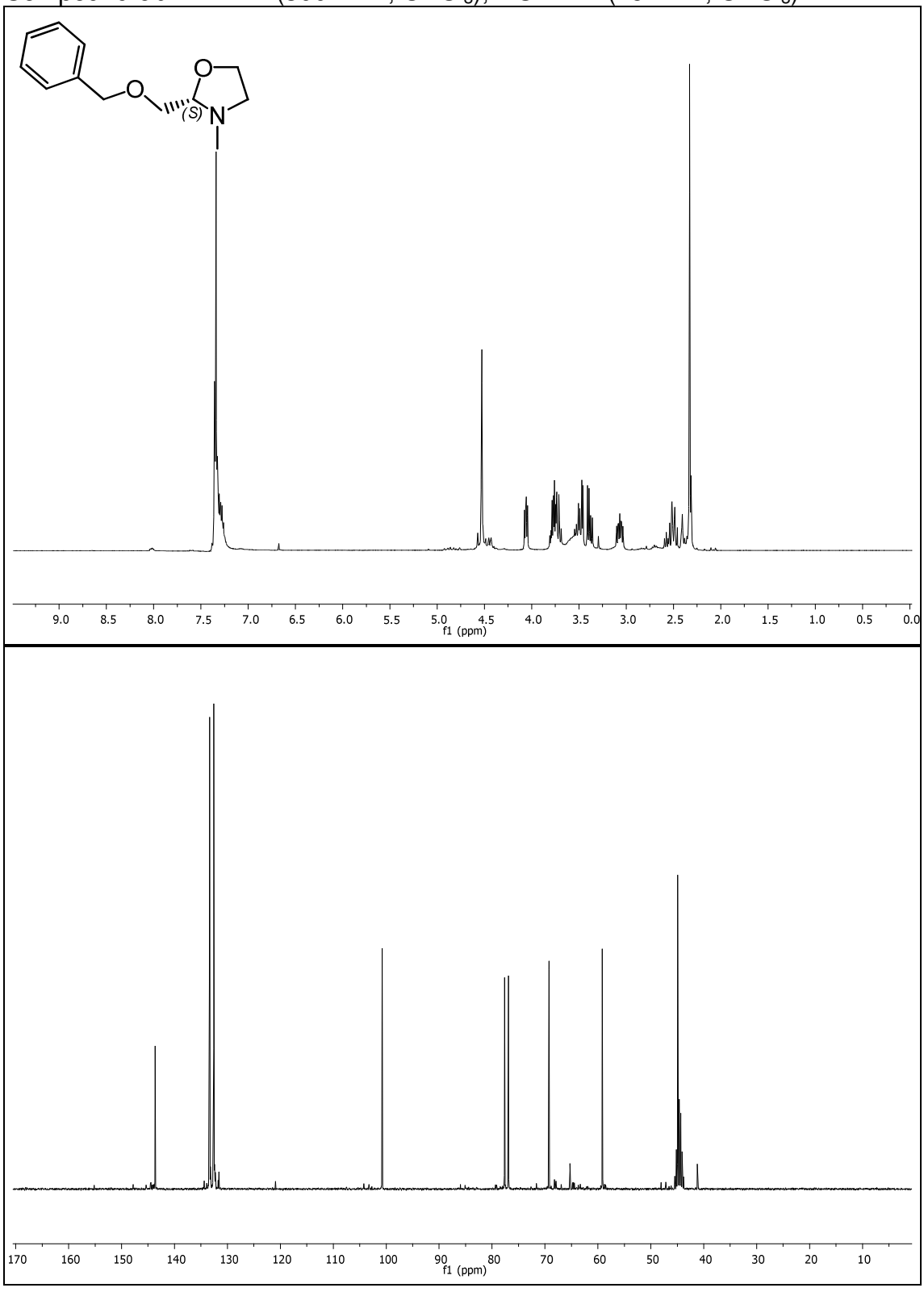
5.5. Supporting Information

Spectra.

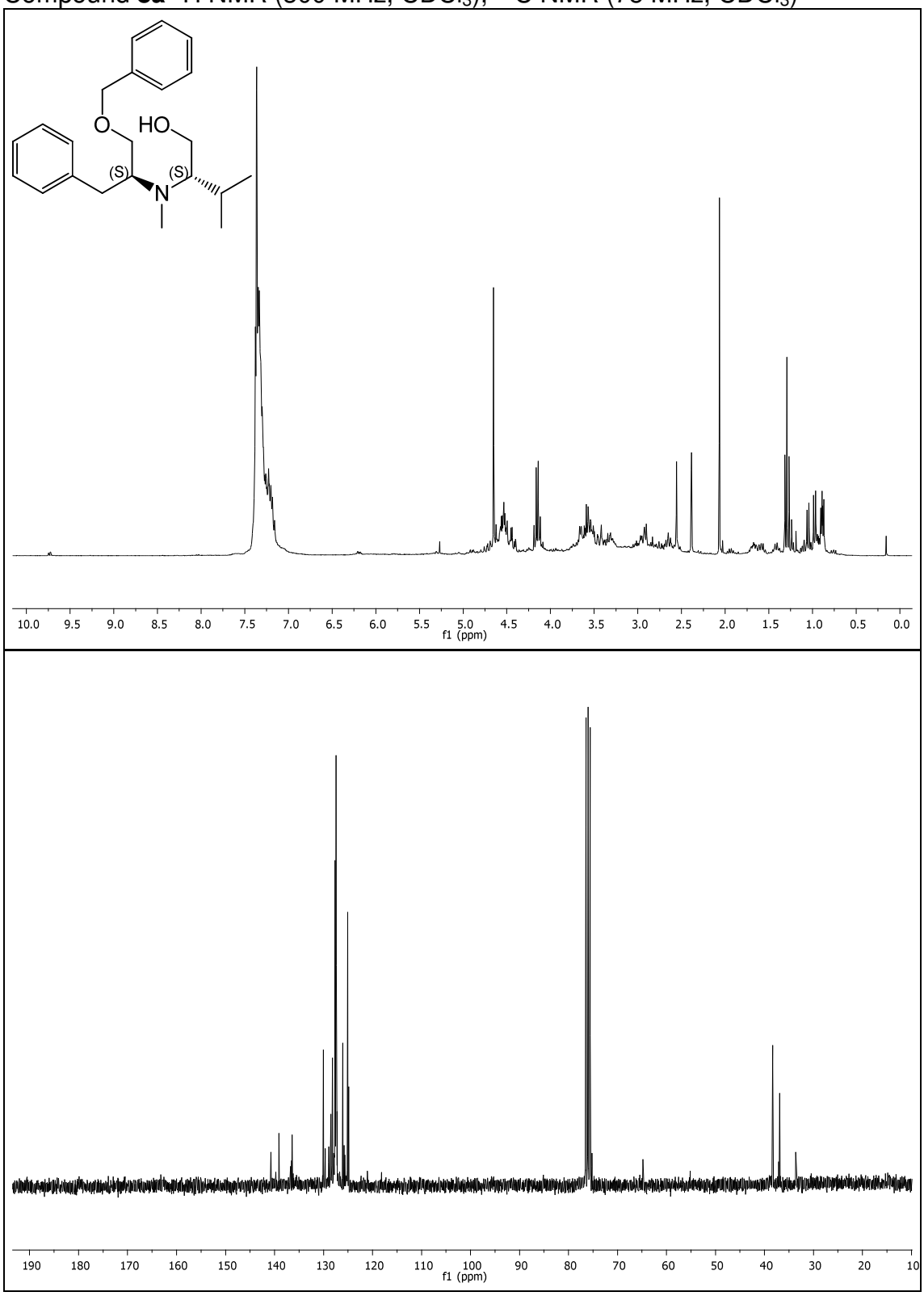
Compound **6a** ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$)



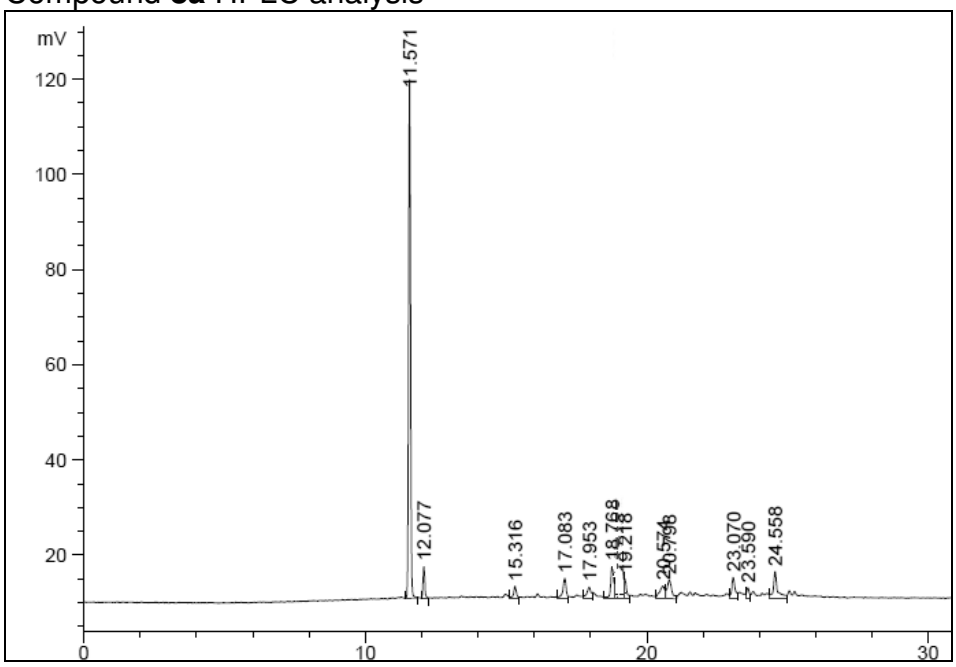
Compound **6b** ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$)



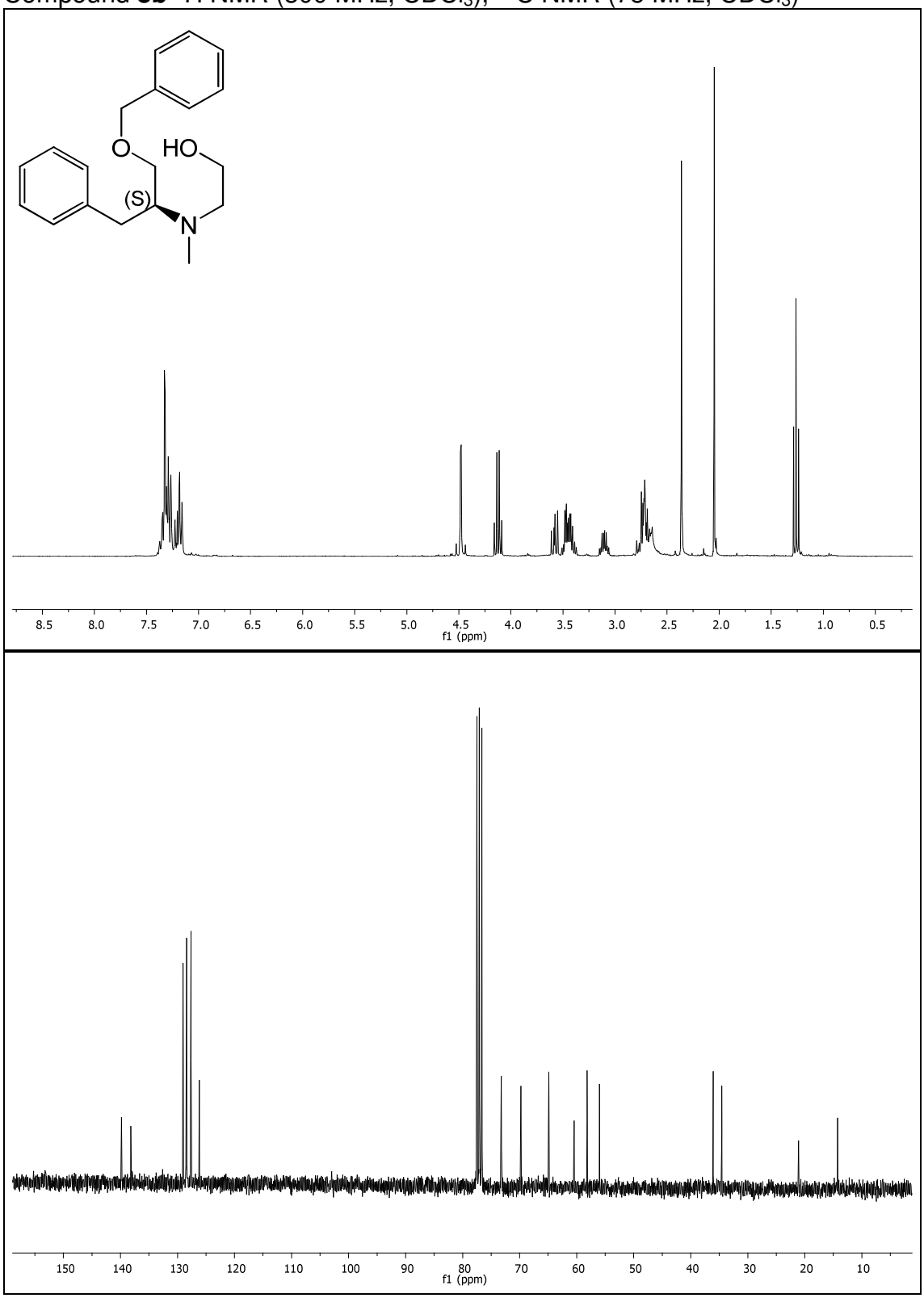
Compound **8a** ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$)



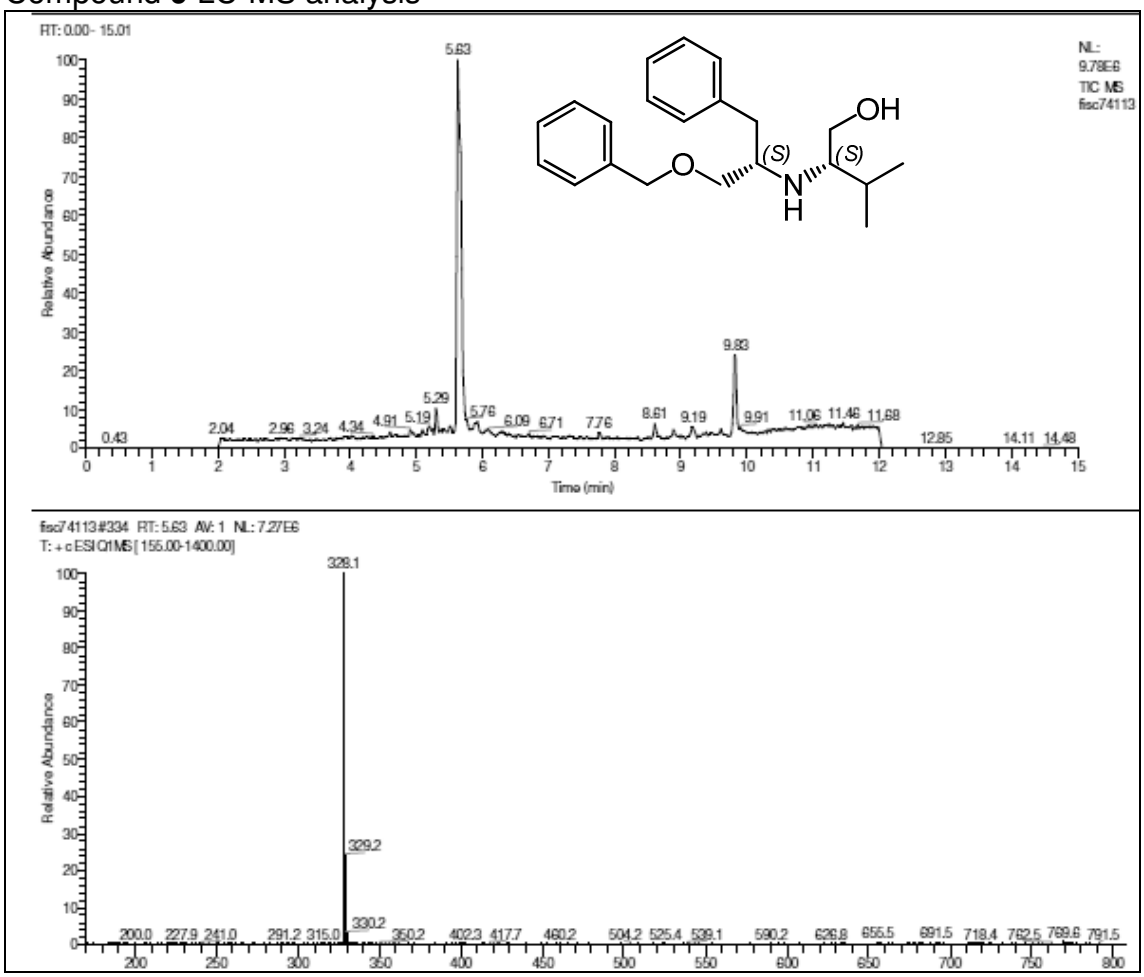
Compound **8a** HPLC analysis



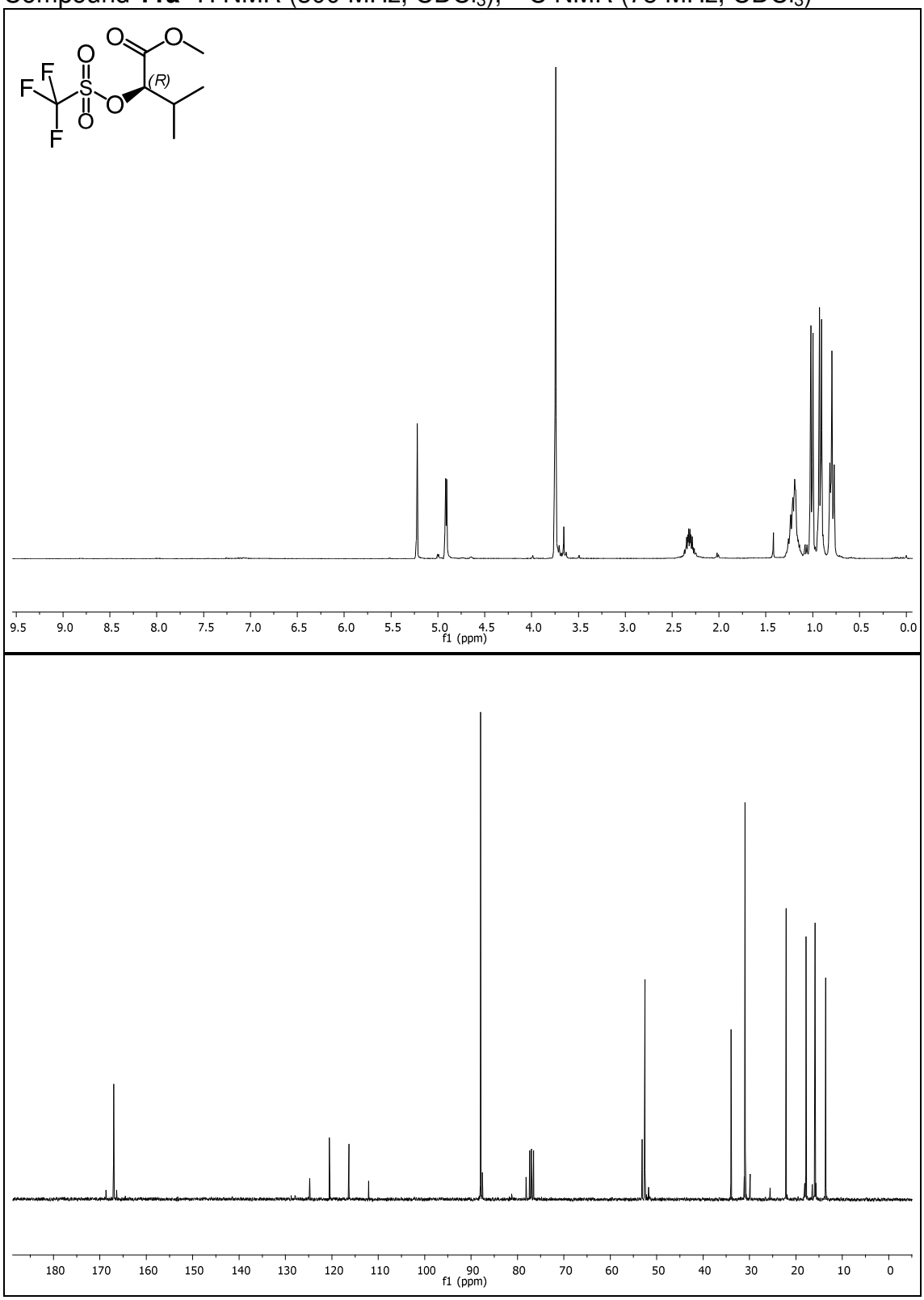
Compound **8b** ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$)



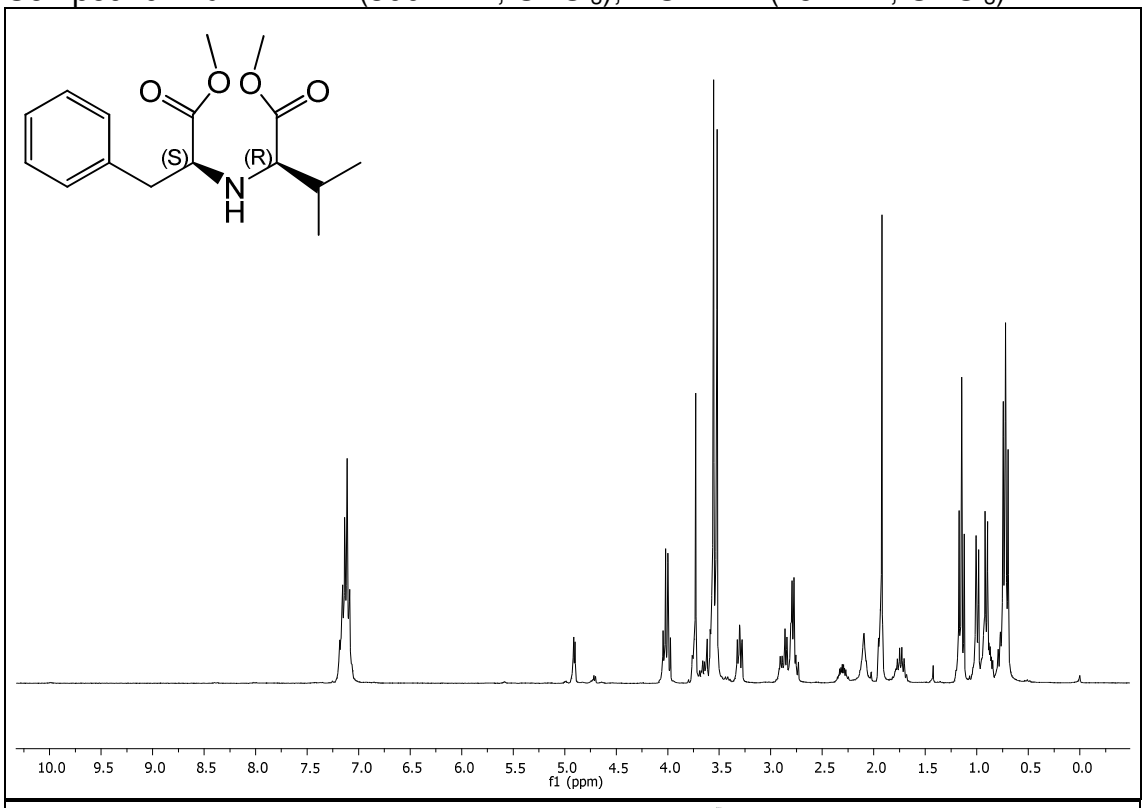
Compound **9** LC-MS analysis

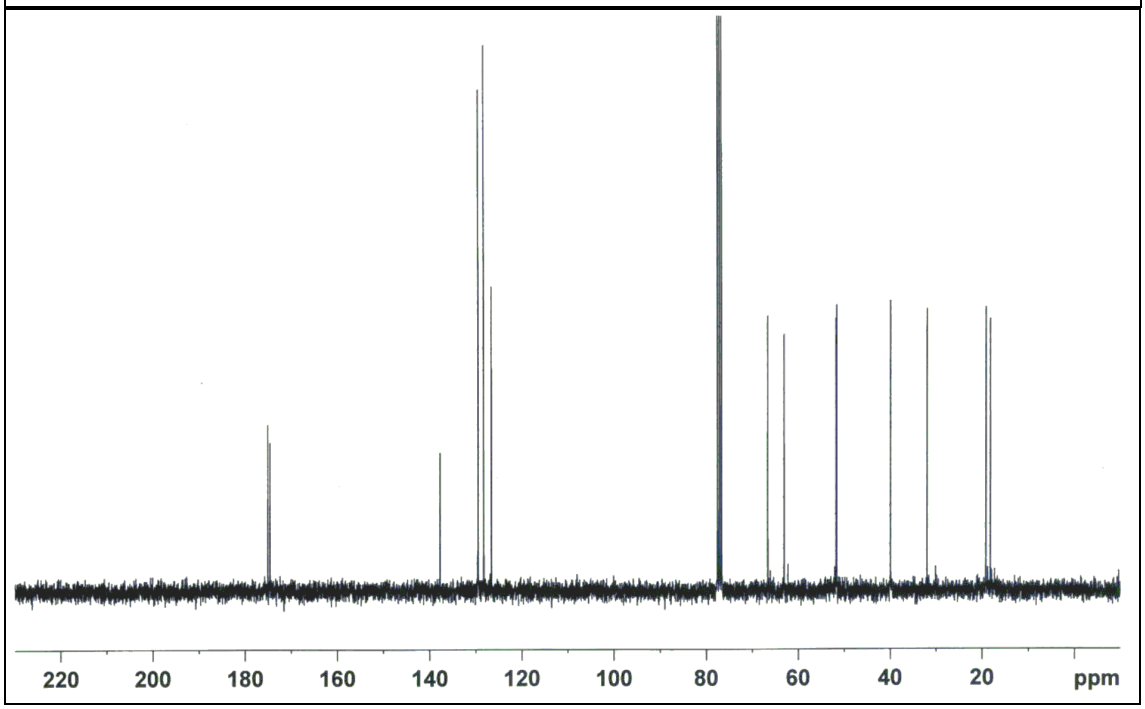


Compound **11a** ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$)

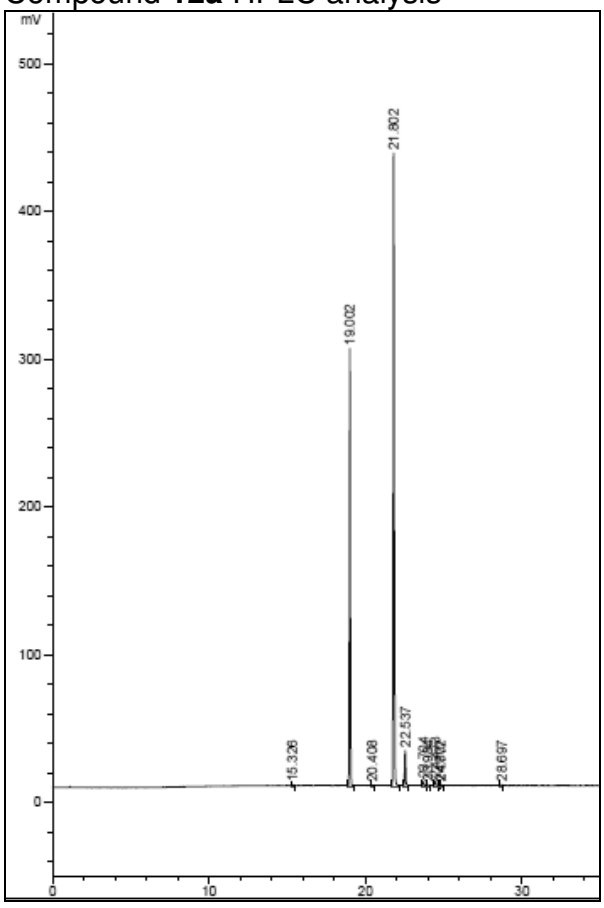


Compound **12a** ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$)

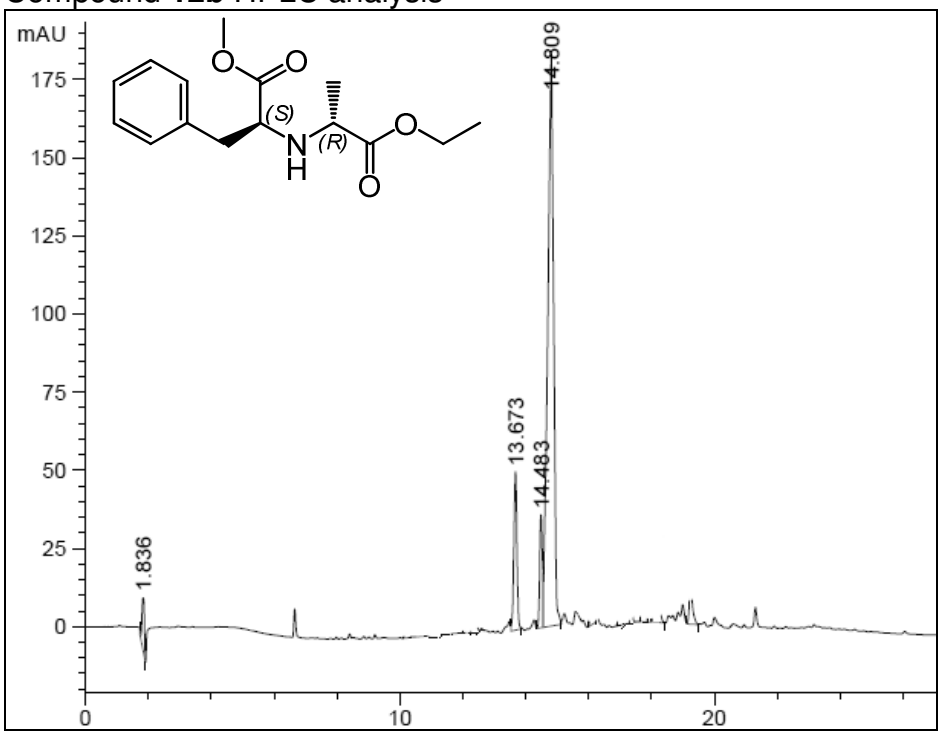




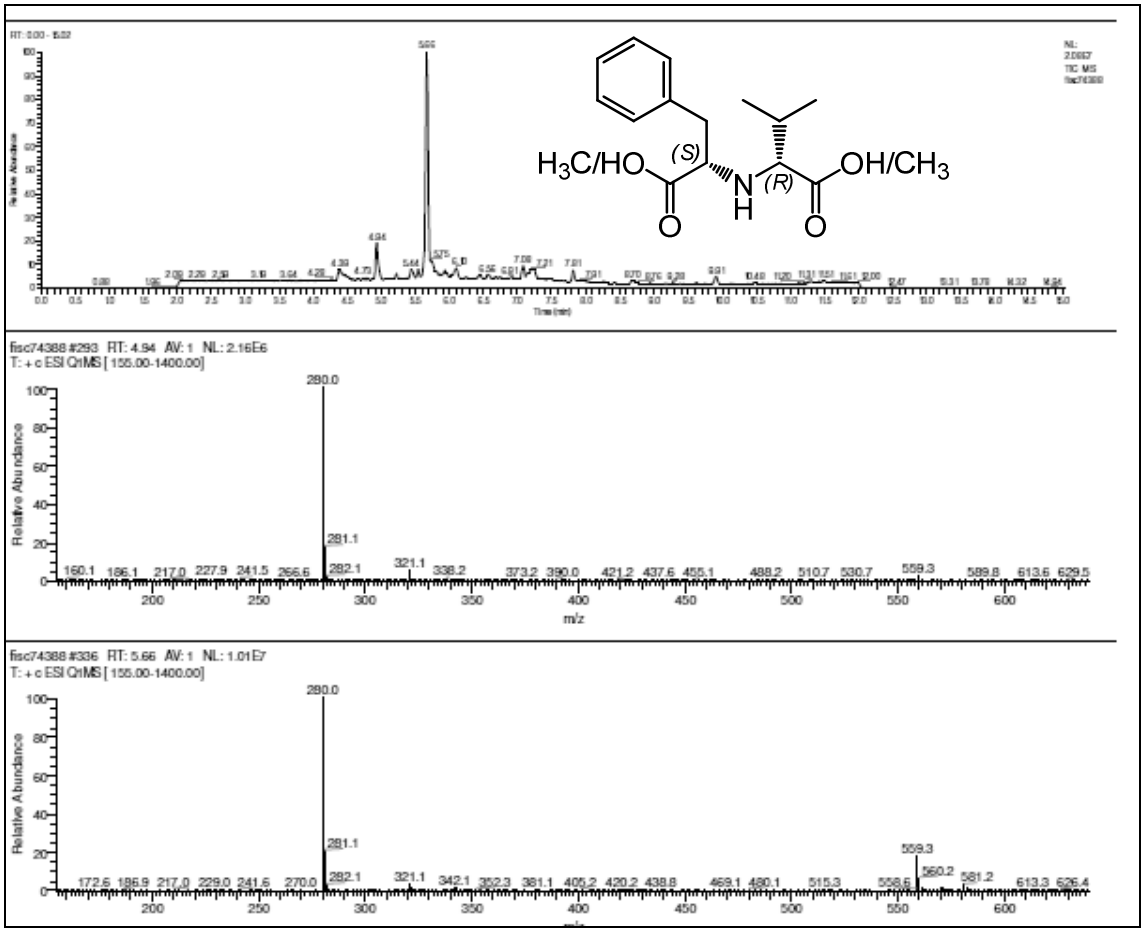
Compound **12a** HPLC analysis



Compound **12b** HPLC analysis



Compound **13a** LC-MS analysis



Inhibition of human angiotensin-converting enzyme (ACE-2) at 10 μM final concentration.

Compound	% inhibition of control values*
8a	3
8b	4
12a	0
12b	3
13b	5

*reference compound: Ac-GG-26-NH_2

5.6. References

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6. Summary

Chapter 1 reviews the copper- and palladium-mediated aryl-*N* bond formations used in the synthesis of biological active compounds. Examples using the palladium-catalysed Buchwald-Hartwig method, the copper-mediated Ullmann-Goldberg method and the Cham-Lan method were compared concerning their reaction conditions, their scope for a wide spectra of substituent and functional groups and their scale-up prospects. The discussed examples show that palladium-catalysed reactions are favoured for large scale applications and tolerate sterical demanding substituents on the coupling partners better than Cham-Lan conditions. Cham-Lan *N*-arylations are particular mild and do not require the addition of ligands, which facilitates the work-up. However, reaction times can be very long. Ullmann- and Buchwald-Hartwig-type reactions have been used in intramolecular reactions giving access to complex ring structures. All three *N*-arylation methods have specific advantages and disadvantages that should be considered when selecting the reaction conditions for a desired *C-N* bond formation in the course of a total synthesis or drug synthesis.

Chapters 2, 3 and 4 deal with the modulation of the membrane located ABC-transporter ABCG2, which is an effective tool for the treatment of multi-drug resistant cancer cells. Starting from the recently discovered tariquidar-derived selective ABCG2 modulator (lead structure), in chapter 2 different fluorescent labelled ABCG2 modulators were synthesised. The modified modulators show emission in the red part of the spectrum and reveal quantum yields up to 31.2 %. Moderate potency was obtained by the replacement of the tetrahydroisoquinoline part of the tariquidar-derived lead-structure by a pyrylium label. All synthesised derivatives showed selectivity for ABCG2 over ABCB1. Studies at the physiological expressed ABCG2 concentrations on rat brain capillaries revealed that the parent lead structure is also influencing the rat ABCG2 transporter, confirming the results of previous *in vitro* experiments with MCF-7/Topo cells and Hoechst 33342 as substrate. Due to lower affinity of the fluorescent labelled compounds, this could not be proven for the fluorescent modulators.

Chapter 3 shows structural variations of the lead structure to improve the drug-like properties and the potency. Incubation in mouse plasma proved the stability

of the ester functionality, but one of the peptide bonds was cleaved, also if N-methylated. Structural changes of different parts of the molecule decreased the potency only moderately as long as there was no negative charge introduced under physiological conditions. The phenylbenzamide group turned out to be essential for the interaction with the transporter.

Chapter 4 discribes photochromic tariquidar-derived compounds as potential ABCG2 modulators which may be useful as tool in mechanistic investigation. The synthetic approach was focused on the replacement of characteristic structural moieties of a potent and selective ABCG2 inhibitor by photochromic dithienylethenes. Photochemical properties, thermostability, cycle performance, photostationary state and photostability at assay conditions are suitable for all four compounds for pharmacological *in vitro* investigations.

Chapter 5 reports the synthesis of secondary amines containing two carbon stereocentres in both α-positions. These are typical substructure of ACE inhibitors and other biological active compounds. The stereoselective synthesis was aspired in different approaches, but industrial applications still mostly apply the separation of diastereomers. Here, two synthetic methods, a S_N2 reaction and an oxazolidine mediated reaction sequence, were compared concerning their selectivity. The oxazolidine intermediate-mediated amine formation gave only one entantiomer for the substituted secondary amine. The synthesised compounds showed weak or no cytotoxicity. Selected compounds were tested as inhibitors of ACE-2, but revealed no biological activity.

7. Zusammenfassung

Kapitel 1 vermittelt einen Überblick über die kupfer- und palladiumvermittelte Aryl-*N* Bindungsbildung die bei den Synthesen biologisch aktiver Substanzen verwendet wird. Beispiele, die die palladiumkatalysierte Buchwald-Hartwig Methode, die kupfervermittelte Ullmann-Goldberg Methode und die Cham-Lan Methode verwenden, wurden bezüglich ihrer Reaktionsbedingungen, ihrer Toleranz gegenüber verschiedener Substituenten und funktionellen Gruppen und ihrer Up-Scale Möglichkeiten verglichen. Die diskutierten Beispiele zeigen, dass die palladiumkatalysierten Reaktionen für Anwendungen im größeren Maßstab bevorzugt werden und dass sie gleichzeitig sterisch anspruchsvolle Substituenten an den Kupplungspartern besser tolerieren als bei Cham-Lan Bedingungen. Cham-Lan *N*-Arylierungen sind besonders mild und benötigen keine Liganden, was die Aufarbeitung deutlich vereinfacht. Jedoch können die Reaktionszeiten sehr lang sein. Ullmann- und Buchwald-Hartwig Reaktionen wurden in intramolekularen Reaktionen zum Aufbau von komplexen Ringstrukturen verwendet. Alle drei *N*-Arylierungsmethoden haben ihre spezifischen Vor- und Nachteile. Dies spielt bei der Wahl der Reaktionsbedingungen für die gewünschte *C-N* Bindungsbildung in der Planung einer Totalsynthese oder einer Wirkstoffsynthese eine wichtige Rolle.

Kapitel 2, 3 und 4 beschäftigen sich mit der Hemmung des membranständigen ABC-Transporters ABCG2, die ein effektives Instrument für die Behandlung multiwirkstoffresistenter Krebszellen ist. Ausgehend von dem kürzlich entdeckten, von Tariquidar abgeleiteten, selektiven ABCG2 Inhibitor (Leitstruktur) wurden in Kapitel 2 verschiedene fluoreszenzmarkierte ABCG2 Inhibitoren hergestellt. Die modifizierten Modulatoren zeigten Emission im roten Bereich des Lichtspektrums und ergaben Quantenausbeuten bis zu 31,2 %. Moderate biologische Aktivität wurde für die von Tariquidar abgeleitete Leitstruktur erhalten, die an Stelle des Tetrahydroisoquinolinteils ein Pyryliumlabel besitzt. Alle hergestellten Derivate zeigten eine gute selektive Wirksamkeit für ABCG2 gegenüber ABCB1. Untersuchungen an ABCG2 in physiologisch expremierten Konzentrationen an Rattengehirnkapillaren machten deutlich, dass die ursprüngliche Leitstruktur auch die ABCG2

Transporter in Ratten beeinflusst und somit die Ergebnisse der vonangehenden *in vitro* Experimente mit MCF7/Topo Zellen und Hoechst 33342 als Substrat bestätigten. Auf Grund der niedrigeren Affinität der fluoreszenzmarkierten Verbindungen konnte dies für die fluoreszenten Modulatoren nicht bewiesen werden.

Kapitel 3 zeigt strukturelle Veränderungen der Leitstruktur zur Verbesserung potentieller Wirkstoffeigenschaften und der Wirksamkeit. Inkubation mit Mäuseplasma bewies die Stabilität der Esterfunktionalität, jedoch wurde eine der Peptidbindungen, trotz Methylierung gespalten. Strukturelle Veränderungen an verschiedenen Teilen des Moleküls verringerte die Wirksamkeit nur geringfügig, solange unter physiologischen Bedingungen keine negative Ladung vorlag. Die Phenylbenzamid-Funktionalität stellte sich als essentiell für die Wechselwirkung mit dem Transporter heraus.

In Kapitel 4 werden von Tariquidar abgeleitete, photochrome Verbindungen als potentielle ABCG2 Modulatoren, die nützlich für mechanistische Untersuchungen sein können, beschrieben. Die synthetische Strategie konzentrierte sich auf den Austausch charakteristischer Strukturteile eines potenten und selektiven ABCG2 Hemmers durch photochrome Dithienylethene. Die photochemischen Eigenschaften, die Thermostabilität, die Lebensdauer, der photostationäre Zustand und die Photostabilität bei den Testbedingungen zeigten für alle vier Substanzen die Eignung für pharmakologische *in vitro* Untersuchungen. Erste pharmakologische Untersuchungen zeigten eine niedrige Affinität der ringgeöffneten Isomere.

In Kapitel 5 wird die Synthese sekundärer Amine mit zwei Stereozentren in beiden α-Positionen vorgestellt. Diese Substrukturen sind typisch für ACE Hemmer und andere biologisch aktive Substanzen. Die stereoselektive Synthese wurde bereits in verschiedenen Ansätzen angestrebt, aber für industrielle Anwendungen wird meistens noch die Auftrennung der Diastereomere verwendet. Hier wurden zwei synthetische Methoden bezüglich ihrer Selektivität verglichen: Eine S_N2 Reaktion und eine Reaktionssequenz über eine Oxazolidin-Zwischenstufe. Dabei ergab die oxazolidin-vermittelte Reaktion nur ein Enantiomer für das substituierte sekundäre Amin. Die hergestellten Substanzen zeigten schwache oder keine Toxizität. Ausgewählte Verbindungen wurden als Hemmstoffe für ACE-2 getestet, zeigten aber keine biologische Aktivität.

8. Abbreviations

ABC	ATP binding cassette
ABCB1	ABC transporter B1, p-gylocoprotein 170
ABCG2	ABC transporter G2, breast cancer resistance protein
ABCP	ACB transporter expressed in placenta
Ac	Acetyl
ADHD	Attention deficit hyperactivity disorder
Aq	Aqueous
ATP	Adenosine triphosphate
ATR	Attenuated total reflection
BBB	Blood brain barrier
BCRP	Breast cancer resistance protein
BINAP	2,2´–Bis(diphenyl-phosphino)-1,1´-binaphthyl
Boc	t-Butyloxycarbonyl
BSA	Bovine serum albumine
Bu	Butyl
Calcein-AM	Calcein-acetoxymethylester
calcd	Calculated
CI	Chemical ionisation
CNS	Central nervous system
CSC	Cancer stem cell
COSY	Correlated spectroscopy
CLSM	Confocal laser scanning microscopy
DAD	Diode array detector
DCM	Dichloromethane
DEPT	Distortionless enhance-ment by polarization transfer
DIPEA	Diisopropylethylamine
DMAP	Dimethylaminopyridine
DME	1,2-Dimethoxyethan
DMEDA	Dimethylethylendiamin
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Desoxyribonucleic acid
DPPF	1,1'-Bis(diphenyl-phosphino)ferrocen
D*t*BPF	Di-*tert*-butyl phosphate
DTE	Dithienylethene
EMEM	Eagels minimum essential medium
eq	Equivalent
EI-MS	Electron-impact ionization mass spectrometry
ESI	Electronspray ionisation
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol

FACS	Fluorescence activated cell
FCS	Fetal calf serum
FTC	Fumitremorgin C
FT	Fourier transformed
HB	Hydrogen bond
HOBt	1-Hydroxy-benzotriazole
HPLC	High pressure liquid chromatography
HR-MS	High resolution mass spectrometry
5-HT	5-Hydroxytryptamin
IC_{50}	Half maximal inhibitory concentration
IR	Infrared spectroscopy
J	Coupling constant
Log P	Partition coefficient between n-octanol and water
MCF-7/Topo	Topotecan resistant human breast cancer cells
MDR	Multidrug resistance
Me	Methyl
MeOH	Methanol
MF	Molecular formula
Mp	Melting point
MMP	matrix metalloprotease
MS	Mass spectrometry
MRP	Multidrug-related protein
MXR	Mitoxantrone resistance-associated protein
MW	Molecular weight
NET	Norepinephrine transporte
NHC	*N*-heterocyclic carbene
NIR	Near infrared
NMR	Nuclear magnetic resonance
NPY	Neuropeptide Y
PBS	Phosphate buffered saline
PE	Petrol ether (hexanes)
P-gp	P-glycoprotein
Ph	Phenyl
PPAR	Peroxisome proliferators-activated receptor
R_f	Retention factor
RFU	Relative fluorescent units
RP	Reversed phase
RT	Room temperature
SAR	Structur activity relationship
SEM	Standard error of the mean
SERGPHOS	5,5′-Bis(diphenyl-phosphino)-4,4′-bi-1,3-benzodioxole
SNP	Single nucleotide polymorphism
S_N2	nucleophilic substitution type 2
TBTU	O-Benzotriazol-1-yl-N,N,N',N'-tetramethyl-uroniumtetrafluoroborate

TFA	Trifluorous acetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMD	Trans membrane domaines
TMEDA	Tetramethyl-ethylendiamin
Topo	Topotecan
t_r	Retention time
UV	Ultraviolett
Vis	Visible
XANTPHOS	4,5-Bis(diphenyl-phosphino)-9,9-dimethylxanthene

9. Appendix

Curriculum Vitae

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Education

10/2007-01/2011	Dissertation: “New Inhibitors for the ABCG2 Transporter”, University of Regensburg
01/2007-09/2007	Diploma thesis: “Synthesis and Test of New ABCG2 Transporter Inhibitors”
10/2002-09/2007	Studies of Chemistry and Medicinal Chemistry, University of Regensburg

Professional Experience/Internships

01/2007-01/2011	Graduate Student (PhD), Institute of Organic Chemistry, University of Regensburg (advisor: Prof. Dr. Burkhard König)
08/2006-09/2006	Student trainee, RUAG Ammotec GmbH, Fürth, Germany
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08/2002-09/2002	Student trainee, H. C. Starck Ceramics GmbH & Co. KG, Selb, Germany
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Teaching Experience

10/2007-12/2010 Teaching assistant in laboratory courses for chemistry, biology and biochemistry, supervisor for bachelor-, master- and diploma students

Poster Presentations

C. Fischer, Kuzmanović N., M. Kühnle, A. Buschauer, G. Bernhardt, B. König, "Fluorescent and photo-switchable ABCG2 modulators", 5th Summer School Medicinal Chemistry, Regensburg 2010

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C. Fischer, M. Egger, M. Kühnle, A. Buschauer, G. Bernhardt, B. König, "Stability studies of an ABCG2 modulator derived from tariquidar", Wissenschaftsforum Chemie, Frankfurt/Main 2009

C. Fischer, M. Kühnle, A. Buschauer, G. Bernhardt, B. König, "Fluorescent labelled ABCG2 inhibitors derived from tariquidar", Frontiers in Medicinal Chemistry, Heidelberg 2009

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C. Fischer, M. Egger, M. Kühnle, A. Buschauer, G. Bernhardt, B. König, "Synthesis of selective ABCG2 inhibitors derived from tariquidar", Frontiers in Medicinal Chemistry, Regensburg 2008

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Fischer, C., König, B., “Palladium- and copper-mediated N-aryl bond formation reactions for the synthesis of biological active compounds”, *Beilstein J. Org. Chem.*, **2011**, *7*, 59-74.

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