Inhibition of ABC Transporters Associated with Multidrug Resistance

Dissertation

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1. New Tariquidar Analogues: Synthesis by Cu(I)-catalyzed N/O-Aryl Coupling and Inhibitory Activity against the ABCB1 Transporter [i]

Less lipophilic and better water soluble tariquidar analogues were synthesized from one central anthranilic acid derived building block via Cu(I)-catalyzed N/O-arylation reactions.[ii] The compounds were tested for their inhibitory activity against the ABCB1 transporter in a flow cytometric calcein-AM efflux assay.[iii] A correlation between their calculated log P values and their activities was observed, with the more lipophilic analogues being as potent as the reference substance tariquidar.

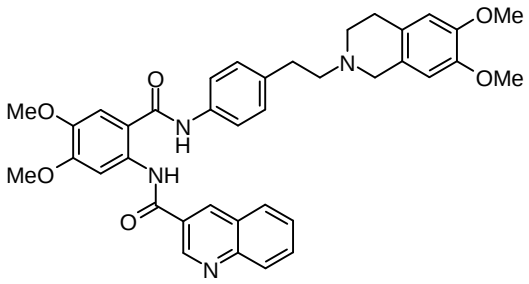
[i] M. Egger, X. Li, C. Müller, G. Bernhardt, A. Buschauer, B. König, *Eur. J. Org. Chem.* **2007**, 2643-2649.
[ii] The synthetic route to bromo tariquidar **3** was developed together with Xuqin Li who prepared N-Boc-protected bromo anthranilic acid **6** as well as the tariquidar analogue **17**.
[iii] The flow cytometric calcein-AM efflux assay was carried out by Christine Müller at the Institute of Pharmacy, University of Regensburg.

1.1. Introduction

The *mdr1* gene product ABCB1 (p-glycoprotein 170), a member of the ABC transporter family of transmembrane proteins, prevents the entry of a vast variety of structurally diverse chemicals into the cell.[1] While protection of the organism against potentially toxic compounds is an important biological function, ABCB1 may also play a critical role in drug treatment. The efflux of cytostatics due to (over)expression of ABC transporters such as ABCB1 is a major limitation in cancer chemotherapy (classical multidrug resistance, MDR, of tumor cells).[1-6] In addition to their contribution to drug resistance, these transporters are highly expressed in the endothelial cells of brain capillaries and represent important components of the blood brain barrier (BBB), preventing the entry of a broad variety of xenobiotics, including anticancer drugs, such as vinca alkaloids, anthracyclines, epipodophyllotoxins and taxanes, into the central nervous system. This leads to very low drug concentrations in the brain and plays a crucial role in the clinical resistance of malignant brain tumors to chemotherapy.[7]

In order to improve drug uptake into the brain, several studies explored the possibility of inhibiting ABCB1 in the capillaries of the BBB.[4-8]. We recently demonstrated that coapplication of the 2nd generation ABCB1 inhibitor valspodar with the anticancer drug paclitaxel, an ABCB1 substrate, increased brain levels of the cytostatic in mice by a factor of 6-8 compared to the treatment with paclitaxel alone.[8] Moreover, in nude mice bearing orthotopically growing human glioblastoma the combination therapy led to a decrease in tumor volume by 90%, whereas application of paclitaxel alone was ineffective. The antitumor effect in vivo could be clearly attributed to increased paclitaxel levels in the brain as a result of inhibited ABCB1-mediated transport at the BBB.[8] However, paclitaxel levels also increased in liver, kidneys and plasma compared to the control, so that systemic paclitaxel toxicity became dose-limiting with valspodar, due to modulation of ABCB1 in liver, kidneys and bone marrow. Using the more potent ABCB1 inhibitor tariquidar (**1**, Figure 1), a higher brain/plasma ratio of paclitaxel was detected in mice.[9] However, despite of high tariquidar concentrations in the brain, paclitaxel brain levels did not increase compared to the valspodar group.[9] The latter result might be explained by the high lipophilicity of tariquidar resulting in its accumulation in the lipid compartment of the brain. Therefore, tariquidar might reach its target, the ABCB1 transporter, at suboptimal concentration. To investigate this

hypothesis we started a project to develop better water soluble tariquidar analogues with more favourable pharmacokinetic properties.



tariquidar (**1**)

Figure 1. Structure of tariquidar (**1**).

The reported activity data of known tariquidar derivatives suggested that modifications of the methoxy groups of the central anthranilic amide are likely to be tolerated. Our retrosynthetic approach uses bromo tariquidar **3** as a precursor that is converted by transition metal catalyzed N- or O-aryl coupling into tariquidar derivatives with different overall polarity (Figure 2). While the traditional Cu-mediated Ullmann coupling reactions required high temperatures and stoichiometric amounts of copper,[10] far more efficient methodologies using catalytic amounts of palladium or copper salts have been developed for the coupling of amines to aryl halides.[11] In most cases though, the reported reaction conditions were optimized for rather simple substrate combinations. Especially for the palladium chemistry the proper choice of the catalyst system is crucial for the success of the reactions,[12] and the combination of palladium source, ligand, their ratio, base and substrate might be very sensitive to variations.[13] We have therefore tested in this work a series of typical palladium and copper catalyzed N-arylation conditions for their application on tariquidar.

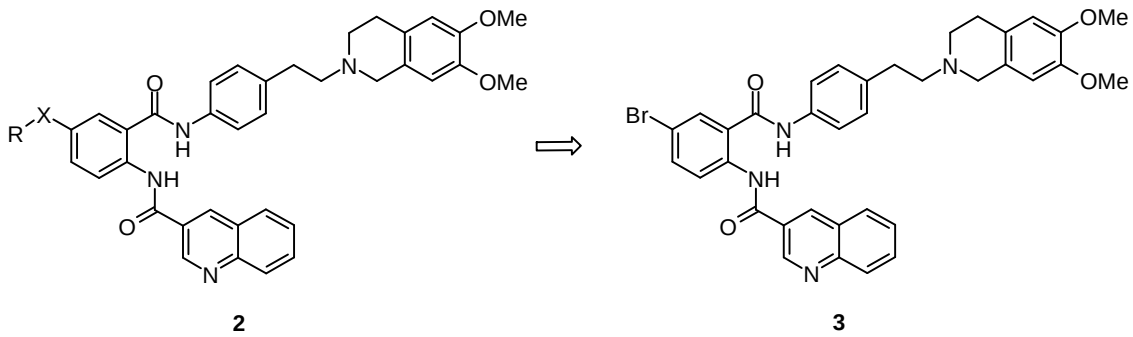
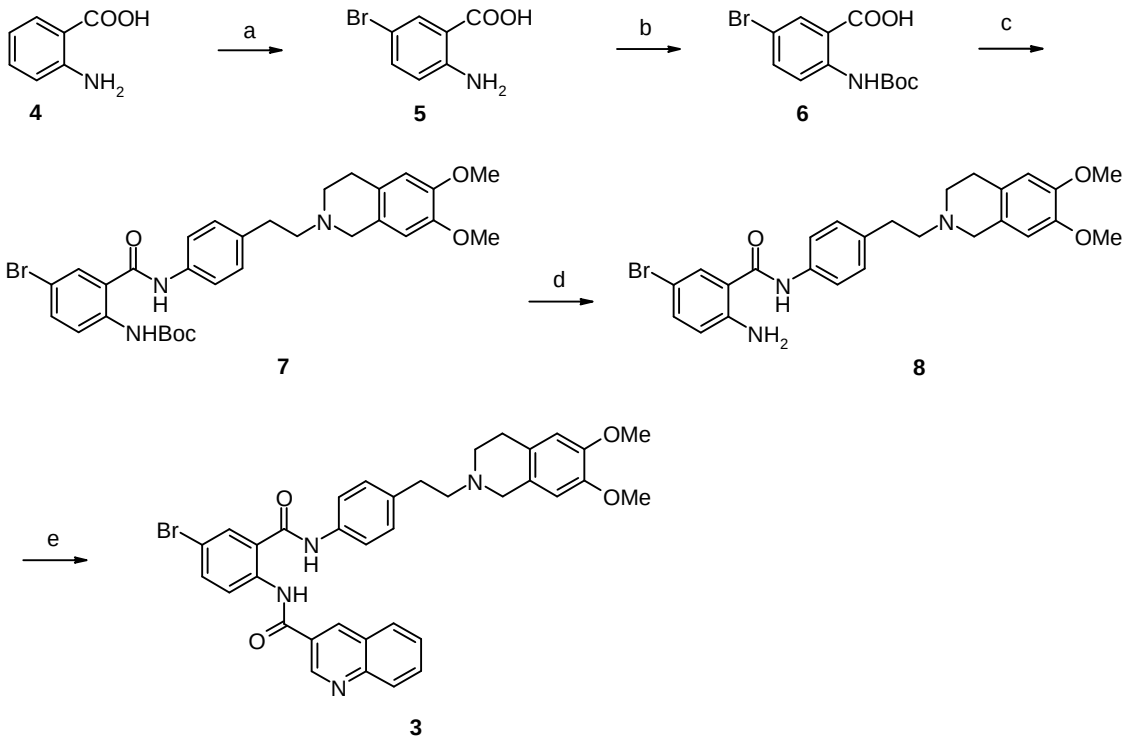


Figure 2. Synthesis of tariquidar derivatives **2** by N- or O-aryl coupling; X = O, N; R = alkyl

1.2. Results and Discussion

Synthesis. The preparation of the bromo tariquidar analogue **3** followed the parent synthesis[14] in coupling the upper aniline part and the lower quinoline part to substituted anthranilic acid as the central moiety. 2-Amino-5-bromobenzoic acid (**5**) was obtained by bromination of anthranilic acid (**4**) with [bmim]Br_3 according to the procedure described in literature in good yield.[15] Boc-protection highly improved the solubility of the intermediate **7** and was crucial for the efficiency of the subsequent coupling reaction. After deprotection and acylation with quinoline-2-carbonyl chloride the bromo tariquidar analogue **3** was obtained in an overall yield of 38% over five reaction steps (Scheme 1).



Scheme 1. Synthesis of bromo tariquidar **3**. Reagents and conditions: a) [bmim]Br_3, CH_2Cl_2, room temp., 40 min, 95%; b) Boc_2O, Na_2CO_3, CH_2Cl_2, room temp. to 40 °C, 60%; c) $ArNH_2$, HBTU, HOBt, DIPEA, CH_2Cl_2, 0 °C to room temp., 24 h, 85%; d) HCl/Et_2O, CH_2Cl_2, 0 °C to room temp., 95%; e) quinoline-3-carbonyl chloride, NEt_3, CH_2Cl_2/DMF, room temp., 70 h, 82%.

A primary (**9**), a secondary cyclic (**10**) and a secondary acyclic (**11**) amine were selected for N-aryl coupling reactions to probe different amine types and obtain tariquidar analogues with increased solubility and decreased lipophilicity (Figure 3).

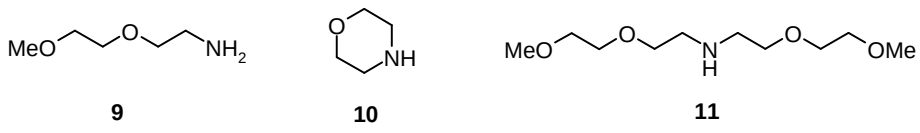
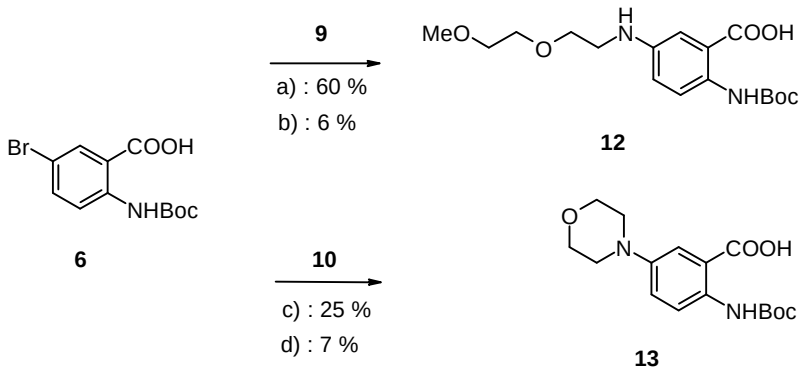


Figure 3. Amines for N-aryl coupling reactions with compound **3**.

To compare different catalyst systems the bromo tariquidar precursor **6** was allowed to react with morpholine (**10**) and the primary amine **9** (Scheme 2) using copper and palladium salts and the ligand systems given in Figure 4. The ligands were adopted from procedures described in literature for the coupling of aryl bromides and primary and secondary aliphatic amines, respectively.[12,16-18]



Scheme 2. Model reactions with aryl bromide **6**. Reagents and conditions: a) CuBr×Me_2S (20 mol-%), **L2** (40 mol-%), K_3PO_4, DMF, 90 °C, 24 h; b) $Pd_2(dba)_3$ (2.5 mol-%), **L4** (4.5 mol-%), NaO^tBu, toluene, 90 °C, 24 h; c) CuI (20 mol-%), **L3** (40 mol-%), K_3PO_4, DMSO, 90 °C, 24 h; d) $Pd_2(dba)_3$ (1 mol-%), **L5** (4 mol-%), K_3PO_4, toluene, 90 °C, 24 h

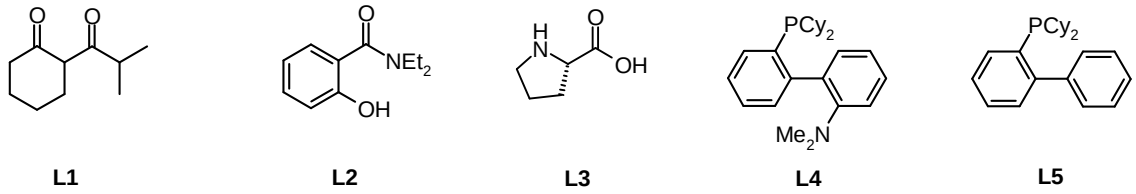
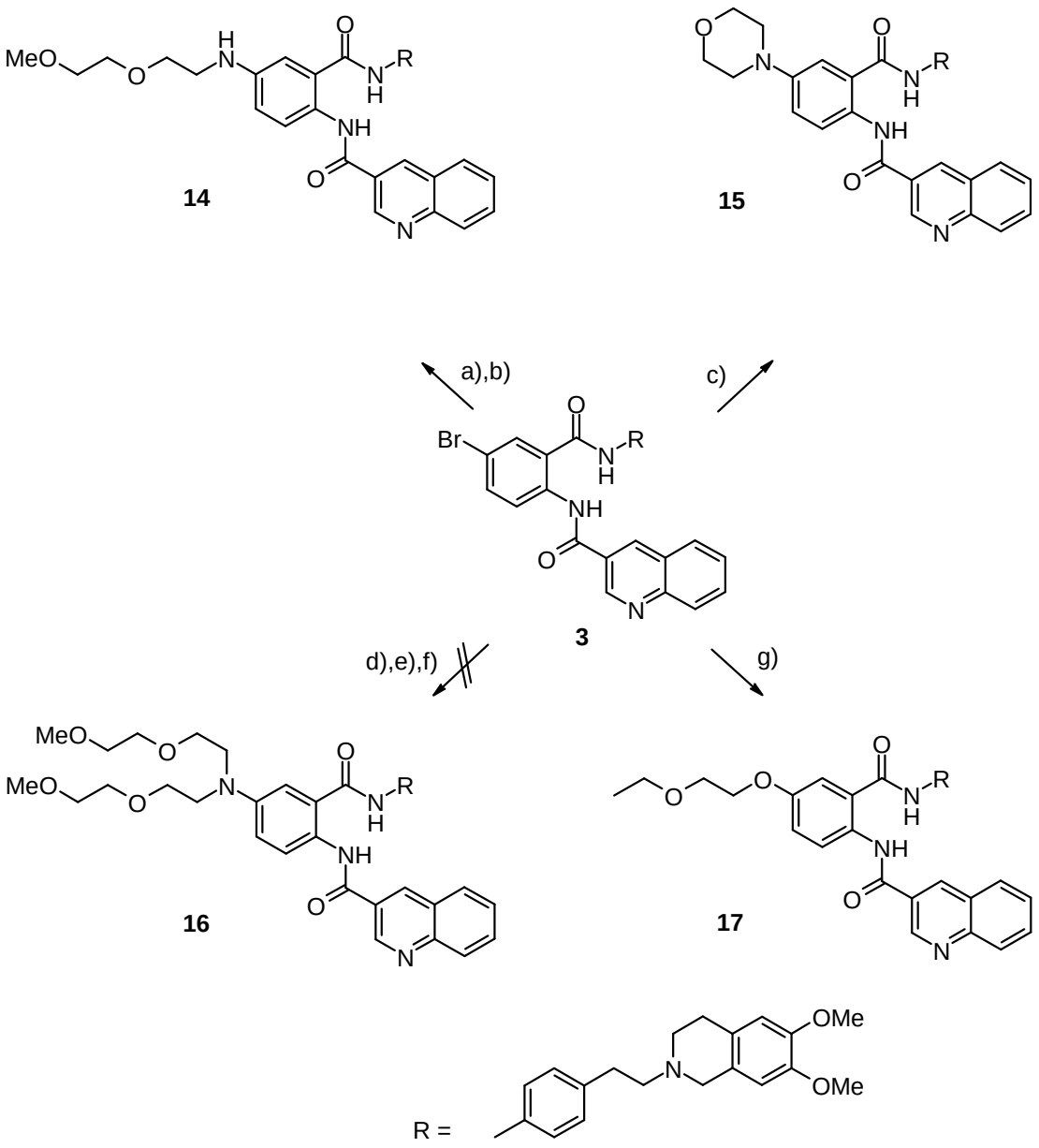


Figure 4. Ligands used for N-aryl coupling reactions.

The *para*-amino substituent in compound **6** disfavours the N-aryl substitution reaction.[19] While the copper-catalyzed reaction for the primary amine still gave moderate coupling yields, the isolated product amounts from the palladium-catalyzed reactions were disappointing. Another drawback of the palladium-catalyzed reactions is the typical use of toluene as solvent, in which compound **3** is only sparingly soluble even at higher temperatures.

Compound **3** was coupled with primary amine **9** using CuI and N,N-diethylsalicylamide (**L2**) or 2-isobutyrylcyclohexanone (**L1**) as ligand,[16,20] yielding tariquidar analogue **14**, which shows improved solubility, in moderate yields. Morpholine (**10**) was introduced as substituent into the tariquidar skeleton in good yields with L-proline (**L3**) as ligand following the conditions recently described by Ma.[18] The most critical substrates for these kinds of coupling reactions are secondary acyclic amines. Several of the above discussed conditions were tested, but none of them gave the desired product in

satisfactory and isolatable amounts (Scheme 3). The use of **3** in a CuCl-catalyzed O-aryl coupling was demonstrated in the reaction with alcohol **19** in an analogous coupling reaction.



Scheme 3. Ullmann-type coupling reactions of **3** with amines **9**-**11**. Reagents and conditions: a) CuBr×Me_2S (20 mol-%), **L2** (40 mol-%), K_3PO_4, DMF, 90°C, 32% (66% of **3** recovered); b) CuI (40 mol-%), **L1** (75 mol-%), K_3PO_4, DMF, 90°C, 40% (41% of **3** recovered); c) CuBr×Me_2S (20 mol-%), **L3** (40 mol-%), K_3PO_4, DMSO, 90°C, 75%; d) CuBr×Me_2S (20 mol-%), **L3** (40 mol-%), K_3PO_4, DMSO, 90°C; e) CuI (40 mol-%), **L1** (80 mol-%), K_3PO_4, DMF, 90°C; f) $Pd_2(dba)_3$ (1.5 mol-%), **L4** (1.5 mol-%), NaO^tBu, toluene, 90°C; g) i) **19**, Na; ii) CuCl (20 mol-%), DMF, 90°C, 52% (46% of **3** recovered).

Flow Cytometric Calcein-AM Efflux Assay (ABCB1 Assay). In Kb-V1 cells calcein-AM is extruded by ABCB1 before non-specific esterases can cleave the ester bonds, and calcein is not accumulated.[21] Therefore, ABCB1 inhibitors can easily be recognized by flow cytometric determination of intracellular calcein-AM levels. Depending on the modulator concentration the change in the calcein-AM efflux or rather in the relative fluorescence intensity of the cells is measured. The assay was performed as recently described by Müller et al.[21]

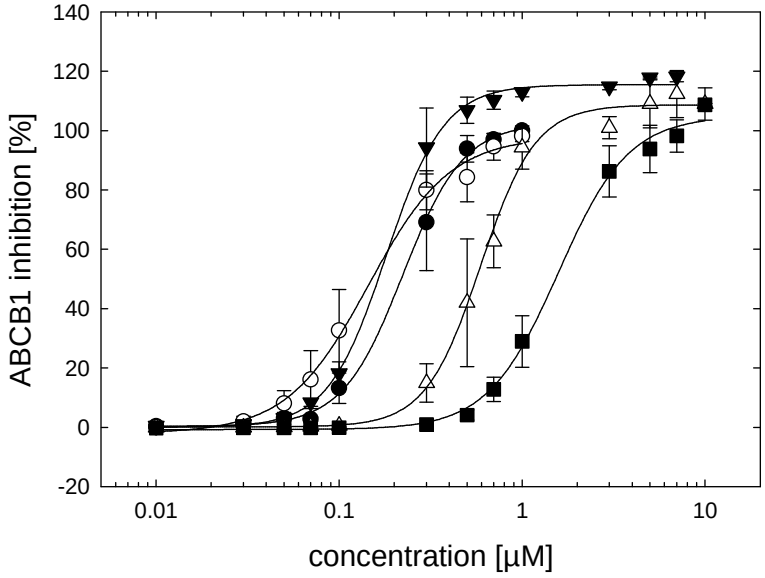


Figure 5. ABCB1 inhibition by tariquidar and the new inhibitors in dependence of their concentrations. Open circle **3**, filled triangle **17**, filled circle **tariquidar**, open triangle **15**, filled square **14**.

Table 1. Calculated properties of the new tariquidar analogues and their biological activities as determined by the calcein-AM efflux assay.

Compound	log P	IC_{50} (nM)	Efficacy (%)	Hill-Coefficient	n
Tariquidar	6.1 ± 1.1	223 ± 8	102	2.6	5
3	7.1 ± 1.0	145 ± 12	98	1.9	3
17	6.0 ± 1.0	181 ± 6	116	2.7	3
15	5.1 ± 1.0	593 ± 21	109	2.9	3
14	4.3 ± 1.1	1 575 ± 98	105	2.3	3

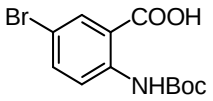
The resulting data of the new tariquidar analogues and their calculated pharmacokinetic parameters (calculations were carried out with ACD Labs software) are shown in Figure 5 and Table 1. Compared to tariquidar both the central building block **3** and the aryl ether analogue **17** show improved activities in the calcein-AM efflux assay, while the two aryl amine analogues **15** and **14** are less potent than the reference substance tariquidar in inhibiting ABCB1. The desired decrease in lipophilicity was achieved in all three derivatives of **3**. In the same order as log P decreases the activity against ABCB1 drops. It is still discussed in literature whether high lipophilicity might be an important criterion (amongst others) for the inhibition of ABC transporters.[22] The results obtained from this small series are in accordance with this hypothesis, assuming that the newly introduced side chains only affect physicochemical parameters .

1.3. Conclusion

In summary, we have shown that modern variants of the Ullmann reaction can be used to prepare tariquidar analogues from bromo tariquidar **3** and suitable amines in one step. Coupling of a primary and a secondary cyclic amine as well as an alcohol was achieved in one metal-catalyzed reaction each. The Ullmann-type coupling reactions using Cu(I) seem to be more generally applicable for these substrate combinations than the Buchwald-Hartwig-aminations using Pd(0). For the Pd-chemistry, the system of Pd source, ligand, base and solvent is probably more critical towards different substrate combinations and might need to be optimized for each reaction. The synthetic route described here allows quick access to new potential MDR modulators. The results from the calcein-AM efflux assay demonstrate that structural changes in the chosen position are indeed tolerated without considerable loss of activity. The series of new ABCB1 modulators is well suited to test the aforementioned BBB selectivity hypothesis in vivo, since the lipophilicities of the compounds range from log P 7 to 4, thereby including the value of tariquidar (log P = 6.1) on one end and that of valspodar (log P = 4.1) on the other end. Further investigations on the new tariquidar-like ABCB1 inhibitors are in progress.

1.4. Experimental Section

General: Commercial reagents and starting materials were purchased from Aldrich, Fluka or Acros and used without further purification. CH_2Cl_2 was distilled from CaH_2, anhydrous DMF was purchased from Fluka. 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). The compounds were detected by UV-light (λ = 254 nm) and a solution of ninhydrine in ethanol. 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 characterisation of the signals: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, dd = double doublet. The relative number 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). Compounds **9, 11** and 2-isobutyrylcyclohexanone (**L1**) were prepared by the reported methods. The products of the model reactions (**12** and **13**) were synthesized under the conditions described above, the experimental procedures were identical with those of the final reactions.

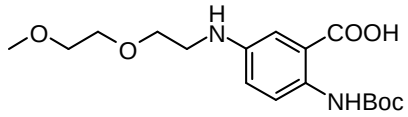


5-Bromo-2-(*tert*-butoxycarbonylamino)benzoic acid (6)

5-Bromoanthranilic acid (**5**) (4.35 g, 20 mmol) was dissolved in anhydrous dichloromethane (150 mL), followed by the addition of Na_2CO_3 (2.36 g, 22 mmol) and di-*tert*-butyl dicarbonate (4.85 g, 22 mmol). The solution was stirred at room temperature for 1 h under nitrogen atmosphere and then heated at reflux temperature for

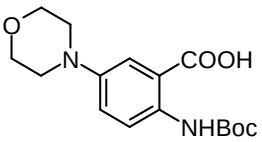
24 h. The mixture was washed with water, 1 M citric acid and brine. The organic phase was dried over Na_2SO_4, evaporated under reduced pressure and separated by column chromatography on silica gel (elution with hexane/acetone 5:1, R_f = 0.35) to provide **6** as a white solid (3.8 g, 60%).

mp = 177-178 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 9H, Boc), 7.62 (dd, ^3J = 9.1 Hz, ^4J = 2.5 Hz, 1H, H-Ar), 8.10 (d, ^4J = 2.5 Hz, 1H, H-Ar), 8.30 (d, ^3J = 9.1 Hz) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (+), 81.9 (C_{quat}), 114.3 (C_{quat}), 118.0 (C_{quat}), 121.4 (+), 134.9 (+), 137.9 (+), 142.7 (C_{quat}), 154.0 (C_{quat}), 170.0 (C_{quat}) – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3330, 2978, 1729, 1682, 1578, 1516 – UV/Vis (MeOH) λ_{max} [nm] (lg ε): 308 (3.514), 253 (4.280) – MS (–p ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 314 (100) [$(M - H^+)^-$], 316 (98) [$(M - H^+)^-$] – Elemental analysis calcd (%) for $C_{12}H_{14}BrNO$ (316.15): C 45.59, H 4.46, N 4.43; found C 45.12, H 4.55, N 4.24.



2-(*tert*-Butoxycarbonylamino)-5-[2-(2-methoxyethoxy)ethylamino]benzoic acid (12)

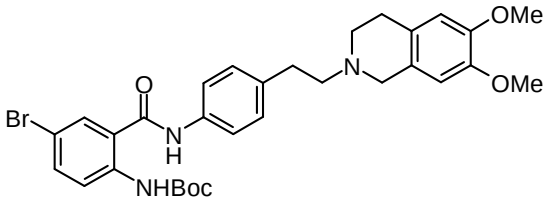
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 9H, Boc), 3.29-3.33 (m, 2H, CH_2), 3.41 (s, 3H, OCH_3), 3.56-3.60 (m, 2H, CH_2), 3.64-3.68 (m, 2H, CH_2), 3.70-3.74 (m, 2H, CH_2), 6.93 (dd, ^3J = 9.1 Hz, ^4J = 3.0 Hz, 1H, H-Ar), 7.35 (d, ^4J = 3.3 Hz, 1H, H-Ar), 8.24 (d, ^3J = 9.1 Hz, 1H, H-Ar), 9.65 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.4 (+), 44.4 (-), 59.1 (+), 69.3 (-), 70.2 (-), 71.9 (-), 80.3 (C_{quat}), 114.7 (C_{quat}), 115.2 (+),120.6 (+), 121.7 (+), 134.5 (C_{quat}), 153.1 (C_{quat}), 172.2 (C_{quat}), 176.8 (C_{quat}) – MS (CI, NH_3): *m/z* (%) = 355 (100) [MH^+].



2-(*tert*-Butoxycarbonylamino)-5-morpholinobenzoic acid (13)

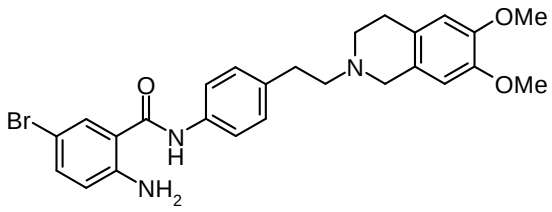
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.53 (s, 9H, Boc), 3.11-3.14 (m, 4H, 2 CH_2), 3.86-3.89 (m, 4H, 2 CH_2), 7.19 (dd, ^3J = 9.3 Hz, ^4J = 3.3 Hz, 1H, H-Ar), 7.58 (d, ^4J = 3.0 Hz,

1H, H-Ar), 8.36 (d, ^3J = 9.3 Hz, 1H, H-Ar), 9.79 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.5 (+), 50.3 (-), 67.1 (-), 80.7 (C_{quat}), 114.6 (C_{quat}) , 118.2 (+), 120.5 (+), 124.2 (+), 136.5 (C_{quat}), 145.9 (C_{quat}), 153.2 (C_{quat}), 171.8 (C_{quat}) – MS (CI, NH_3): *m/z* (%) = 323 (83) [MH^+], 266 (100) [$MH^+ – (C_4H_9)$].



***tert*-Butyl-4-bromo-2-({4-[2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]phenyl}carbamoyl)phenylcarbamate (7)**

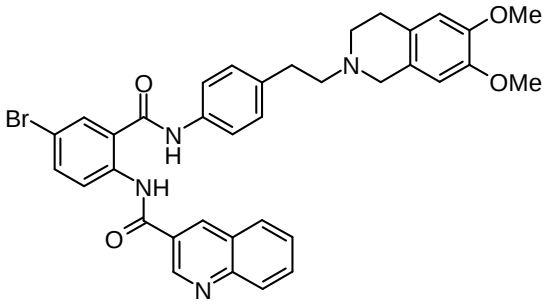
Compound **6** (886 mg, 2.8 mmol), HOBt (419 mg, 3.1 mmol) and HBTU (1166 mg, 3.1 mmol) were added to an ice-cooled solution of DIPEA (0.9 mL, 5.2 mmol) in CH_2Cl_2 (10 mL). The solution was stirred for 10 min and 4-[2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]phenylamine (812 mg, 2.6 mmol) was added in small portions. The reaction mixture was allowed to warm to room temperature and stirred for 24 h, diluted with CH_2Cl_2 and the organic phase was washed with water, saturated aqueous solution of $NaHCO_3$ (3 ×) and dried over $MgSO_4$. After evaporation of the solvent the crude product was purified by flash chromatography on silica gel (elution with EtOAc/1% NEt_3, R_f = 0.27) to obtain a pale yellow solid (1.3 g, 85%).
mp = 196 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.49 (s, 9H, Boc), 2.75-2.95 (m, 8H, 4 CH_2), 3.66 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.54 (s, 1H, H-Ar), 6.61 (s, 1H, H-Ar), 7.26-7.29 (m, 2H, H-Ar, *AA'*BB'), 7.46-7.49 (m, 2H, H-Ar, AA'*BB'*), 7.56 (dd, ^4J = 2.5 Hz, ^3J = 9.1 Hz, 1H, H-Ar), 7.68 (d, ^4J = 2.2 Hz, 1H, H-Ar), 7.74 (bs, 1H, CONH), 8.31 (d, ^3J = 9.1 Hz, 1H, H-Ar), 9.72 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $[D_6]$DMSO): δ = 27.8 (+), 28.2 (-), 32.4 (-), 50.5 (-), 55.0 (-), 55.3 (+), 55.3 (+), 59.5 (-), 80.1 (C_{quat}), 109.8 (+), 111.6 (+), 113.3 (C_{quat}), 120.9 (+), 121.3 (+), 123.4 (C_{quat}), 125.8 (C_{quat}), 126.5 (C_{quat}), 128.7 (+), 131.0 (+), 134.5 (+), 136.1 (C_{quat}), 136.5 (C_{quat}), 138.1 (C_{quat}), 146.7 (C_{quat}), 147.0 (C_{quat}), 151.9 (C_{quat}), 165.5 (C_{quat}) – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3321, 2974, 2933, 1728, 1512, 1156 – UV/Vis (MeOH) λ_{max} [nm] (lg ε): 281 (4.107), 256 (4.300) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 610 (100) [MH^+], 612 (97) [MH^+].



2-Amino-5-bromo-N-(4-{-[6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl]ethyl}-phenyl)benzamide (8)

Compound **7** (1.3 g, 2.2 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled in an ice-bath. After addition of HCl/Et_2O the mixture was stirred overnight, concentrated in vacuo and the precipitate was suspended in CH_2Cl_2 and washed with saturated aqueous solution of $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed to obtain the pure product as a white solid (1.0 g, 95%).

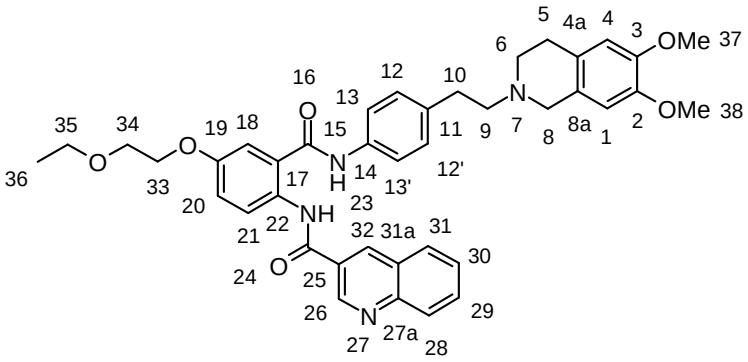
mp = 157 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.74-2.94 (m, 8H, 4 CH_2), 3.65 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.51 (bs, 2H, NH_2), 6.54 (s, 1H, H-Ar), 6.61 (s, 1H, H-Ar), 6.61 (d, ^3J = 8.8 Hz, 1H, H-Ar), 7.23-7.26 (m, 2H, H-Ar, *AA'*BB'), 7.32 (dd, ^4J = 2.2 Hz, ^3J = 8.8 Hz, 1H, H-Ar), 7.46-7.49 (m, 2H, H-Ar, AA'*BB'*), 7.56 (d, ^4J = 2.2 Hz, 1H, H-Ar), 7.65 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6, 33.4, 51.1, 55.7, 55.9, 55.9, 60.1, 107.8, 109.5, 111.4, 117.8, 119.1, 121.0, 126.1, 126.3, 129.3, 129.7, 135.3, 135.6, 136.9, 147.2, 147.6, 147.9, 166.3 – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3470, 3373, 3302, 1638, 1596, 1520, 818 – UV/Vis (CH_2Cl_2) λ_{max} [nm] (lg ε): 346 (3.366), 264 (3.808), 229 (4.067) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 510 (100) [MH^+], 512 (98) [MH^+].



N-(4-Bromo-2-{[4-(2-{6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}ethyl)phenyl]-carbamoyl}phenyl)quinoline-3-carboxamide (3)

Compound **8** (702 mg, 1.4 mmol) and NEt_3 (0.4 mL, 3 mmol) were dissolved in a mixture of CH_2Cl_2 and anhydrous DMF (10 mL/1 mL). Quinoline-3-carbonyl chloride hydrochloride (477 mg, 2.1 mmol) was added in small portions and the mixture was stirred at room temperature for 3 d. After dilution with CH_2Cl_2 the organic phase was washed with saturated aqueous solution of $NaHCO_3$ and dried over $MgSO_4$. Evaporation of the solvent and purification by flash chromatography on silica gel (elution with acetone/hexanes 1:1, 1% NEt_3, R_f = 0.27) yielded the product as a pale yellow solid (761 mg, 82%).

mp = 204-207 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.76-2.97 (m, 8H, 4 CH_2), 3.67 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.54 (s, 1H, H-Ar), 6.61 (s, 1H, H-Ar), 7.31 (d, ^3J = 8.5 Hz, 2H, H-Ar, *AA'*BB'), 7.57 (dd, ^4J = 2.2 Hz, ^3J = 8.8 Hz, 1H, H-Ar), 7.61-7.67 (m, 1H, H-Ar), 7.63 (d, ^3J = 8.5 Hz, 2H, H-Ar, AA'*BB'*), 7.78 (d, ^4J = 2.2 Hz, 1H, H-Ar), 7.81-7.87 (m, 1H, H-Ar), 7.99-8.02 (m, 1H, H-Ar), 8.16-8.19 (m, 1H, H-Ar), 8.47 (bs, 1H, CONH), 8.62 (d, ^3J = 8.8 Hz, 1H, H-Ar), 8.78 (d, ^4J = 2.2 Hz, 1H, H-Ar), 9.52 (d, ^4J = 2.2 Hz, 1H, H-Ar), 12.07 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.7 (-), 33.5 (-), 51.1 (-), 55.7 (-), 55.9 (+), 55.9 (+), 60.1 (-), 109.5 (+), 111.4 (+), 116.1 (C_{quat}), 121.0 (+), 123.1 (C_{quat}), 123.6 (+), 126.1 (C_{quat}), 126.5 (C_{quat}), 126.7 (C_{quat}), 126.9 (C_{quat}), 127.6 (+), 129.2 (+), 129.5 (+), 129.5 (+), 130.0 (+), 131.7 (+), 135.2 (C_{quat}), 135.4 (+), 136.2 (+), 137.8 (C_{quat}), 138.3 (C_{quat}), 147.2 (C_{quat}), 147.6 (C_{quat}), 148.7 (+), 149.5 (C_{quat}), 164.1 (C_{quat}), 166.1(C_{quat}) – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2932, 1677, 1597, 1512, 1464, 829 – UV/Vis (CH_2Cl_2) λ_{max} [nm] (lg ε): 287 (4.333), 238 (4.637) – HRMS calcd. for $C_{36}H_{33}BrN_4O_4$ [$M^{\cdot+}$]: 664.1685, found: 664.1692 – Elemental analysis calcd (%) for $C_{36}H_{33}BrN_4O_4$ (665.59): C 64.96, H 5.00, N 8.42; found C 64.37, H 4.99, N 8.11.

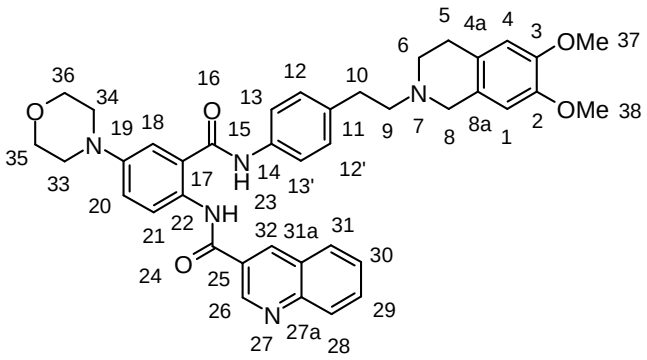


N-(2-{[4-(2-{6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}ethyl)phenyl]carbamoyl}-4-{2-ethoxyethoxy}phenyl)quinoline-3-carboxamide (17)

A 25 mL three-neck flask was evacuated, flushed with nitrogen (3 cycles) and charged with 2-ethoxyethanol (2 mL, 21 mmol) and Na (10 mg, 0.4 mmol). After the formation of H_2 had ceased, DMF (0.5 mL), CuCl (4 mg, 0.04 mmol) and compound **3** (122 mg, 0.2 mmol) were added under nitrogen. The flask was equipped with a condenser and the reaction mixture was stirred at 90 °C for 24 h. After cooling, the mixture was diluted with CH_2Cl_2, washed with water and saturated aqueous solution of $NaHCO_3$ (3×), and the organic phase was dried over $MgSO_4$. The solvent was evaporated and the remaining solid was purified by flash-chromatography on silica gel (elution with acetone/hexanes 1:1, R_f = 0.31) to obtain a white solid (64 mg, 52%).

mp = 124-126 °C – ^{1}H-NMR (400 MHz, CD_2Cl_2; HSQC, HMBC, COSY, ROESY): δ = 1.18 (t, ^3J = 7.2 Hz, 3H, CH_3, H(36)), 2.76-2.84 (m, 6H, 3 CH_2, H(9/4/5)), 2.90-2.93 (m, 2H, CH_2, H(10)), 3.51 (q, ^3J = 7.2 Hz, 2H, OCH_2, H(35)), 3.62 (s, 2H, NCH_2, H(8)), 3.64 (t, ^3J = 4.8 Hz, 2H, OCH_2, H(34)), 3.78 (s, 3H, OCH_3, H(38)), 3.78 (s, 3H, OCH_3, H(37)), 4.07 (t, ^3J = 4.8 Hz, 2H, OCH_2, H(33)), 6.54 (s, 1H, H(1)-Ar), 6.60 (s, 1H, H(4)-Ar), 7.10 (dd, ^3J = 9.2 Hz, ^4J = 2.7 Hz, 1H, H(20)-Ar), 7.28 (d, ^4J = 2.7 Hz, 1H, H(18)-Ar), 7.31 (d, ^3J = 8.3 Hz, 2H, H(12/12')-Ar), 7.62 (d, ^3J = 8.3 Hz, 2H, H(13/13')-Ar), 7.62-7.66 (m, 1H, H(30)-Ar), 7.80-7.84 (m, 1H, H(29)-Ar), 7.99-8.02 (m, 1H, H(31)-Ar), 8.13-8.15 (m, 1H, H(28)-Ar), 8.38 (bs, 1H, CONH(15)), 8.66 (d, ^3J = 9.2 Hz, 1H, H(21)-Ar), 8.75 (d, ^4J = 2.3 Hz, 1H, H(32)-Ar), 9.43 (d, ^4J = 2.3 Hz, 1H, H(26)-Ar), 11.80 (bs, 1H, CONH(23)) – ^{13}C-NMR (100 MHz, CD_2Cl_2): δ = 15.3 (+, C(36)), 29.0 (-, C(5)), 33.5 (-, C(10)), 51.4 (-, C(6)), 55.9 (-, C(8)), 56.2 (+, C(38)), 56.2 (+, C(37)), 60.2 (-, C(9)), 67.1 (-, C(35)), 68.5 (-, C(33)), 69.1 (-, C(34)), 110.2 (+,

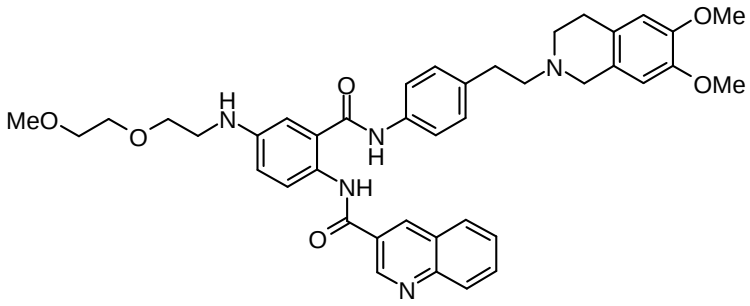
C(1)), 112.1 (+, C(4)), 114.2 (+, C18)), 118.3 (+, C(20)), 121.3 (+, C(13/13')), 123.1 (C_{quat}, C(17)), 123.6 (+, C(21)), 126.7 (C_{quat}, C(4a)), 127.2 (C_{quat}, C(25)), 127.4 (C_{quat}, C(8a)), 127.7 (+, C(30)), 127.8 (C_{quat}, C(31a)), 129.5 (+, C(31)), 129.8 (+, C(12/12')), 129.8 (+, C(28)), 131.6 (+, C(29), 133.3 (C_{quat}, C(22)), 135.8 (C_{quat}, C(14)), 135.9 (+, C(32)), 138.2 (C_{quat}, C(11)), 147.8 (C_{quat}, C(2)), 148.1 (C_{quat}, C(3)), 149.0 (+, C(26)), 149.8 (C_{quat}, C(27a)), 154.9 (C_{quat}, C(19)), 163.8 (C_{quat}, C(24)), 167.5 (C_{quat}, C(16)) – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2931, 1633, 1597, 1517, 1408, 1127 – UV/Vis (CH_2Cl_2) λ_{max} [nm] (lg ε): 282 (4.176), 238 (4.550) – HRMS calcd. for $C_{40}H_{42}N_4O_6$ [$M^{\cdot+}$]: 674.3104, found: 674.3106.



N-(2-{[4-(2-{6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}ethyl)phenyl]carbamoyl}-4-morpholinophenyl)quinoline-3-carboxamide (15)

A 5 mL screw-capped glass vial with stirring bar was charged with CuBr-dimethylsulfide complex (10 mg, 0.05 mmol) and put in a small Schlenk tube. The reaction vessel was heated and evacuated until dimethylsulfide was removed completely and the white solid had turned green. After cooling the vial was evacuated and backfilled with argon (3 cycles) and L-proline (12 mg, 0.1 mmol), compound **3** (154 mg, 0.2 mmol), K_3PO_4 (106 mg, 0.5 mmol), morpholine (40 µL, 0.5 mmol) and anhydrous DMSO (2 mL) were added under argon. The vial was closed and the mixture was stirred at 90 °C for 44 hours, cooled and diluted with CH_2Cl_2. The organic phase was washed with water and saturated aqueous solution of $NaHCO_3$ and dried over $MgSO_4$. After evaporation of the solvent the remaining solid was purified by flash-chromatography on silica gel (elution with EtOAc/EtOH 4:1, R_f = 0.29) to give a yellow solid (116 mg, 75%).

mp = 238 °C – ^{1}H-NMR (600 MHz, CD_2Cl_2; HSQC, HMBC, COSY, ROESY): δ = 2.87-3.00 (m, 8H, 4 CH_2, H(5,6/9,10), 3.15 (m, 4H, 2 CH_2, H(33/34), 3.77 (s, 3H, OCH_3, H(38)), 3.78 (s, 3H, OCH_3, H(37)), 3.77-3.79 (m, 6H, NCH_2, H(8), 2 CH_2, H(35/36)), 6.55 (s, 1H, H-Ar, H(1)), 6.62 (s, 1H, H-Ar, H(4)), 7.11 (dd, ^{3}J = 9.0 Hz, ^{4}J = 2.6 Hz, 1H, H-Ar, H(20)), 7.26-7.30 (m, 3H, H-Ar, H(12/12'), H(18)), 7.61-7.67 (m, 3H, H-Ar, H(13/13'), H(30)), 7.81-7.85 (m, 1H, H-Ar, H(29)), 8.00-8.03 (m, 1H, H-Ar, H(31)), 8.11-8.14 (m, 1H, H-Ar, H(28)), 8.51 (d, ^{3}J = 9.0 Hz, 1H, H-Ar, H(21)), 8.75-8.77 (m, 1H, H-Ar, H(32)), 9.30 (bs, 1H, CONH, H(15)), 9.38-9.40 (m, 1H, H-Ar, H(26)), 11.70 (bs, 1H, CONH, H(23)) – ^{13}C-NMR (150 MHz, CD_2Cl_2): δ = 27.5 (C(5)), 32.5 (C(10)), 49.8 (C(33/34)), 50.9 (C(6)), 54.9 (C(8)), 56.1 (C(38)), 56.2 (C(37)), 60.8 (C(9)), 67.0 (C(35/36)), 110.1 (C(1)), 111.9 (C(4)), 115.0 (C(18)), 119.9 (C(20)), 121.8 (C(13/13')), 123.4 (C(21)), 123.8 (C17)), 124.5 (C(8a)), 125.6 (C4a)), 127.4 (C(31a)), 127.9 (C(25)), 127.9 (C(30)), 129.2 (C(28)), 129.5 (C(31)), 129.6 (C(12/12')), 131.6 (C(22)), 131.7 (C(14)), 131.9 (C(29)), 136.2 (C(32)), 136.5 (C(11)), 147.9 (C(2)), 148.1 (C(19)), 148.6 (C(3)), 148.9 (C(26)), 149.4 (C(27a)), 163.7 (C(24)), 168.4 (C(16)) – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2933, 1655, 1598, 1516, 1228, 1123 – UV/Vis (CH_2Cl_2) λ_{max} [nm] (lg ε): 283 (4.245), 236 (4.562) – HRMS calcd. for $C_{40}H_{41}N_5O_5$ [$M^{\cdot+}$]: 671.3108, found: 671.3122.



N-(2-{[4-(2-{6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}ethyl)phenyl]carbamoyl}-4-{2-[2-methoxyethoxy]ethylamino}phenyl)quinoline-3-carboxamide (14)

A 5 mL screw-capped glass vial with stirring bar was charged with CuI (20 mg, 0.1 mmol), compound **3** (176 mg, 0.3 mmol) and K_3PO_4 (106 mg, 0.5 mmol). The vial was put in a small Schlenk tube, evacuated and filled with argon (3 cycles). 2-Isobutyrylcyclohexanone (34 mg, 0.2 mmol), 2-(2-methoxyethoxy)ethylamine (60 mg,

0.5 mmol) and dry DMF were added by syringe under argon, the vial was capped and the reaction mixture was stirred at 90 °C for 48 h. After cooling the solution was diluted with CH_2Cl_2, washed with water and a saturated aqueous solution of $NaHCO_3$ and the organic phase was dried over $MgSO_4$. Evaporation of the solvent and purification of the crude product by flash-chromatography on silica gel (elution with EtOAc/MeOH 4:1, 1% NEt_3, R_f = 0.25) gave **19** as a yellow solid (74 mg, 40%).
mp = 96 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.82-3.00 (m, 8H, 4 CH_2), 3.16 (t, ^3J = 5.1 Hz, 2H, CH_2), 3.33 (s, 3H, OCH_3), 3.44-3.51 (m, 6H, 3 CH_2), 3.73 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.54 (s, 1H, H-Ar), 6.61 (s, 1H, H-Ar), 6.72 (dd, ^3J = 9.1 Hz, ^{4}J = 2.7 Hz, 1H, H-Ar), 6.92 (d, ^4J = 2.7 Hz, 1H, H-Ar), 7.28 (d, ^3J = 8.5 Hz, 2H, H-Ar, AA'BB'), 7.59-7.65 (m, 1H, H-Ar), 7.67 (d, ^3J = 8.5 Hz, 2H, H-Ar, AA'BB'), 7.78-7.83 (m, 1H, H-Ar), 7.96-7.99 (m, 1H, H-Ar), 8.14-8.17 (m, 1H, H-Ar), 8.42 (d, ^3J = 9.1 Hz, 1H, H-Ar), 8.69 (bs, 1H, CONH), 8.73 (d, ^4J = 2.2 Hz, 1H, H-Ar), 9.49 (d, ^4J = 2.2 Hz, 1H, H-Ar), 11.60 (bs, 1H, CONH) – ^{13}C-NMR (100 MHz, CD_2Cl_2): δ = 24.4, 30.4, 49.8, 52.6, 54.3, 56.2, 56.2, 56.3, 59.0, 68.3, 70.3, 72.2, 109.8, 111.7, 118.7, 122.1, 122.2, 123.0, 123.4, 123.4, 127.6, 128.0, 128.1, 128.2, 128.4, 129.5, 129.6, 129.6, 132.6, 133.0, 137.4, 137.5, 147.9, 147.9, 148.2, 149.0, 149.7, 163.2, 168.0 – IR (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2930, 1599, 1516, 1252, 1226 – UV/Vis (MeOH) λ_{max} [nm] (lg ε): 281 (4.150), 236 (4.519) – HRMS calcd. for $C_{41}H_{45}N_5O_6$ [$M^{\cdot+}$]: 703.3370, found: 703.3376.

Cell line and culture conditions. Kb-V1 cells, an ABCB1 overexpressing subclone[23] of Kb cells (ATCC CCL-17), were maintained in Dulbecco´s modified Eagle´s medium (Sigma, Deisenhofen, Germany) supplemented with 10 % FCS (Biochrom, Berlin, Germany) and 300 ng/mL vinblastine. Cells were maintained in a water saturated atmosphere (95% air/5% carbon dioxide) at 37 °C in 75-cm² culture flasks (NUNC, Wiesbaden, Germany) and serially passaged following trypsinization using 0.05 % trypsin/0.02 % EDTA (Roche Diagnostics, Mannheim, Germany). *Mycoplasma* contamination was routinely monitored by polymerase chain reaction (Venor® GeM, Minerva Biolabs GmbH, Berlin, Germany), and only *Mycoplasma*-free cultures were used.

1.5. References

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2. Potent and Selective Inhibitors of Breast Cancer Resistance Protein (ABCG2) Derived from the p-Glycoprotein (ABCB1) Modulator Tariquidar [i]

The efflux pumps ABCB1 (p-gp, MDR1) and ABCG2 (BCRP) are expressed to a high extent by endothelial cells at the blood-brain barrier (BBB) and other barrier tissues, and are involved in drug resistance of tumor (stem) cells. Based on the findings that slight structural modifications of the aromatic tariquidar core resulted in potent ABCG2 inhibition a series of ABCG2 inhibitors was prepared and found to be highly selective over ABCB1 and ABCC2. [ii] Chemosensitivity assays against MCF-7/Topo cells revealed that a non-toxic inhibitor completely reverted ABCG2-mediated topotecan resistance at concentrations >100 nM, whereas another analogue showed ABCG2 independent cytotoxicity.[iii] ABCG2 inhibitors might be useful for cancer treatment with respect to reversal of multidrug resistance, overcoming the BBB and targeting of tumor stem-cells.

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[ii] The calcein-AM efflux assays (ABCB1) and the mitoxantrone efflux assays (ABCG2) were carried out by Matthias Kühnle and Christine Müller at the Institute of Pharmacy, University of Regensburg. The CMFDA accumulation assays (ABCC2) were done by Anne Mahringer at the Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg.

[iii] The kinetic chemosensitivity assays were performed by Matthias Kühnle and Christine Müller at the Institute of Pharmacy, University of Regensburg.

2.1. Introduction

ABC transporters use the energy of ATP-hydrolysis to transport a broad variety of substrates across the cell membrane. These efflux transport proteins include ABCB1 (p-glycoprotein 170, P-gp), ABCC2 (multidrug resistance related protein 2)[1,2] and ABCG2 (BCRP, ABCP, MXR),[3,4] and appear to play a protective role in normal tissues.[5] For example, in the placenta ABCG2 appears to reduce the passage of substrates from the mother to the fetus and, as reasoned from mice studies,[6] decreases the concentration of certain substrates in the fetal circulation. A high ABCG2 expression at the luminal surface of the endothelium of microvessel also suggests a tutelary function at the blood-brain barrier. In the therapy of CNS diseases the aforementioned physiological functions often lead to low drug concentrations in the brain.

Numerous anticancer agents are ABCB1 and ABCG2 substrates, which are actively pumped out by the transporters located at the blood-brain barrier. This is one of the primary causes of the failure of chemotherapy in the treatment of malignant brain tumors.[7] Furthermore, ATP-binding cassette (ABC) transporters play a more distinctive role in conjunction with multidrug resistance (MDR). It is estimated that multidrug resistant tumors account for up to half of all cancer-related deaths.[8, 9] MDR is caused by the overexpression of efflux transporters such as ABCB1 and ABCG2, located in the plasma membrane of cancer cells and actively extruding a vast number of structurally unrelated compounds, including many commonly used anticancer drugs. Since its discovery in 1998,[10, 11] ABCG2 has been the subject of many investigations concerning its role in MDR, and overexpression of the transporter is associated with high-level resistance to a large number of cytostatics.

In addition, ABCG2 transporters have recently attracted interest with respect to a new concept of tumor development and progression, the so-called cancer stem cell hypothesis. The classical stochastic model of tumor development and progression assumes that all cancer cells are tumor initiating and participate in the tumor growth. By contrast, the cancer stem cell concept is based on the idea that only a small side-population of cancer cells proliferate, in analogy to the hematopoietic stem cells in the bone marrow. The cells of this side-population divide slowly, are capable of long-term

self-renewal and express ABCG2.[12-14] Such cells have been found in numerous established tumor cell lines as well as in tumor biopsies[15-17] and might be responsible for the long-term failure of many cancer chemotherapies. As a result of their ABCG2-mediated drug resistance and slow proliferation, they are inefficient targets for classical cytostatic drugs.

Thus, in many respects, the potent and selective inhibition of ABCG2 might be a promising approach in cancer therapy. The reversal of the multidrug resistance mediated by ABCG2 and the specific targeting of the stem-cell like side-population are conceivable applications. Specific inhibition of ABCG2 in combination with a cytostatic, which is a substrate of ABCG2, might eradicate the tumor stem-cell population, provided that ABCG2 expression is actually a characteristic of these cells. Another interesting strategy might be the co-administration of potent ABCG2 inhibitors with drugs that are substrates of the efflux pump to increase drug levels in the brain.[18] The latter has already been proven by the combination of the cytostatic paclitaxel, an ABCB1 substrate, and the ABCB1 inhibitors valspodar,[19] elacridar and tariquidar.[20] (**3** and **4**, Figure 1).

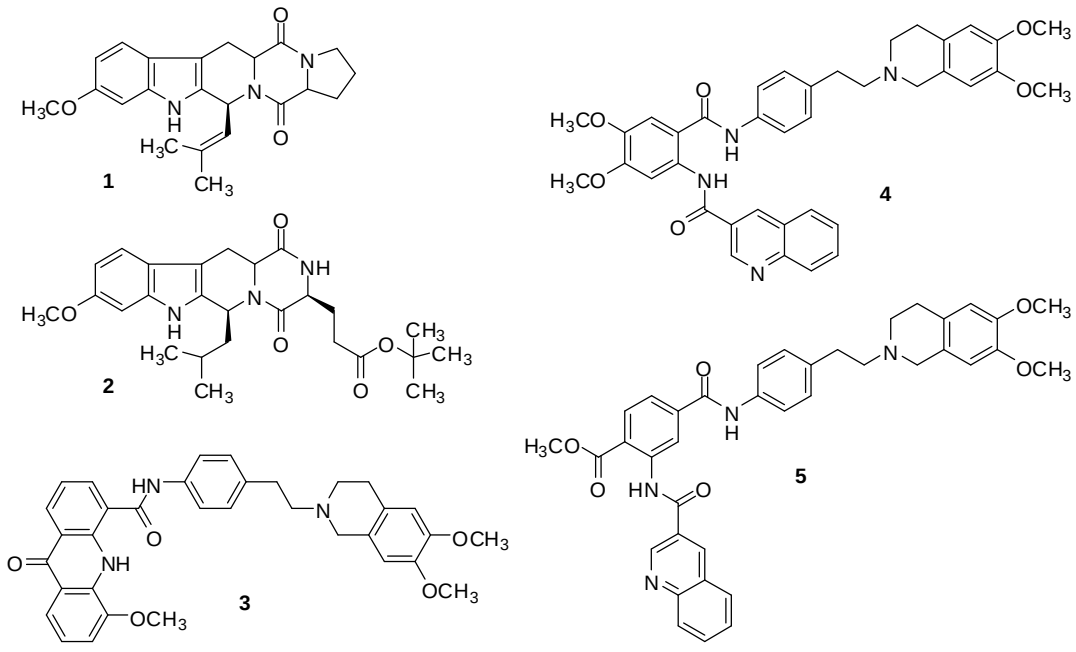


Figure 1. Structures of the ABCG2 modulators fumitremorgin C (**1**) and Ko143 (**2**), the dual ABCB1/ABCG2 inhibitor elacridar (**3**), the ABCB1 inhibitor tariquidar (**4**) and the initial ABCG2 selective lead structure **5** derived from tariquidar.

Due to the relatively recent discovery of the ABCG2 transporter only a few ABCG2 inhibitors have been reported so far.[21-25] Fumitremorgin C (FTC, **1**, Figure 1), a diketopiperazine isolated from the fermentation broth of *Aspergillus fumigatus*, was reported first.[26] However, its neurotoxicity precluded its use in in vivo experiments. The most potent ABCG2 inhibitor known so far is the FTC analogue Ko143 (**2**).[27] Its low cytotoxicity made it promising for in vivo studies, and the bioavailability of orally administered topotecan could be increased by a combination with Ko143.[27] Novobiocin, a coumermycin derivative and inhibitor of the prokaryotic enzyme gyrase was also identified as an ABCG2 inhibitor. In cytotoxicity and flow cytometric assays, micromolar concentrations of novobiocin overcame ABCG2-mediated resistance to mitoxantrone, topotecan and SN-38, the active metabolite of irinotecan.[28] Several ABCB1 inhibitors have also been reported to modulate ABCG2. It was demonstrated that elacridar (GF120918, **3**)[29] acts as an ABCG2 inhibitor, as does – although with lower potency - the ABCB1 inhibitor tariquidar (XR 9576, **4**)[30] and the tariquidar and elacridar analogue WK-X-34.[31, 32]

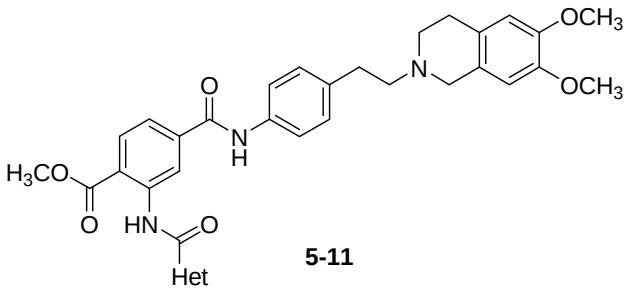
A very recent structure-activity relationship study on tariquidar analogues revealed preferential inhibition of ABCB1 compared to ABCG2. In these compounds the anthranilic acid portion was either acylated with different hetaroyl residues at the *o*-amino group or was replaced with (hetero)aromatic carboxylic acids.[33] During our work on tariquidar-like compounds as ABCB1 modulators[20,34] we discovered that, surprisingly, minimal structural changes at the benzamide core of tariquidar (**4,** Figure 1) resulted in a potent and selective ABCG2 inhibitor (**5**, Figure 1 and Chart 1). Based on this finding we prepared a series of new analogues that were characterized with respect to their activity and selectivity and are presented in this work.

2.2. Results and Discussion

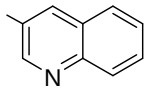
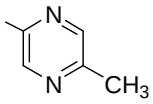
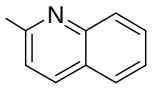
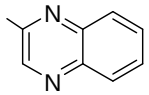
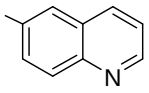
Synthesis. The design of the compounds described in this work was based on the serendipitous observation that compound **5** (Figure 1 and Chart 1) was only weakly active against ABCB1 but was found to be a potent ABCG2 inhibitor instead. Compared to the third generation ABCB1 inhibitor and anthranilic acid derivative tariquidar (**4**) the substitution pattern of carboxylic acid and amine functionalities at the

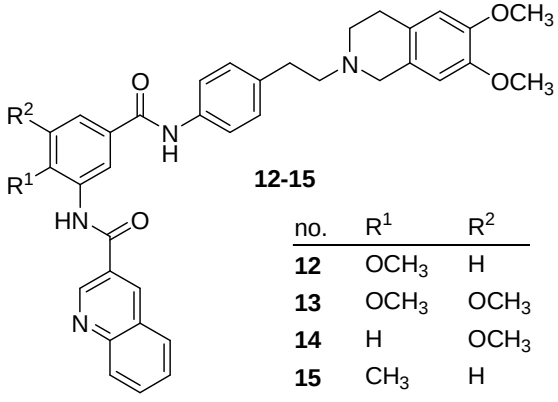
central aromatic core of **5** was changed and the two methoxy groups were replaced by an ester group. Since this change apparently had a strong impact on the selectivity for ABCG2 versus ABCB1 (Table 1) we decided to prepare two small series of compounds in which either the 3-quinolinecarboxylic acid moiety was replaced by various heteroaromatic systems or the ester group was exchanged by methoxy and methyl substituents at different positions (Chart 1).

Chart 1. Variation of the lead structure **5** (subseries **5-11** and **12-15**).



5-11

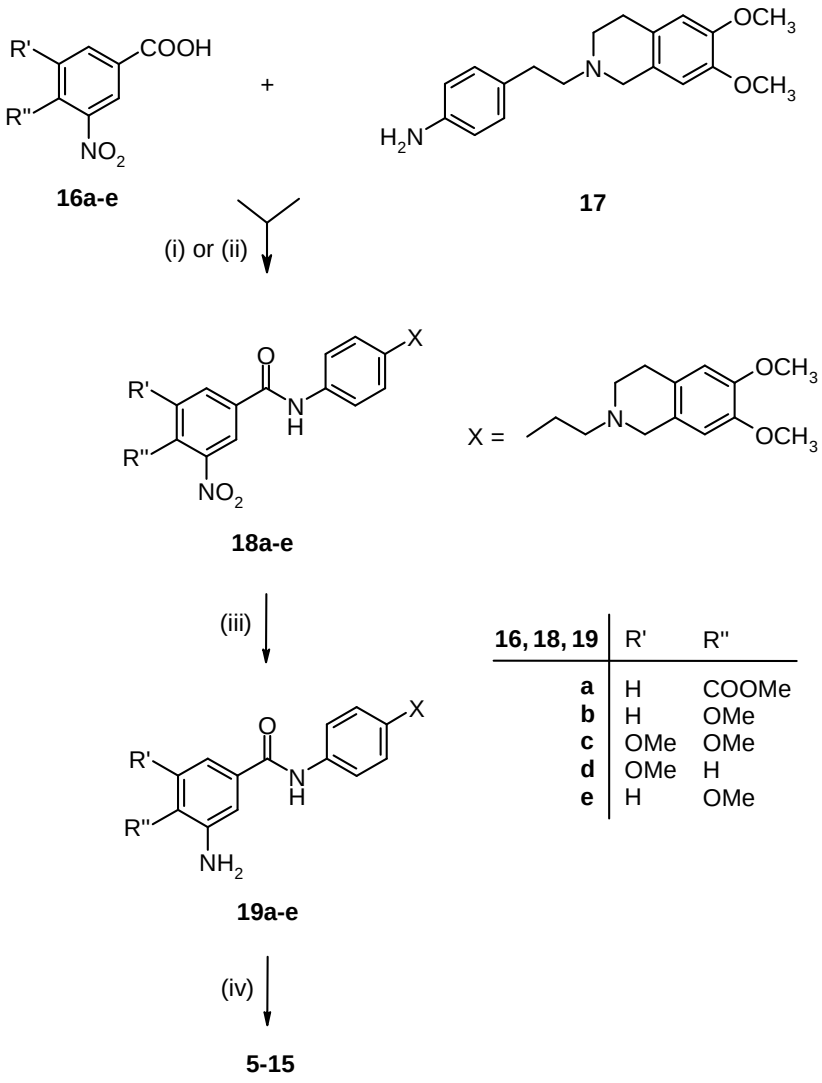
no.	Het	no.	Het
5		**9**	
6		**10**	
7		**11**	
8			



12-15

no.	R^1	R^2
12	OCH_3	H
13	OCH_3	OCH_3
14	H	OCH_3
15	CH_3	H

The general synthetic route for the title compounds (Scheme 1) comprises the formation of an amide bond between 3-nitrobenzoic acid analogues **16a-e** and the aromatic amine **17,** which was synthesized as described before.[20] Subsequent hydrogenation of the nitro group followed by acylation of the resulting aromatic amines **19a-e** with different heteroaromatic carbonyl chlorides yielded the compounds **5-15**.



16, 18, 19	R'	R''
a	H	COOMe
b	H	OMe
c	OMe	OMe
d	OMe	H
e	H	OMe

Scheme 1. General synthetic route for compounds **5-15**. Reagents and conditions: (i) (1) $SOCl_2$; (2) **17**, NEt_3, CH_2Cl_2; (ii) HBTU, HOBt, DIPEA, **17**, CH_2Cl_2; (iii) H_2, Pd/C, EtOAc/MeOH; (iv) HetC(O)Cl · HCl, NEt_3, CH_2Cl_2/DMF.

Inhibition of the ABC transporters ABCG2, ABCB1 and ABCC2. The synthesized modulators **5-15** and the reference compounds **1-4** were investigated for inhibition of ABCG2 and ABCB1 by flow cytometry in a mitoxantrone efflux assay and a calcein-AM efflux assay. In MCF-7/Topo cells red fluorescent mitoxantrone is not accumulated but extruded by the ABCG2 transporter. Therefore, ABCG2 inhibitors can easily be recognized by the flow cytometric determination of intracellular mitoxantrone levels. Changes in the mitoxantrone efflux caused by different concentrations of the ABCG2 modulators can be measured by the relative fluorescence intensity of the cells. Similarly, in ABCB1 overexpressing KBv1 cells, the accumulation of calcein, a fluorescent substrate of ABCB1, can be quantified by flow cytometric analysis. In the presence of ABCB1 inhibitors higher intracellular calcein levels lead to increased relative fluorescence intensities of the cells. The modulation of ABCC2 was investigated on ABCC2-overexpressing MDCK-cells by incubation with chloromethyl-fluorescein-diacetate (CMFDA) in the absence and presence of increasing concentrations of test compounds. After intracellular formation of the ABCC2 substrate glutathione methylfluorescein, (GSMF), the extent of intracellular fluorescence was monitored with a plate reader in a concentration-dependent manner.

To classify the new compounds with respect to their inhibitory potency against the targets ABCG2, ABCB1 and ABCC2 known modulators were investigated as references (Table 1). Whereas fumitremorgin C (**1**) shows only an IC_{50} value in the micromolar range against ABCG2, its analogue Ko143 (**2**) is a highly potent ABCG2 modulator with a maximal inhibitory effect of 82% referred to the control. Both compounds were inactive against the ABCB1 transporter suggesting that the diketopiperazine partial structure confers some selectivity against ABCG2. However, the acridone carboxamide derivative elacridar[29] (**3**) strongly inhibits both transporters without a preference to one of the two targets, whereas tariquidar (**4**) was about equipotent with elacridar at ABCB1 but about four times less potent at ABCG2. Elacridar and tariquidar have the same N-substituent (dimethoxytetrahydroquinolinyl-ethylphenyl) at acridone carboxamide and benzamide, respectively. As shown very recently, the ABCG2 preference of acridone carboxamides can be improved by replacing the amide substituent in elacridar with methoxyphenylethyl residues.[22] Here we report that the preference for one of the ABC transporters depends on the core amide moiety in tariquidar analogues with kept N-substituent.

Table 1. ABCG2, ABCB1 and ABCC2 inhibition of the reference compounds **1-3** and the new inhibitors **5-15** determined in the mitoxantrone (ABCG2), the calcein-AM efflux (ABCB1) and the CMFDA accumulation (ABCC2) assay.

Compound	**ABCG2**	**ABCG2**	**ABCB1**	**ABCC2**
	IC_{50} [nM] [a]	MIE [%] [a, b]	IC_{50} [nM] [c]	IC_{50} [nM]
1 Fumitremorgin C	> 11 000	100	inactive [d]	inactive [d]
2 Ko143	225 ± 33	82 ± 5	inactive [d]	> 50,000
3 Elacridar	250 ± 45	46 ± 2	193 ± 18	inactive [d]
4 Tariquidar	916 ± 197	39 ± 3	223 ± 8	inactive [d]
5	119 ± 22	41 ± 3	9,450 ± 417	inactive [d]
6	60 ± 10	56 ± 6	> 29,000 [e]	>20,000
7	183 ± 50	55 ± 3	> 34,000	n.d.
8	179 ± 35	25 ± 2	inactive [d]	n.d.
9	552 ± 125	44 ± 2	> 57,000 [e]	> 20,000
10	632 ± 222	38 ± 3	> 20,000 [e]	> 50,000
11	1,015 ± 403	47 ± 4	> 14,000 [e]	> 20,000
12	317 ± 131	63 ± 7	> 6,000 [e]	inactive [d]
13	858 ± 210	36 ± 3	> 17,000	inactive [d]
14	977 ± 244	43 ± 6	> 15,000	inactive [d]
15	1,990 ± 355	52[e]	> 15,000 [e]	> 50,000
16 MK571[f]	inactive	-	inactive	> 1000
17 LTC_4 [g]	inactive	-	Inactive	< 150

[a] Mean values ± SEM, calculated from two to three independent experiments. [b] Maximal inhibitory effects (MIE) [%] are expressed as inhibition caused by the highest concentration of the compound tested (7 or 10 µM, respectively) relative to the inhibitory effect caused by 10 µM fumitremorgin C (100% inhibition). [c] Mean values ± SEM, calculated from two to five independent experiments. [d] No effect up to 50 µM Fumitremorgin C: 0.8 % inhibition at 10 µM. [e] N= 1. IC_{50} values were calculated using SIGMA PLOT 9.0, four parameter logistic curve fitting. [f] MK571: 3-(((3-(2-(7-chloroquinoline-2-yl)ethenyl)-phenyl)((3-dimethylamino-3-oxopropyl)thio)methyl)thio) propanoic acid. [g] LTC_4: leukotriene C_4.

Minimal structural changes at the benzamide core of tariquidar resulted in a change from ABCB1 to ABCG2 inhibition. The shift of the quinoline-3-carboxamido substituent from position 2 (tariquidar, **4**) to position 3 of the benzamide moiety (**13**) proved to be key to increase the selectivity for ABCG2 over ABCB1: whereas the moderate inhibition of ABCG2 was maintained (**13**: IC_{50} 858 nM, **4**: IC_{50} 916 nM), the inhibition of ABCB1 decreased by a factor of >75 (IC_{50} >17,000 vs. 223 nM for **13** and **4**, respectively). An additional step towards potent and selective ABCG2 inhibitors was the introduction of an ester instead of the methoxy groups. Comparing the methyl ester **5** with tariquidar (**4**) and **13**, the structural modification on the one hand led to a strong increase in the modulatory potency against ABCG2 and on the other hand the affinity against the original target ABCB1 was dramatically reduced. Similar to the lead structure **5**, the substances **6-11** were potent inhibitors of ABCG2 with up to 500-fold lower activity at ABCB1. None of the novel tariquidar derivatives (up to a concentration of 50 µM) exerted any inhibitory effect on ABCC2 (MRP2).

Obviously, the shift of the hetarylcarboxamido substituent from the 2- to the 3-position at the benzamide core of tariquidar dramatically changes the selectivity of the compounds for ABCG2 over ABCB1. All compounds with bicyclic hetarylcarboxamides in position 3 (**5-8**) were highly potent ABCG2 modulators (IC_{50} values within the range of the known ABCG2 inhibitor Ko143) superior to the substances with monocyclic heteroaromatic moieties (**6-8**). The most potent inhibitor (IC_{50}: 60 ± 10 nM) was obtained with a quinoline-2-carboxamido substituent (**6**). Although the methoxy-substituted compounds **12-14** showed some selectivity for ABCG2 these compounds were less potent than **5**. The decrease in activity was most pronounced with 5-methoxy substitution (**14**). Presumably, the carbonyl oxygen of the ester group in **5-11** contributes to the interaction with ABCG2. As the esters **5-11** may not be considered drug-like due to susceptibility towards enzymatic hydrolysis, compound **5** was exemplarily converted to the carboxylic acid. The cleavage product turned out to be about 80 times less potent than the parent compound **5** as an inhibitor of ABCG2, i. e., higher potency resides in the esters.

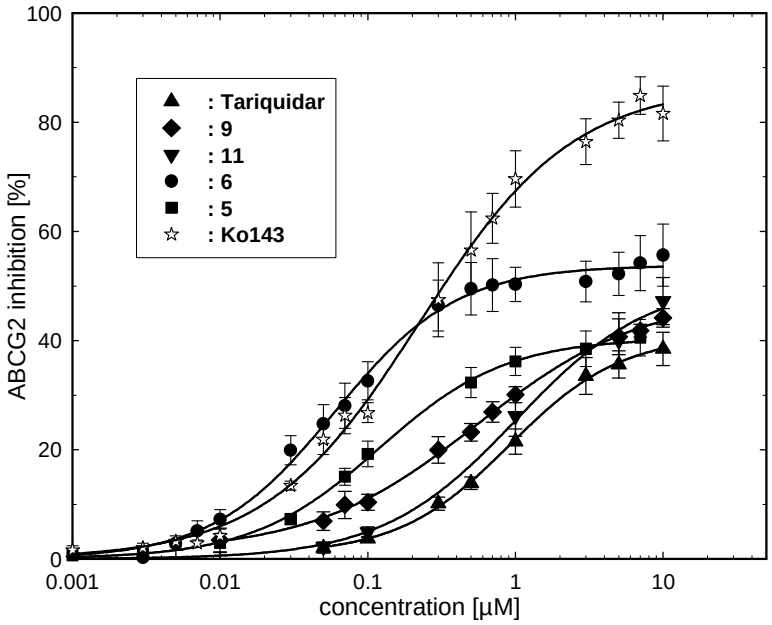


Figure 2. Concentration-response curves of ABCG2 inhibition by selected tariquidar analogues (**5**, **6**, **9** and **11**) and the reference compounds Ko143 and tariquidar.

As shown in Figure 2 the quinoline-3-carboxamide **5** and the structural isomer **6** were about as potent as Ko143 (**2**), the most potent ABCG2 modulator described so far. However, the maximum inhibition obtained by Ko143 was not reached. Although the lower efficacy compared to fumitremorgin C appears to be characteristic of the new ABCG2 inhibitors related to **3**,[22] **4** and **5**, the low water solubility of the tariquidar analogues presented in this work might contribute to this phenomenon. Stability investigations, as well as the search for more stable bioisosteric replacements of the ester group and compounds with higher water solubility are subject of ongoing studies.

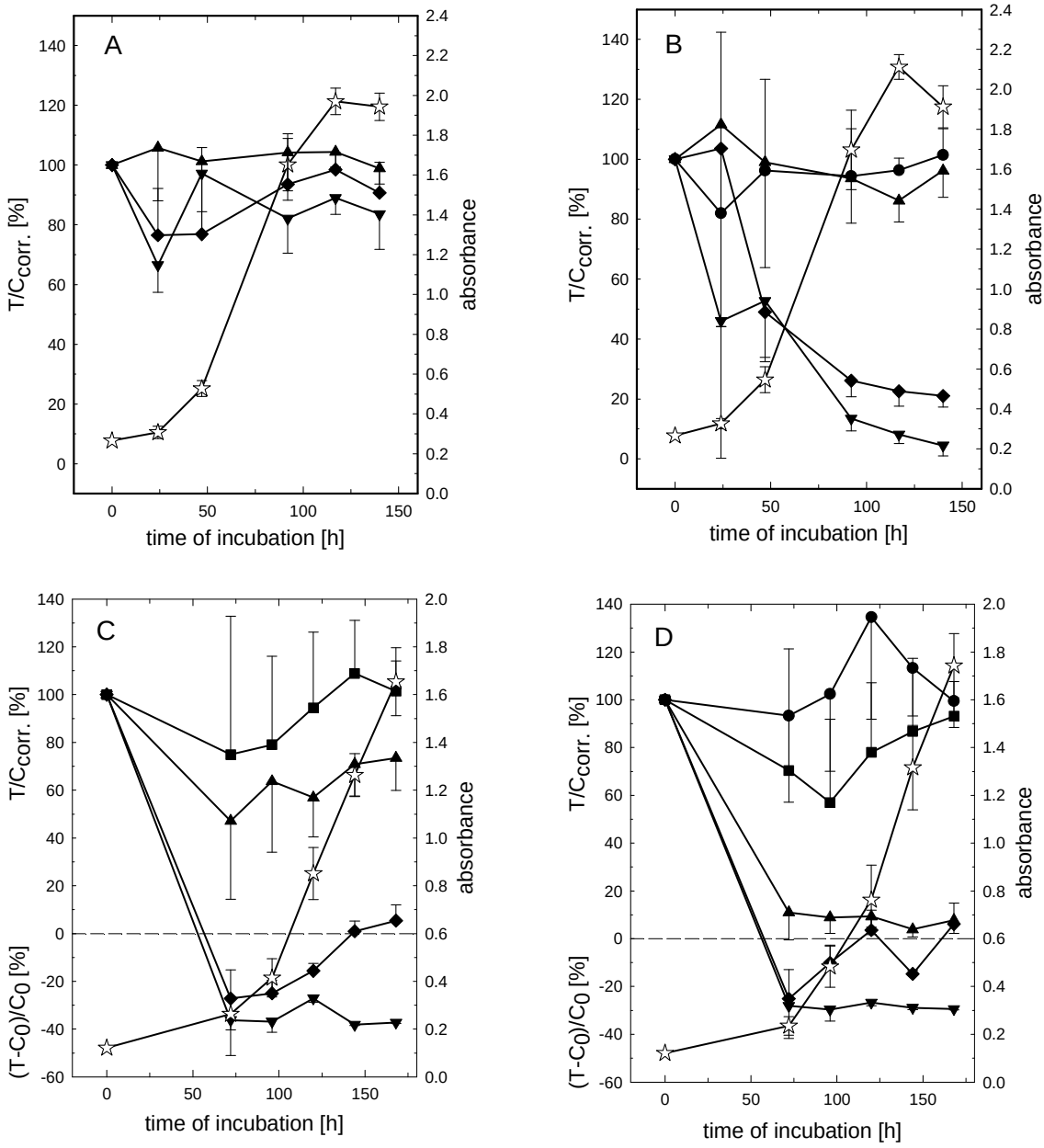


Figure 3. Effect of compound **6** alone (A) and in combination with 100 nM topotecan (B) on proliferating MCF-7/Topo cells (long-term drug exposure). Vehicle *(open stars)*, 100 nM topotecan *(filled circles)* and **6** at different concentrations: 10 nM *(filled triangles)*, 100 nM *(filled diamonds)* and 500 nM *(filled inverted triangles)*. Effect of compound **5** alone (C) and in combination with topotecan (D) on proliferating MCF-7/Topo cells (permanent incubation with the test compounds). Vehicle *(open stars)*, 100 nM topotecan *(filled circles)* and **5** at different concentrations: 1 nM *(filled squares)*, 10 nM *(filled triangles)*, 100 nM *(filled diamonds)* and 500 nM *(filled inverted triangles)*.

Cytotoxicity and reversal of drug resistance. Based on the results of the mitoxantrone efflux assay the ability of the two most potent modulators (**5** and **6**) to overcome multidrug resistance mediated by ABCG2 was investigated using a kinetic chemosensitivity assay. For this purpose ABCG2 positive topotecan-resistant MCF-7 breast cancer cells (MCF-7/Topo cells) were incubated with the new inhibitors alone and in combination with topotecan at a non-toxic concentration. The results are shown in Figure 3.

Incubation of MCF-7/Topo breast cancer cells with **6** alone resulted only in a weak cytostatic effect up to concentrations of 500 nM (Figure 3A). However, compound **6** at a concentration of 100 nM combined with a non-toxic concentration of topotecan (100 nM) resulted in a strong cytostatic effect. The effective inhibition of ABCG2 led to a total reversal of the ABCG2 mediated topotecan resistance (Figure 3B). Surprisingly, at a concentration of 100 nM compound **5** had a cytostatic effect against proliferating MCF-7/Topo cells (Figure 3C), which was only slightly enhanced by the combination with topotecan (Figure 3D). Chemosensitivity assays with proliferating ABCG2 negative U-373 MG glioblastoma cells indicate that the toxicity of **5** is independent from ABCG2 modulation. In addition, quiescent U-373 MG cells were not affected by **5,** indicating that the antiproliferative effect is cell cycle dependent (data not shown).

Specific modulators are desired as pharmacological tools for the functional analysis and characterization of the ABCG2 transporter as the mechanism of action and the selection and binding of substrates is far from being understood. Some anticancer drugs, e.g. topotecan, are substrates of different ABC transporters. This might be one of the reasons why the reversal of MDR by inhibition of one of these proteins often fails in the clinics,[35, 36] suggesting a potential therapeutic value of dual inhibitors of ABC transporters.[37] Selective inhibition should be superior to dual ABCB1/ABCG2 inhibition, if the coadministered drug is preferentially transported by one specific efflux pump. Additionally, combination therapy could improve the pharmacokinetics of transported drugs, thereby increasing oral bioavailability,[38] plasma half-lives and brain penetration.[18, 39]

2.3. Conclusion

Starting from the ABCB1 preferring dual ABCB1/ABCG2 modulator tariquidar structural modifications at the benzamide core were performed. The synthesized compounds are among the most potent and selective ABCG2 inhibitors known so far. Such compounds could be useful in combination with cytostatics that are ABCG2 substrates in order to overcome drug resistance of tumor cells or to modulate ABCG2 at the blood-brain barrier and thereby improve the outcome of cancer chemotherapy of malignant brain tumors. Kinetic chemosensitivity assays revealed two main effects of the new compounds. Firstly, weakly toxic modulators such as compound **6** were able to reverse the ABCG2 mediated topotecan resistance in MCF-7/Topo breast cancer cells. Secondly, compound **5** showed additional cytotoxicity. The latter is an unexpected interesting aspect since ABCG2 modulators with intrinsic antiproliferative activity may offer a new chemotherapeutic approach addressing the tumor stem cell concept.[40]

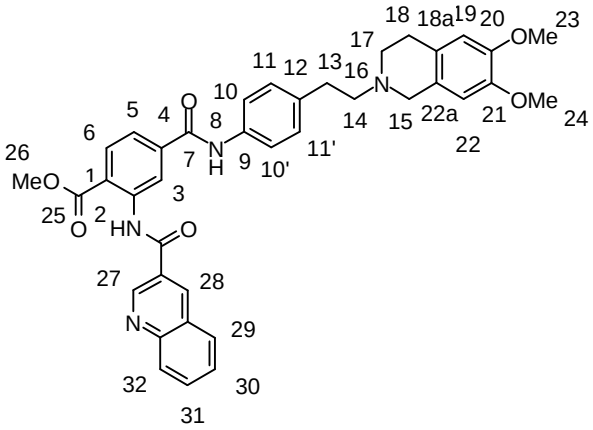
2.4. Experimental Section

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 number 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).

General procedure A for the preparation of carbonyl chlorides. The corresponding (hetero-) aromatic carboxylic acid was suspended in $SOCl_2$ (10-15 mL) and heated to reflux for two hours. Excess $SOCl_2$ was removed under reduced pressure and the resulting white solid was dried under vacuum.

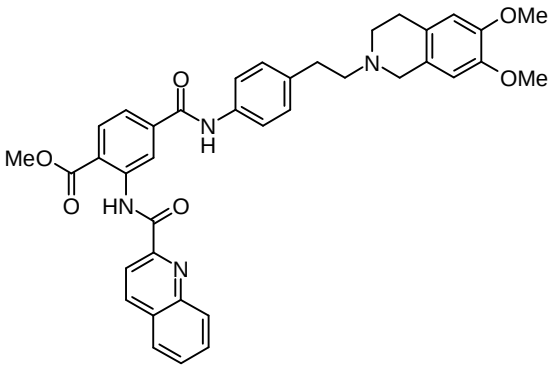
General procedures B and C for the preparation of the amide bonds. (B) The aromatic amine (**17**, **19a-d**) (1 eq) and NEt_3 (3 eq) were dissolved in CH_2Cl_2 and the aromatic carbonyl chloride (1.5 eq) derived from the corresponding acid via general procedure A was added in small portions. The solution was stirred at room temperature for 24 hours, diluted with CH_2Cl_2, washed with water and saturated aqueous solution of Na_2CO_3 (3 ×), dried over $MgSO_4$ and concentrated to give the crude product which was purified by flash chromatography on silica gel or recrystallisation. (C) The aromatic carboxylic acid (1.1 eq), DIPEA (2 eq), HOBt (1.2 eq) and HBTU (1.2 eq) were dissolved in CH_2Cl_2 at 0 °C and stirred for 20 min. The amine (1 eq) was added in small portions and the solution was allowed to warm to room temperature and stirred for 24 h. The solution was diluted with CH_2Cl_2, washed with water (2×) and saturated aqueous solution of Na_2CO_3 (3×), dried over $MgSO_4$ and concentrated to give the crude product which was purified by flash chromatography on silica gel.

General procedure D for the reduction of the nitro group. The corresponding nitro compound was dissolved in a mixture of ethyl acetate and methanol, palladium on activated charcoal (10 % m/m) was added and the solution was stirred under 5 bar H_2 atmosphere overnight. The catalyst was filtered off and the solvents were removed to obtain the amines in quantitative yields.



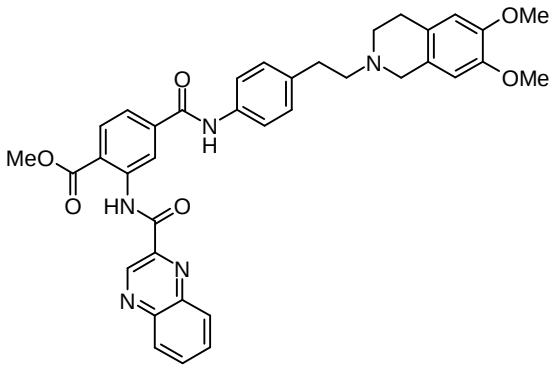
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(quinolin-3-ylcarbonylamino)benzoate (5).

The compound was synthesized following general procedure B and recrystallized from methanol to obtain compound **5** as a white solid (0.20 g, 58%); mp = 191 °C (decomposition) – ^{1}H-NMR (400 MHz, [D$_6$]-DMSO; COSY): δ = 2.66–2.89 (m, 8H, 4 CH_2, H(13/14), H(17/18)), 3.55 (s, 2H, CH_2, H(15)), 3.70 (2 s, 6H, CH_3, H(23)/H(24)), 3.91 (s, 3H, $COOCH_3$), 6.64 (s, 1H, H(22)-Ar), 6.65 (s, 1H, H(19)-Ar), 7.26 (d, ^{3}J = 8.5 Hz, 2H, AA'BB', H(11/11')), 7.71 (d, ^{3}J = 8.5 Hz, 2H, AA'BB', H(10/10')), 7.74-7.78 (m, 1H, H(30/31)-Ar), 7.85 (dd, ^{3}J = 8.2 Hz, ^{4}J = 1.5 Hz, 1H, H(5)-Ar), 7.92-7.96 (m, 1H, H(31/30)-Ar), 8.09 (d, ^{3}J = 8.2 Hz, 1H, H(6)-Ar), 8.15 (d, ^{3}J = 8.2 Hz, 1H, H(29/32)-Ar), 8.21 (d, ^{3}J = 7.8 Hz, 1H, H(32/29)-Ar), 8.81 (d, ^{4}J = 1.5 Hz, 1H, H(3)-Ar), 8.99 (d, ^{4}J = 2.1 Hz, 1H, H(27/28)-Ar), 9.41 (d, ^{4}J = 2.1 Hz, 1H, H(28/27)-Ar), 10.45 (s, 1H, CONH), 11.57 (s, 1H, CONH) – ^{13}C-NMR (100 MHz, [D$_6$]-DMSO): δ = 28.8 (-), 32.9 (-), 48.9 (-), 53.2 (+), 55.5 (-), 55.9 (+), 56.0 (+), 60.0 (-), 109.9 (+), 111.7 (+), 120.4 (+), 121.7 (C_{quat}), 122.1 (+), 122.8 (+), 125.8 (C_{quat}), 126.4 (C_{quat}), 126.5 (C_{quat}), 126.8 (C_{quat}), 127.7 (+), 128.7 (+), 128.8 (+), 129.3 (+), 130.5 (+), 131.7 (+), 136.0 (+), 136.1 (C_{quat}), 136.6 (C_{quat}), 138.8 (C_{quat}), 139.6 (C_{quat}), 146.8 (C_{quat}), 147.0 (C_{quat}), 148.3 (+), 148.7 (C_{quat}), 163.7 (C_{quat}), 164.2 (C_{quat}), 167.1 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3414, 2921, 1650 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 288 (4.508), 240 (4.967) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 323 (30) [MH_2^{2+}], 645 (100) [MH^+] – Elemental analysis calcd (%) for $C_{38}H_{36}N_4O_6$ (644.73): C 70.79, H 5.63, N 8.69; found C 69.98, H 5.68 N 8.27.



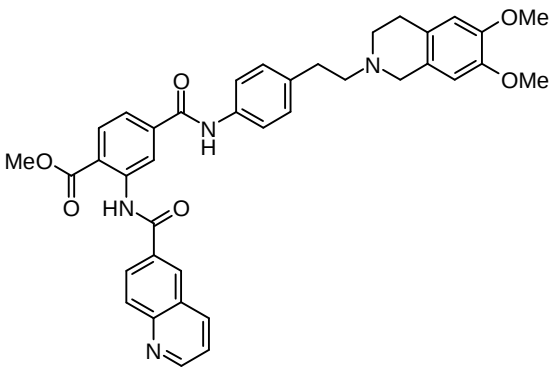
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(quinoline-2-carbonylamino)benzoate (6).

The compound was synthesized following general procedure C and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$, R_f = 0.36) to obtain a pale yellow solid (70 mg, 10%). mp = 176°C (decomposition) – ^{1}H-NMR (300 MHz, CD_2Cl_2): δ = 2.85–3.02 (m, 8H, 4 CH_2), 3.78 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.07 (s, 3H, $COOCH_3$), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 7.24-7.27 (m, 2H, H-Ar, *AA*'BB'), 7.64-7.67 (m, 2H, H-Ar, AA'*BB*'), 7.62-7.67 (m, 1H, H-Ar), 7.69 (dd, ^3J = 8.2 Hz, ^4J = 1.6 Hz, 1H, H-Ar), 7.77-7.84 (m, 1H, H-Ar), 7.88-7.91 (m, 1H, H-Ar), 8.17 (d, ^3J = 8.2 Hz, 1H, H-Ar), 8.28-8.36 (m, 3H, H-Ar), 8.34 (bs, 1H, CONH), 9.45 (d, ^4J = 1.6 Hz, 1H, H-Ar), 12.31 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD): δ = 27.8 (-), 32.9 (-), 50.7 (-), 52.8 (+), 55.0 (-), 55.9 (+), 56.0 (+), 59.4 (-), 109.4 (+), 111.3 (+), 117.9 (+), 118.7 (C_{quat}), 118.8 (+), 120.8 (+), 122.5 (+), 123.3 (C_{quat}), 124.8 (C_{quat}), 125.4 (C_{quat}), 127.7 (+), 128.5 (+), 129.3 (+), 129.4 (C_{quat}), 130.2 (+), 130.4 (+), 131.9 (+), 136.1 (C_{quat}), 137.8 (+), 140.1 (C_{quat}), 140.8 (C_{quat}), 146.6 (C_{quat}), 147.5 (C_{quat}), 147.8 (C_{quat}), 149.5 (C_{quat}), 163.9 (C_{quat}), 164.8 (C_{quat}), 167.4 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3303, 2940, 2831, 1697, 1655, 1570, 1519 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 309 (4.273), 289 (4.316), 245 (4.776) – HRMS calcd. for $C_{38}H_{37}N_4O_6$ [$M^{\cdot+}$]: 645.2713, found: 645.2709.



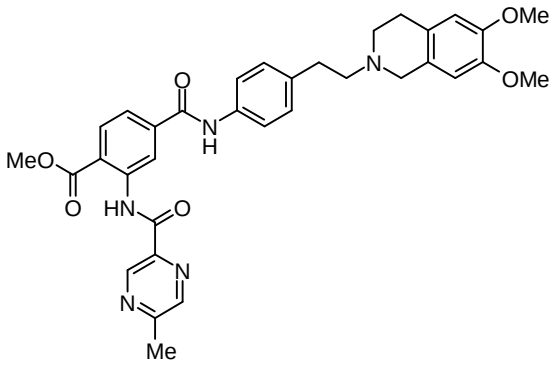
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(quinoxaline-2-carbonylamino)benzoate (7).

The compound was synthesized following general procedure B and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$, R_f = 0.31) to obtain a pale yellow solid (40 mg, 14%); mp = 163 °C (decomposition) – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.76-2.96 (m, 8H, 4 CH_2), 3.67 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.08 (s, 3H, $COOCH_3$), 6.65 (s, 1H, H-Ar), 6.61 (s, 1H, Ar-H), 7.26-7.28 (m, 2H, H-Ar, AA'BB'), 7.64-7.67 (m, 2H, H-Ar, AA'BB'), 7.71 (dd, ^3J = 8.2 Hz, ^4J = 1.6 Hz, 1H, H-Ar), 7.83-7.93 (m, 2H, H-Ar), 8.18-8.21 (m, 1H, H-Ar), 8.20 (d, ^3J = 8.2 Hz, 1H, H-Ar), 8.26-8.29 (m, 1H, H-Ar), 8.27 (bs, 1H, CONH), 9.40 (d, ^4J = 1.6 Hz, 1H, H-Ar), 9.71 (s, 1H, H-Ar), 13.17 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.7 (-), 33.5 (-), 51.1 (-), 52.9 (+), 55.7 (-), 55.9 (+), 55.9 (+), 60.1 (-), 109.5 (+), 111.3 (+), 117.9 (+), 118.6 (C_{quat}), 120.7 (+), 122.9 (+), 126.1 (C_{quat}), 126.4 (C_{quat}), 129.3 (+), 129.4 (+), 130.2 (+), 131.0 (+), 131.9 (+), 132.1 (+), 135.8 (C_{quat}), 137.0 (C_{quat}), 140.3 (C_{quat}), 140.4 (C_{quat}), 140.5 (C_{quat}), 143.5 (C_{quat}), 143.8 (+), 144.0 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 162.7 (C_{quat}), 164.6 (C_{quat}), 167.5 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3310, 2834, 1688, 1652 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 288 (4.503) – HRMS calcd. for $C_{37}H_{36}N_5O_6$ [$M^{\cdot+}$]: 646.2666, found: 646.2648.



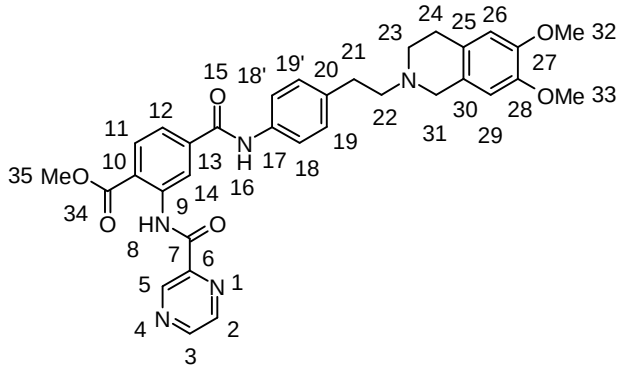
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(quinoline-6-carbonylamino)benzoate (8).

The compound was synthesized following general procedure B and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$, R_f = 0.27) to obtain a pale yellow solid (40 mg, 14%); mp > 200 °C (decomposition) – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.75-2.95 (m, 8H, 4 CH_2), 3.67 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.02 (s, 3H, $COOCH_3$), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 7.24-7.27 (m, 2H, H-Ar, AA'BB'), 7.48-7.52 (m, 1H, H-Ar), 7.63-7.66 (m, 2H, H-Ar, AA'BB'), 7.72 (dd, ^3J = 8.2 Hz, ^4J = 1.6 Hz, 1H, H-Ar), 8.18 (d, ^3J = 8.2 Hz, 1H, H-Ar), 8.22-8.33 (m, 3H, H-Ar), 8.35 (bs, 1H, CONH), 8.52 (d, ^4J = 1.6 Hz, 1H, H-Ar), 9.01-9.03 (m, 1H, H-Ar), 9.39-9.40 (m, 1H, H-Ar), 12.32 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (-), 33.5 (-), 51.0 (-), 53.0 (+), 55.7 (-), 55.9 (+), 55.9 (+), 60.1 (-), 109.5 (+), 111.3 (+), 117.4 (C_{quat}), 117.8 (+), 120.7 (+), 122.1 (+), 122.7 (+), 126.1 (C_{quat}), 126.4 (C_{quat}), 127.0 (+), 127.7 (C_{quat}), 128.6 (+), 129.3 (+), 130.4 (+), 131.6 (+), 132.1 (C_{quat}), 135.9 (C_{quat}), 136.9 (C_{quat}), 137.4 (+), 140.7 (C_{quat}), 141.6 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 149.6 (C_{quat}), 152.5 (+), 164.6 (C_{quat}), 165.2 (C_{quat}), 168.5 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3296, 2951, 1679, 1578, 1521 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 316 (4.365), 246 (4.731) – HRMS calcd. for $C_{38}H_{37}N_4O_6$ [MH^+]: 645.2713, found: 645.2729.



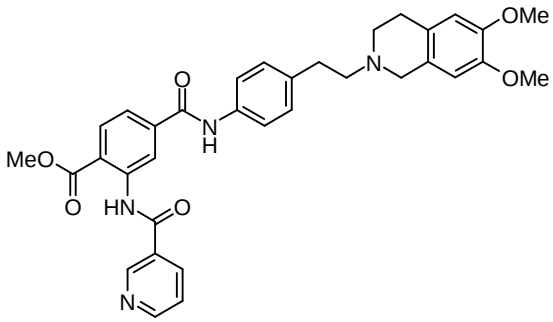
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(5-methylpyrazine-2-carbonylamino)benzoate (9).

The compound was synthesized following general procedure C and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$, R_f = 0.31) to obtain a pale yellow solid (0.15 g, 41%); mp = 182 °C (decomposition) – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.69 (s, 3H, CH_3), 2.88–3.09 (m, 8H, 4 CH_2), 3.84 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.02 (s, 3H, $COOCH_3$), 6.54 (s, 1H, H-Ar), 6.61 (s, 1H, H-Ar), 7.22-7.25 (m, 2H, H-Ar, AA'BB'), 7.63-7.66 (m, 2H, H-Ar, AA'BB'), 7.74 (dd, ^3J = 8.5 Hz, ^4J = 1.7 Hz, 1H, H-Ar), 8.19 (d, ^3J = 8.5 Hz, 1H, H-Ar), 8.31 (bs, 1H, CONH), 8.59 (d, ^5J = 0.3 Hz, 1H, H-Ar), 9.33 (d, ^5J = 0.3 Hz, 1H, H-Ar), 9.40 (d, ^4J = 1.7 Hz, 1H, H-Ar), 12.92 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.0 (+), 28.7 (-), 33.5 (-), 51.1 (-), 52.9 (+), 55.7 (-), 55.9 (+), 55.9 (+), 60.2 (-), 109.5 (+), 111.4 (+), 117.9 (+), 118.5 (C_{quat}), 120.7 (+), 123.0 (+), 126.1 (C_{quat}), 126.5 (C_{quat}), 129.3 (+), 131.9 (+), 135.7 (C_{quat}), 137.1 (C_{quat}), 140.4 (C_{quat}), 140.7 (C_{quat}), 142.1 (C_{quat}), 142.9 (+), 143.9 (+), 144.0 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 157.7 (C_{quat}), 164.5 (C_{quat}), 167.7(C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3288, 2940, 2828, 1691, 1650, 1572 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 288 (4.129), 244 (4.345) – HRMS calcd. for $C_{34}H_{35}N_5O_6$ [$M^{\cdot+}$]: 609.2587, found: 609.2590.



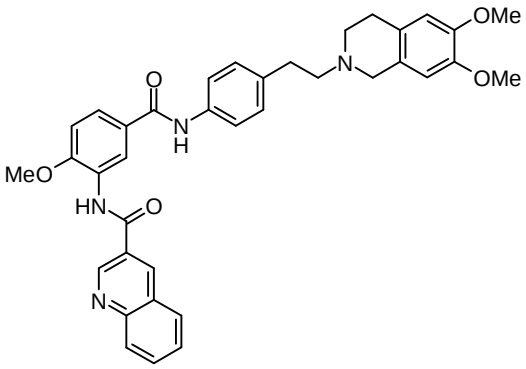
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(pyrazine-2-carbonylamino)benzoate (10).

The compound was synthesized following general procedure C and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$, R_f = 0.31) to obtain a pale yellow solid (0.12 g, 25%); mp > 200 °C (decomposition) – ^{1}H-NMR (300 MHz, $CDCl_3$; HSQC, HMBC, COSY, ROESY): δ = 2.76-2.96 (m, 8H, 4 CH_2, H(21/22), H(23/24)), 3.68 (s, 2H, H(31)), 3.85 (s, 3H, CH_3, H(33)), 3.85 (s, 3H, CH3, H(32)), 4.04 (s, 3H, CH_3, H(35)), 6.55 (s, 1H, H(29)-Ar), 6.61 (s, 1H, H(26)-Ar), 7.27-7.30 (m, 2H, AA'BB', H(19/19')-Ar), 7.61-7.64 (m, 2H, AA'BB', H(18/18')-Ar), 7.77 (dd, ^3J = 8.3 Hz, ^4J = 1.7 Hz, 1H, H(12)-Ar), 8.07 (bs, 1H, N(16)-H), 8.23 (d, ^3J = 8.3 Hz, 1H, H(11)-Ar), 8.76 (dd, ^3J = 2.5 Hz, ^4J = 1.5 Hz, 1H, H(3)-Ar), 8.84 (dd, ^3J = 2.5 Hz, ^5J = 0.3 Hz, 1H, H(2)-Ar), 9.43 (d, ^4J = 1.7 Hz, 1H, H(14)-Ar), 9.51 (dd, ^4J = 1.5 Hz, ^5J = 0.3 Hz, 1H, H(5)-Ar), 13.04 (bs, 1H, N(8)-H) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.7 (-, C(24)), 33.5 (-, C(21)), 51.1 (-, C(23)), 53.0 (+, C(35)), 55.7 (-, C(31)), 55.9 (+, C(33)), 55.9 (+, C(32)), 60.2 (-, C(22)), 109.4 (+, C(29)), 111.3 (+, C(26)), 118.0 (+, C(14)), 118.4 (C_{quat}, C(10)), 120.7 (+, C(18/18')), 123.0 (+, C(12)), 126.1 (C_{quat}, C(25)), 126.4 (C_{quat}, C(30)), 129.4 (+, C(19/19')), 131.9 (+, C(11)), 135.8 (C_{quat}, C(20)), 137.1 (C_{quat}, C(17)), 140.5 (C_{quat}, C(13)), 140.6 (C_{quat}, C(9)), 143.1 (+, C(3)), 144.7 (C_{quat}, C(6)), 144.9 (+, C(5)), 147.2 (C_{quat}, C(28)), 147.5 (C_{quat}, C(27)), 147.7 (+, C(2)), 162.4 (C_{quat}, C(7)), 164.6 (C_{quat}, C(15)), 167.7 (C_{quat}, C(34)) – IR (KBr) [cm^{-1}]: ν = 3310, 3225, 2928, 2832, 1691, 1651, 1571, 1516 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 289 (4.501), 244 (4.722) – HRMS calcd. for $C_{33}H_{33}N_5O_6$ [$M^{\cdot+}$]: 595.2431, found: 595.2414.



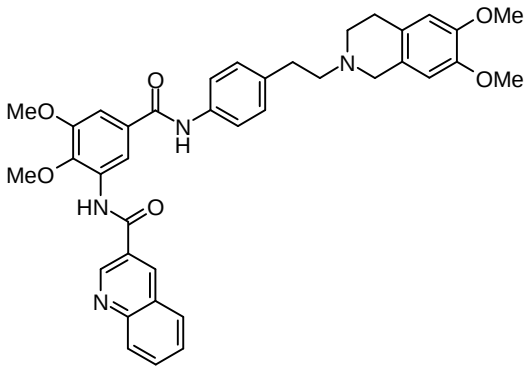
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-(pyridine-3-carbonylamino)benzoate (11).

The compound was synthesized following general procedure B and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$, R_f = 0.24) to obtain a pale yellow solid (0.28 g, 78%); mp = 159 °C (decomposition) – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.75–2.95 (m, 8H, 4 CH_2), 3.66 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.00 (s, 3H, $COOCH_3$), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 7.23-7.26 (m, 2H, AA'BB'), 7.46-7.50 (m, 1H, H-Ar), 7.61-7.64 (m, 2H, AA'BB'), 7.74 (dd, ^3J = 8.5 Hz, ^4J = 1.6 Hz, 1H, H-Ar), 8.19 (d, ^3J = 8.2 Hz, 1H, H-Ar), 8.28-8.32 (m, 1H, H-Ar), 8.38 (s, 1H, CONH), 8.80-8.82 (m, 1H, H-Ar), 9.28-9.29 (m, 1H, H-Ar), 9.31 (d, ^4J = 1.6 Hz, 1H, H-Ar), 12.24 (s, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (-), 33.4 (-), 51.0 (-), 53.0 (+), 55.7 (-), 55.8 (+), 55.9 (+), 60.1(-), 109.4 (+), 111.3 (+), 117.3 (C_{quat}), 117.9 (+), 120.8 (+), 122.8 (+), 123.7 (+), 126.1 (C_{quat}), 126.4 (C_{quat}), 129.3 (+), 131.6 (+), 134.9 (+), 135.9 (C_{quat}), 136.9 (C_{quat}), 140.8 (C_{quat}), 141.3 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 148.9 (+), 150.5 (C_{quat}), 152.9 (+), 164.0 (C_{quat}), 164.7 (C_{quat}), 168.5 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3291, 2935, 1688, 1656, 1580, 1515 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 310 (4.133), 279 (4.266), 242 (4.494) – HRMS calcd. for $C_{34}H_{34}N_4O_6$ [$M^{\cdot+}$]: 594.2478, found: 594.2473.



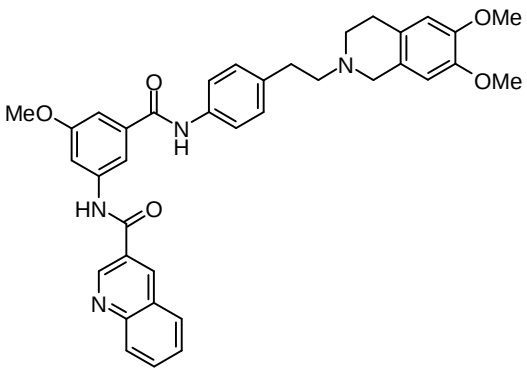
N-(5-((4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-methoxyphenyl)quinoline-3-carboxamide (12).

The compound was prepared following general procedure B and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$ R_f = 0.18) to obtain a pale yellow solid (0.34 g, 79%); mp = 131 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.76-2.95 (m, 8H, 4 CH_2), 3.68 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.55 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 7.06 (d, ^3J = 8.8 Hz, 1H, H-Ar), 7.23-7.26 (m, 2H, H-Ar, AA'BB'), 7.61-7.64 (m, 2H, H-Ar, AA'BB'), 7.65-7.70 (m, 1H, H-Ar), 7.84-7.89 (m, 2H, H-Ar), 7.98-8.00 (m, 1H, H-Ar), 8.19-8.21 (m, 2H, H-Ar/CONH), 8.71-8.73 (m, 2H, H-Ar/CONH), 9.01-9.02 (m, 1H, H-Ar), 9.39-9.40 (m, 1H, H-Ar) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (-), 33.4 (-), 51.0 (-), 55.6 (-), 55.9 (+), 55.9 (+), 56.3 (+), 60.1 (-), 109.46 (+), 110.3 (+), 111.3 (+), 117.5 (+), 120.6 (+), 125.6 (+), 126.1 (C_{quat}), 126.3 (C_{quat}), 126.9 (C_{quat}), 127.0 (C_{quat}), 127.3 (C_{quat}), 127.8 (C_{quat}), 127.9 (+), 128.9 (+), 129.3 (+), 129.5 (+), 131.8 (+), 135.9 (+), 136.3 (C_{quat}), 136.4 (C_{quat}), 147.2 (C_{quat}), 147.6 (C_{quat}), 147.9 (+), 149.5 (C_{quat}), 150.8 (C_{quat}), 163.8 (C_{quat}), 165.0 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3282, 2939, 2836, 1670, 1591, 1515 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 275 (4.347), 242 (4.634) – HRMS calcd. for $C_{37}H_{37}N_4O_5$ [$M^{\cdot+}$]: 617.2764, found: 617.2751.



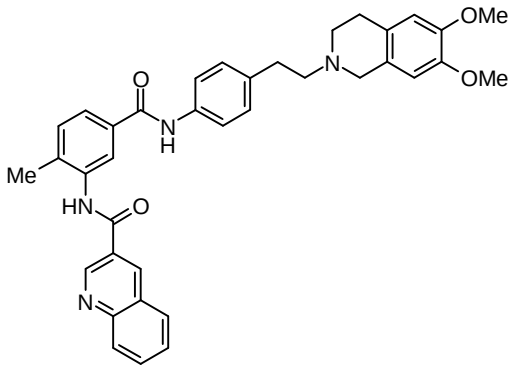
N-(5-((4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2,3-dimethoxyphenyl)quinoline-3-carboxamide (13).

The compound was prepared following general procedure B and purified by flash chromatography on silica gel (EtOAc/MeOH 8:2 R_f = 0.27) to obtain a pale yellow solid (237 mg, 61%); mp = 181 °C (decomposition) – ^{1}H-NMR (300 MHz, [D_6]-DMSO): δ = 2.63-2.84 (m, 8H, 4 CH_2), 3.55 (s, 2H, NCH_2), 3.70 (s, 6H, OCH_3), 3.87 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.64 (s, 1H, H-Ar), 6.66 (s, 1H, H-Ar), 7.23-7.26 (m, 2H, H-Ar, AA'BB'), 7.53 (d, ^4J = 1.9 Hz, 1H, H-Ar), 7.66-7.69 (m, 2H, H-Ar, AA'BB'), 7.71-7.77 (m, 1H, H-Ar), 7.89-7.94 (m, 1H, H-Ar), 8.03 (d, ^4J = 1.9 Hz, 1H, H-Ar), 8.12-8.20 (m, 2H, H-Ar), 8.99 (d, ^4J = 1.9 Hz, 1H, H-Ar), 9.38 (d, ^4J = 2.2 Hz, 1H, H-Ar), 10.17 (bs, 1H, CONH), 10.22 (bs, 1H, H-Ar) – ^{13}C-NMR (75 MHz, [D_6]-DMSO): δ = 28.2 (-), 32.4 (-), 50.5 (-), 55.0 (-), 55.3 (+), 55.3 (+), 56.1 (+), 59.5 (-), 60.3 (+), 109.3 (+), 109.8 (+), 111.6 (+), 117.2 (+), 120.5 (+), 125.8 (C_{quat}), 126.4 (C_{quat}), 126.5 (C_{quat}), 127.0 (C_{quat}), 127.4 (+), 128.7 (+), 128.7 (+), 129.2 (+), 129.9 (C_{quat}), 131.0 (C_{quat}), 131.4 (+), 135.8 (C_{quat}), 136.1 (+), 136.9 (C_{quat}), 144.4 (C_{quat}), 146.8 (C_{quat}), 147.0 (C_{quat}), 148.5 (C_{quat}), 149.0 (+), 152.1 (C_{quat}), 164.4 (C_{quat}), 164.5 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3416, 2927, 1668, 1595, 1520 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 284 (4.527), 242 (4.778) – HRMS calcd. for $C_{38}H_{39}N_4O_6$ [$M^{\cdot+}$]: 647.2870, found: 647.2859.



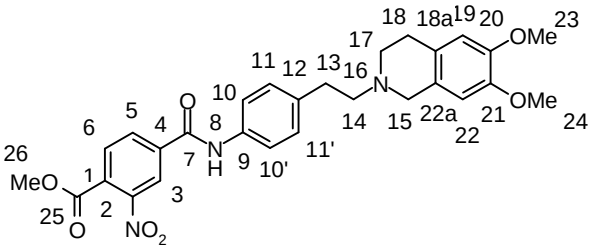
N-(3-((4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-5-methoxyphenyl)quinoline-3-carboxamide (14).

The compound was prepared following general procedure B and crystallized from methanol to obtain as a pale yellow solid (154 mg, 91%); mp = 188-196 °C – ^{1}H-NMR (300 MHz, [D_6]-DMSO): δ = 2.64-2.84 (m, 8H, 4 CH_2), 3.56 (s, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.64 (s, 1H, H-Ar), 6.66 (s, 1H, H-Ar), 7.24-7.26 (m, 2H, H-Ar, AA'BB'), 7.29-7.30 (m, 1H, H-Ar), 7.67-7.70 (m, 2H, H-Ar, AA'BB'), 7.72-7.77 (m, 2H, H-Ar), 7.89-7.95 (m, 2H, H-Ar), 8.12-8.18 (m, 2H, H-Ar), 9.00 (d, ^{4}J = 1.9 Hz, 1H, H-Ar), 9.39 (d, ^{4}J = 2.2 Hz, 1H, H-Ar), 10.23 (bs, 1H, CONH), 10.79 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, [D_6]-DMSO): δ = 27.2 (-), 31.4 (-), 50.1 (-), 54.0 (-), 55.3 (+), 55.4 (+), 55.4 (+), 58.5 (-), 108.3 (+), 108.8 (+), 109.7 (+), 111.5 (+), 112.3 (+), 120.5 (+), 125.1 (C_{quat}), 126.3 (C_{quat}), 127.2 (C_{quat}), 127.5 (C_{quat}), 127.5 (+), 128.7 (+), 129.2 (+), 129.2 (+), 131.4 (+), 136.1 (+), 136.6 (C_{quat}), 137.1 (C_{quat}), 140.1 (C_{quat}), 147.0 (C_{quat}), 147.3 (C_{quat}), 148.5 (C_{quat}), 149.0 (+), 149.0 (C_{quat}), 159.2 (C_{quat}), 164.2 (C_{quat}), 165.1 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3264, 2938, 1651, 1594, 1518 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 281 (4.360), 241 (4.683) – HRMS calcd. for $C_{37}H_{37}N_4O_5$ [$M^{\cdot+}$]: 617.2764, found: 617.2772.



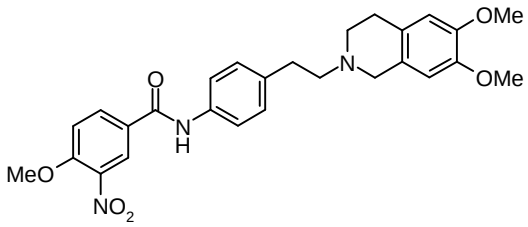
N-(5-((4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-methylphenyl)quinoline-3-carboxamide (15).

The compound was prepared following general procedure B and purified by flash chromatography on silica gel (5% MeOH/$CHCl_3$ R_f = 0.18) to obtain a pale yellow solid (230 mg, 51%); mp = 143°C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.31 (s, 3H, CH_3), 2.70–2.89 (m, 8H, 2*2 CH_2), 3.64 (s, 2H, N-CH_2), 3.83 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.53 (s, 1H, H-Ar), 6.59 (s, 1H, H-Ar), 7.15-7.18 (m, 2H, *AA'*BB'), 7.22 (d, ^3J = 8.2 Hz, 1H, H-Ar), 7.55-7.58 (m, 2H, AA'*BB'*), 7.58-7.63 (m, 2H, H-Ar), 7.78-7.84 (m, 1H, H-Ar), 7.88-7.91 (m, 1H, H-Ar), 8.12-8.15 (m, 2H, H-Ar), 8.46 (bs, 1H, CONH), 8.59 (bs, 1H, CONH), 8.76 (d, ^4J = 1.9 Hz, 1H, H-Ar), 9.39 (d, ^4J = 2.2 Hz, 1H, H-Ar) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 (+), 28.2 (-), 32.3 (-), 50.5 (-), 55.0 (-), 55.3 (+), 55.3 (+), 59.5 (-), 109.8 (+), 111.6 (+), 120.3 (+), 125.3 (+), 125.8 (C_{quat}), 125.8 (+), 126.4 (C_{quat}), 126.5 (C_{quat}), 127.0 (C_{quat}), 127.5 (+), 128.7 (+), 128.7 (+), 129.2 (+), 130.4 (+), 131.4 (+), 132.8 (C_{quat}), 135.7 (C_{quat}), 136.1 (+), 136.9 (C_{quat}), 137.7 (C_{quat}), 139.9 (C_{quat}), 146.7 (C_{quat}), 147.0 (C_{quat}), 148.5 (C_{quat}), 149.0 (+), 164.1 (C_{quat}), 164.5 (C_{quat}) – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 282 (4.442), 240 (4.857) – IR (KBr) [cm^{-1}]: ν = 3275, 2933, 2835, 1652, 1601, 1516 – HRMS calcd. for $C_{37}H_{36}N_4O_4$ [$M^{\cdot+}$]: 600.2737, found: 600.2727.



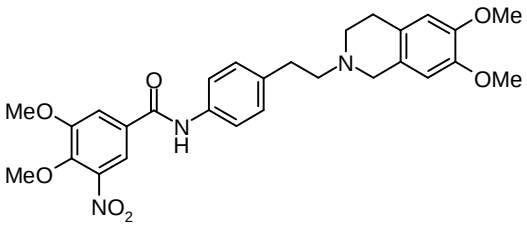
Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-aminocarbonyl)-2-nitrobenzoate (18a).

The compound was synthesized following general procedure B and recrystallized from methanol to obtain a pale yellow solid (1.44 g, 71%); mp = 101 °C – ^{1}H-NMR (600 MHz, $CDCl_3$; HSQC, HMBC, COSY): δ = 2.73-2.90 (m, 8H, 4 CH_2, H(13/14), H(17/18)), 3.64 (s, 2H, CH_2, H(15)), 3.81 (s, 3H, OCH_3, H(24)), 3.82 (s, 3H, OCH_3, H(23)), 3.93 (s, 3H, OCH_3, H(26)), 6.53 (s, 1H, H(22)-Ar), 6.60 (s, 1H, H(19)-Ar), 7.21-7.22 (m, 2H, AA'BB', H(11/11')), 7.55-7.56 (m, 2H, AA'BB', H(10/10')), 7.80 (d, ^{3}J = 8.0 Hz, 1H, H(6)-Ar), 8.17 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.7 Hz, 1H, H(5)-Ar), 8.17 (s, 1H, CONH), 8.39 (d, ^{4}J = 1.7 Hz, 1H, H(3)-Ar) – ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 28.7 (-, C(18)), 33.4 (-, C(13)), 51.0 (-, C(17)), 53.6 (+, C(26)), 55.7 (-, C(15)), 55.8 (+, C(24)), 55.9 (+, C(23)), 60.0 (-, C(14)), 109.5 (+, C(22)), 111.4 (+, C(19)), 121.0 (+, C(10/10')), 122.7 (+, C(3)), 126.2 (C_{quat}, C(18a)), 126.6 (C_{quat}, C(22a)), 129.3 (+, C(11/11')), 129.7 (C_{quat}, C(1)), 130.2 (+, C(6)), 131.7 (+, C(5)), 135.3 (C_{quat}, C(9)), 137.7 (C_{quat}, C(12)), 138.5 (C_{quat}, C(4)), 147.2 (C_{quat}, C(21)), 147.5 (C_{quat}, C(20)), 147.9 (C_{quat}, C(2)), 162.7 (C_{quat}, C(7)), 165.3 (C_{quat}, C(25)) – IR (KBr) [cm^{-1}]: ν = 2924, 1736, 1676, 1534, 1516 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 283 (4.245), 239 (4.160) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 520 (100) [MH^+] – Elemental analysis calcd (%) for $C_{28}H_{29}N_3O_7$ (519.56): C 64.73, H 5.63, N 8.09; found C 64.03, H 5.70 N 7.99.



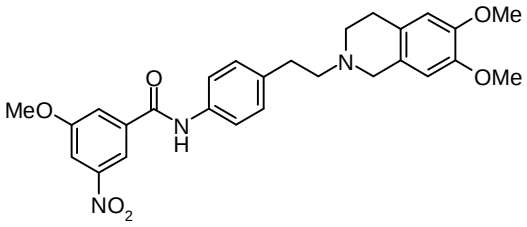
N-(4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-4-methoxy-3-nitrobenzamide (18b).

The compound was prepared following general procedure C and purified by flash chromatography on silica gel ($CHCl_3$/MeOH 9:1, R_f = 0.36) to yield the product as a pale yellow solid (0.80 g, 81%); mp = 179 °C (decomposition) – ^{1}H-NMR (300 MHz, [D_6]-DMSO): δ = 2.64-2.83 (m, 8H, 4 CH_2), 3.54 (s, 2H, NCH_2), 3.69 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.63 (s, 1H, H-Ar), 6.66 (s, 1H, H-Ar), 7.23-7.26 (m, 2H, AA'BB'), 7.52 (d, ^3J = 8.8 Hz, 1H, H-Ar), 7.64-7.67 (m, 2H, AA'BB'), 8.28 (dd, ^3J = 8.8 Hz, ^4J = 2.2 Hz, 1H, H-Ar), 8.52 (d, ^4J = 2.2 Hz, 1H, H-Ar), 10.30 (s, 1H, CONH) – ^{13}C-NMR (75 MHz, [D_6]-DMSO): δ = 24.4 (-), 28.9 (-), 48.8 (-), 51.2 (-), 55.4 (+), 55.5 (+), 55.8 (-), 57.1 (+), 109.5 (+), 111.4 (+), 114.2 (+), 119.8 (C_{quat}), 120.7 (+), 123.2 (C_{quat}), 124.5 (+), 126.4 (C_{quat}), 128.8 (+), 132.3 (C_{quat}), 133.9 (+), 137.6 (C_{quat}), 138.6 (C_{quat}), 147.6 (C_{quat}), 148.2 (C_{quat}), 154.2 (C_{quat}), 162.8 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3240, 2936, 2835, 1659, 1618, 1514 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 278 (4.150), 259 (4.157), 242 (4.255) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 492 (100) [MH^+] – Elemental analysis calcd (%) for $C_{27}H_{29}N_3O_6$ (491.55): C 65.98, H 5.95, N 8.55; found C 65.57, H 6.06 N 8.59.



N-(4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-3,4-dimethoxy-5-nitro-benzamide (18c).

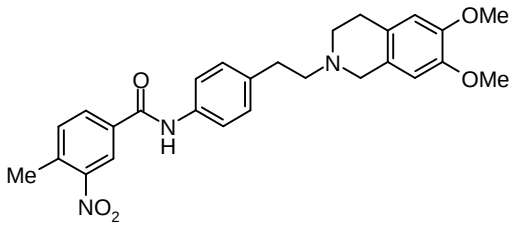
The compound was prepared following general procedure C and crystallized from EtOAc/MeOH 9:1 to obtain a pale yellow solid (610 mg, 91%); mp = 99-103 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.73-2.93 (m, 8H, 4 CH_2), 3.64 (s, 2H, NCH_2), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.53 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 7.23-7.25 (m, 2H, H-Ar, AA'BB'), 7.55-7.58 (m, 2H, H-Ar, AA'BB'), 7.75 (d, ^{4}J = 1.9 Hz, 1H, H-Ar), 7.80 (d, ^{4}J = 2.2 Hz, 1H, H-Ar), 8.00 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (-), 33.4 (-), 51.0 (-), 55.7 (-), 55.9 (+), 55.9 (+), 56.7 (+), 60.1 (-), 62.2 (+), 109.4 (+), 111.3 (+), 113.8 (+), 115.5 (+), 120.7 (+), 126.1 (C_{quat}), 126.4 (C_{quat}), 129.4 (+), 130.4 (C_{quat}), 135.5 (C_{quat}), 137.3 (C_{quat}), 144.1 (C_{quat}), 145.7 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 154.6 (C_{quat}), 163.2 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 2940, 1663, 1609, 1518 – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 522 (100) [MH^+].



N-(4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-3-methoxy-5-nitro-benzamide (18d).

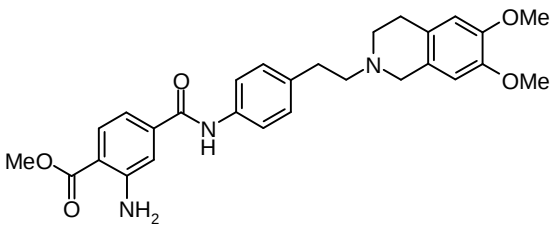
The compound was prepared following general procedure C and purified by flash chromatography on silica gel (EtOAc/MeOH 9:1, R_f = 0.29) to obtain a pale yellow solid (0.80 g, 81%); mp = 119-121 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.75-2.95 (m, 8H, 4 CH_2), 3.66 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.95 (s, 3H,

OCH_3), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 7.25-7.28 (m, 2H, H-Ar, AA'BB'), 7.55-7.58 (m, 2H, H-Ar, AA'BB'), 7.79-7.80 (m, 1H, H-Ar), 7.88-7.89 (m, 1H, H-Ar), 8.03 (bs, 1H, CONH), 8.23-8.25 (m, 1H, H-Ar) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (-), 33.4 (-), 51.0 (-), 55.6 (-), 55.9 (+), 55.9 (+), 56.3 (+), 60.0 (-), 109.4 (+), 111.3 (+), 111.8 (+), 113.5 (+), 113.9 (C_{quat}), 119.7 (+), 120.7 (+), 126.1 (C_{quat}), 126.3 (C_{quat}), 129.4 (+), 135.5 (C_{quat}), 137.3 (C_{quat}), 137.5 (C_{quat}), 147.2 (C_{quat}), 147.6 (C_{quat}), 149.1 (C_{quat}), 160.6 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 2936, 1735, 1608, 1521 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 282 (4.117), 242 (4.419) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 492 (100) [MH^+].



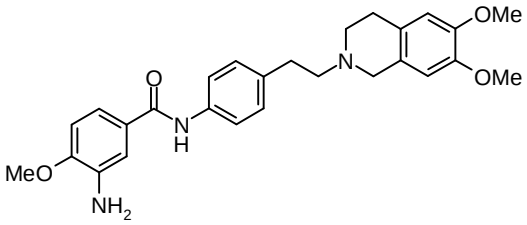
N-(4-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)-4-methyl-3-nitrobenz-amide (18e).

The compound was prepared following general procedure C and purified by flash chromatography on silica gel (EtOAc/MeOH 8:2, R_f = 0.49) to yield the product as a pale yellow solid (94%); mp = 124°C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.62 (s, 3H, CH_3), 2.74-2.94 (m, 8H, 2*2 CH_2), 3.67 (s, 2H, N-CH_2), 3.81 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.51 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 7.20 (d, ^3J = 8.5 Hz, 2H, *AA*'BB'), 7.40 (d, ^3J = 8.2 Hz, 1H, H-Ar), 7.61 (d, ^3J = 8.5 Hz, 2H, AA'*BB*'), 8.08 (dd, ^3J = 8.0 Hz, ^4J = 1.9Hz, 1H, H-Ar), 8.50 (d, ^4J = 1.9 Hz, 1H, H-Ar), 8.91 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.6 (+), 28.4 (-), 33.3 (-) 51.0 (-), 55.5 (-), 55.9 (+), 55.9 (+), 59.9 (-), 109.4 (+), 111.3 (+), 120.9 (+), 123.5 (+), 125.9 (C_{quat}), 129.2 (+), 131.9 (+), 133.2 (+), 134.2 (C_{quat}), 136.0 (C_{quat}), 136.6 (C_{quat}), 137.0 (C_{quat}), 147.3 (C_{quat}), 147.6 (C_{quat}), 148.9 (C_{quat}), 165.7 (C_{quat}) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 476 (100) [MH^+], 951 (8) [$2MH^+$] – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 279 (4.132), 241 (4.211) – IR (KBr) [cm^{-1}]: ν = 3315, 2933, 2837, 1647, 1599, 1520 – Elemental analysis calcd (%) for $C_{27}H_{29}N_3O_5$ (475.55): C 68.20, H 6.15, N 8.84; found C 68.02, H 6.18 N 8.78.



Methyl 2-amino-4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)-phenyl)aminocarbonyl)benzoate (19a).

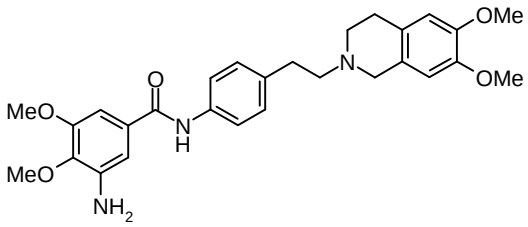
The compound was synthesized following general procedure D and obtained as a pale yellow solid in quantitative yield; mp = 104-106 °C – ^{1}H-NMR (300 MHz, [D_6]-DMSO): δ = 2.63-2.82 (m, 8H, 4 CH_2), 3.53 (s, 2H, NCH_2), 3.69 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.82 (s, 3H, $COOCH_3$), 6.63 (s, 1H, H-Ar), 6.65 (s, 1H, H-Ar), 6.85 (bs, 2H, NH_2), 7.01 (dd, ^{4}J = 1.7 Hz, ^{3}J = 8.5 Hz, 1H, H-Ar), 7.22 (d, ^{3}J = 8.5 Hz, 2H, AA'BB'), 7.29 (d, ^{4}J = 1.7 Hz, 1H, H-Ar), 7.65 (d, ^{3}J = 8.5 Hz, 2H, AA'BB'), 7.80 (d, ^{3}J = 8.3 Hz, 1H, H-Ar), 10.23 (s, 1H, CONH) – ^{13}C-NMR (100 MHz, [D_6]-DMSO): δ = 25.5, 29.8, 49.3, 51.6, 52.2, 55.5, 55.6, 56.8, 109.7, 110.6, 111.6, 113.3, 116.1, 120.5, 124.0, 128.7, 128.8, 130.8, 133.3, 137.5, 140.1, 147.5, 148.0, 151.0, 165.3, 167.4 – IR (KBr) [cm^{-1}]: ν = 3334, 2934, 1737, 1663 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 283 (5.201), 239 (5.763) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 490 (100) [MH^+].



3-Amino-*N*-(4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)-phenyl)-4-methoxy-benzamide (19b).

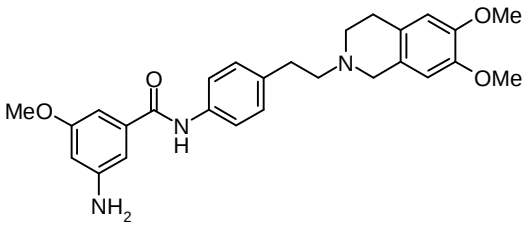
The compound was prepared following general procedure D and was isolated in quantitative yield; mp = 178°C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.75-2.94 (m, 8H, 4 CH_2), 3.67 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 6.82 (d, ^{3}J = 8.5 Hz, 1H, H-Ar), 7.20-7.24 (m,

4H, H-Ar), 7.54 (d, ^3J = 8.5 Hz, 2H, H-Ar, AA'BB'), 7.71 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, [D_6]-DMSO): δ = 28.2 (-), 32.2 (-), 50.5 (-), 55.0 (-), 55.3 (+), 59.6 (-), 109.3 (+), 109.8 (+), 111.6 (+), 113.0 (+), 116.0 (+), 120.1 (+), 125.8 (C_{quat}), 126.5 (C_{quat}), 127.5 (C_{quat}), 128.6 (+), 135.1 (C_{quat}), 137.3 (C_{quat}), 146.7 (C_{quat}), 147.0 (C_{quat}), 148.8 (C_{quat}), 165.6 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3311, 2936, 2839, 1648, 1612, 1514 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 279 (4.160), 265 (4.166), 241 (4.284) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 462 (100) [MH^+], 923 (5) [$2MH^+$].



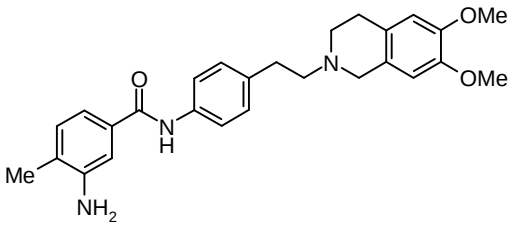
3-Amino-*N*-(4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)-phenyl)-4,5-dimethoxybenzamide (19c).

The compound was prepared following general procedure D and isolated in quantitative yield; mp = 176-179 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.74-2.93 (m, 8H, 4 CH_2), 3.66 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.99 (bs, 2H, NH_2), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 6.83 (d, ^4J = 1.9 Hz, 1H, H-Ar), 6.88 (d, ^4J = 2.2 Hz, 1H, H-Ar), 7.22-7.25 (m, 2H, H-Ar, AA'BB'), 7.52-7.55 (m, 2H, H-Ar, AA'BB'), 7.68 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.7 (-), 33.5 (-), 51.1 (-), 55.7 (-), 55.9 (+), 55.9 (+), 56.0 (+), 60.0 (+), 60.2 (-), 101.8 (+), 107.0 (+), 109.5 (+), 111.4 (+), 120.3 (+), 126.1 (C_{quat}), 126.5 (C_{quat}), 129.3 (+), 131.1 (C_{quat}), 136.2 (C_{quat}), 136.6 (C_{quat}), 138.5 (C_{quat}), 140.6 (C_{quat}), 147.2 (C_{quat}), 147.5 (C_{quat}), 153.0 (C_{quat}), 165.7 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 2983, 1730, 1686, 1576, 1520 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 324 (3.788), 255 (4.345) – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 492 (100) [MH^+], 983 (15) [$2M+H^+$].



3-Amino-*N*-(4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)-phenyl)-5-methoxybenzamide (19d).

The compound was prepared following general procedure D and isolated in quantitative yield; mp = 153-155 °C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.72-2.92 (m, 8H, 4 CH_2), 3.65 (s, 2H, NCH_2), 3.78 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.34-6.35 (m, 1H, H-Ar), 6.54 (s, 1H, H-Ar), 6.60 (s, 1H, H-Ar), 6.72-6.75 (m, 2H, H-Ar), 7.20-7.23 (m, 2H, H-Ar, AA'BB'), 7.52-7.55 (m, 2H, H-Ar, AA'BB'), 7.84 (bs, 1H, CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.6 (-), 33.4 (-), 51.1 (-), 55.4 (+), 55.7 (-), 55.9 (+), 55.9 (+), 60.1 (-), 102.6 (+), 103.9 (+), 106.4 (+), 109.5 (+), 111.4 (+), 120.4 (+), 126.2 (C_{quat}), 126.5 (C_{quat}), 129.3 (+), 136.1 (C_{quat}), 136.6 (C_{quat}), 137.4 (C_{quat}), 147.3 (C_{quat}), 147.6 (C_{quat}), 148.2 (C_{quat}), 161.0 (C_{quat}), 165.9 (C_{quat}) – IR (KBr) [cm^{-1}]: ν = 3451, 2934, 2836, 1665, 1591, 1520 – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 282 (4.142) – MS (CI-MS, NH_3): m/z (%) = 208 (100) [$C_{12}H_{18}O_2N^+$], 462 (73) [MH^+].



3-Amino-*N*-(4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)-phenyl)-4-methylbenz-amide (19e).

The compound was prepared following general procedure D and isolated in quantitative yield; mp = 144°C (decomposition) – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.20 (s, 3H, CH_3), 2.76-2.95 (m, 8H, 2*2 CH_2), 3.69 (s, 2H, N-CH_2), 3.84 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.53 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 7.21-7.26 (m, 2H, *AA*'BB'), 7.10-7.13 (m, 2H, H-Ar), 7.21-7.22 (m, 1H, H-Ar), 7.54-7.57 (m, 2H, AA'*BB*'), 7.84 (bs, 1H,

CONH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.5 (+), 28.4 (-), 33.2 (-) 51.0 (-), 55.5 (-), 55.9 (+), 55.9 (+), 59.9 (-), 109.4 (+), 111.3 (+), 113.7 (+), 116.3 (+), 120.3 (+), 125.9 (C_{quat}), 126.3 (C_{quat}), 129.3 (+), 130.6 (+), 133.8 (C_{quat}), 136.0 (C_{quat}), 136.3 (C_{quat}), 145.1 (C_{quat}), 147.3 (C_{quat}), 147.6 (C_{quat}), 165.9 (C_{quat}) – UV/Vis ($CHCl_3$) λ_{max} [nm] (lg ε): 277 (4.206), 255 (4.256), 242 (4.289) – IR (KBr) [cm^{-1}]: ν = 3324, 2936, 2835, 1648, 1573, 1519 – MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 446 (100) [MH^+], 891 (11) [$2MH^+$].

HPLC analysis of key target compounds. 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) unless otherwise stated (see HPLC of compounds **8** and **12**). 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, 30 min 95% B. Compounds were detected with a diode array detector (DAD, 220 nm) and an evaporative light scattering detector (ELSD (PL-ELS 2100 Ice), gas flow 1.6 L/min, evaporator 30 °C, nebulizer 30 °C). Compounds were dissolved either in DMSO or a mixture of chloroform/acetonitrile.

Table. Purities (%) of key target compounds **5-15**

Compound	UV (220 nm)	ELSD
5	94	99
6	100	100
7	100	100
8[a]	80[a]	-
9	98	100
10	96	100
11	93	100
12[a]	90[a]	-
13	94	99
14	94	100
15	100	100

[a]HPLC of compounds 8 and 12. Column: Luna RP-18 (2), (3µm) (150 mm x 4.6 mm), Detector: UV (detection wavelength: 210 nm). Gradient elution was done with water (0.05% TFA) (solvent A) and acetonitrile (solvent B) at a constant flow rate of 0.8 mL/min (30 °C). A gradient profile with the following proportions of solvent B was applied: 0 min, 15% B; 19 min, 60% B; 20 min, 95% B; 24.5 min, 95% B; 25 min, 15% B; 38 min, 15% B. Prior to injection DMSO stock solutions of compound 8 and compound 12 were diluted with a mixture of 85% ammoniumacetate buffer (0.2 M) and 15% acetonitrile to a final DMSO concentration of 2%.

Drugs and chemicals used for assays. Mitoxantrone stocks were obtained by diluting Novantron® (Wyeth Pharma, Muenster, Germany) in 70% ethanol to a concentration of 2 mM. Test compounds were dissolved in DMSO (Merck, Darmstadt, Germany) at a concentration of 10 mM. Fumitremorgin C (gift of Dr. Susan Bates, NIH) was also dissolved in DMSO and diluted to a concentration of 1 mM. A 10 mM stock solution of Ko143 in DMSO was kindly provided by Dr. A. H. Schinkel (Netherlands Cancer Institute). All stocks were stored at -20 °C. Topotecan stocks were prepared by diluting Hycamtin® (GlaxoSmithKline, München, Germany) in 70% ethanol to a concentration of 0.1 mM and stored at 4 °C. Tariquidar (free base) was synthesized according to the literature with slight modifications.[20] Elacridar (GF 120918·HCl) was kindly provided by GlaxoSmithKline (Research Triangle Park, North Carolina, United States). Calcein-AM, purchased from Biotrend (Köln, Germany), was dissolved in DMSO (Merck, Darmstadt, Germany) to achieve a final concentration of 1 mM. The aliquoted stock solutions were stored at -20 ° C. The 1 mM stock solution of vinblastine (vinblastine sulphate, Sigma, Munich, Germany) was prepared in 70% ethanol.

Cell lines and culture condition. 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 3 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 (FCS, Biochrom , Berlin, Germany) and topotecan at a concentration of 0.55 µM.

KBv1 cells, an ABCB1 overexpressing subclone of Kb cells (ATCC CCL-17), were maintained in Dulbecco's modified Eagle's medium (Sigma, Deisenhofen, Germany) supplemented with 10% FCS (Biochrom, Berlin, Germany) and 270 ng/mL vinblastine. U-373 MG cells, an ABCB1 and ABCG2 non-expressing human glioblastoma cell line, were cultured 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) and 5% fetal calf serum (Biochrom, Berlin, Germany). All cells were cultured in a water-saturated atmosphere (95% air/5% CO_2) at 37 °C in 75 cm^2 and 175 cm^2 culture flasks (NUNC, Wiesbaden, Germany and Greiner, Frickenhausen, Germany) respectively. Mycoplasma contamination was routinely monitored by polymerase chain reaction (Venor GeM, Minerva Biolabs GmbH, Berlin, Germany), and only mycoplasma free cultures were used for testing. ABCC2 overexpressing MDCKII-MRP2 cells transfected with human ABCC2 were a kind gift from Prof. Dr. P. Borst (Netherland Cancer Institute, Amsterdam, NL). The cells were grown in Dulbecco´s Modified Eagle medium supplemented with 5% FCS (Biochrom, Berlin, Germany).

Modulation of ABCG2 (BCRP, ABCP, MXR): determination in the flow cytometric mitoxantrone efflux assay. The assay was essentially performed as described.[34] Briefly, 3 to 5 days after passaging, the ABCG2 overexpressing MCF-7/Topo cells were trypsinized and resuspended in culture medium at 25 °C. After adjusting the cells to a number of 1×10^6 per mL with culture medium, mitoxantrone was added to the cell suspension to achieve a concentration of 20 µM. Different concentrations of the test compound, solvent and fumitremorgin C (final concentration 10 µM) were added, respectively. The cell suspensions were vortexed and incubated for 30 min at 37 °C / 5% CO_2 to allow maximal mitoxantrone uptake into the cells. After one washing step with 0.8 mL of ice-cold PBS the cells of the fumitremorgin C sample were resuspended in 0.5 mL of PBS and placed on ice in the dark until the measurement to avoid mitoxantrone efflux (determination of the 100% mitoxantrone uptake). All other samples were resuspended in 1 mL of drug-free culture medium and incubated for 1 h at 37 °C / 5% CO_2 in which an equilibrium of mitoxantrone could be reached between the cytoplasm and the surrounding medium. Subsequently, after the medium

was removed by centrifugation, the cell pellets were rinsed once with 0.8 mL of ice-cold PBS and finally resuspended in 0.5 mL of PBS for the flow cytometry. A FACS calibur™ (Becton Dickinson, Heidelberg, Germany) was used to analyze the fluorescence intensity of the cells. Mitoxantrone accumulation in the cells was monitored by using an excitation wavelength of 635 nm whereas emission was detected at a wavelength of 661 nm. A minimum of 20000 events was collected per sample, and the events were gated according to forward scatter and sideward scatter to exclude clumps and debris. Analysis of the raw data was performed with the WinMDI 2.8 software. The geometric means were calculated from the fluorescence intensity histogram and related to the controls. Afterwards, the mean values of 3 independent measurements were plotted against the concentration of the test compounds. Addition of increasing concentrations of the modulator led to sigmoidal concentration response curves. IC_{50} values were calculated using SIGMA PLOT 9.0, four parameter logistic curve fitting. Errors were calculated as standard error of the mean.

Modulation of ABCB1 (p-gp): determination in the flow cytometric calcein-AM assay. The assay was performed as described before.[34]

Modulation of ABCC2 (MRP2): determination in the CMFDA assay. Cells were kept in culture in 24-well plates. They were washed twice with Krebs-Ringer buffer (KRB) at 37 ^{o}C prior to experiments, and then incubated with 1 µM CMFDA in absence or in presence of the test compounds at increasing concentrations. Typically, the modulators were dissolved in DMSO. Control experiments confirmed that the cell monolayers tolerated up to 1% DMSO in the incubation medium without functional impairment. All monolayers were incubated for 90 min at 37 °C and under constant circular shaking at 50 rpm. Subsequently, the culture plates were placed on ice, medium in the apical compartment was removed and the cells were washed twice with ice-cold KRB. The cells were lysed by incubation with 200 µL of 1% Triton X-100 in KRB for 30 min under constant shaking. Finally, the culture plates were subjected to fluorescence quantification in a plate reader (Tecan Safire XFLUOR4, Tecan Safire, Crailsheim, Germany) with filter settings of λ_{ex} of 485 nm and λ_{em} of 516 nm. The extent of transport inhibition in presence of ABCC2 modulators was calculated from fluorescence intensities using the software GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA).

Chemosensitivity assays. The assays were performed as described previously.[34] In brief: tumor cell suspensions (100 µL/well) were seeded into 96-well flat bottomed microtitration plates (Greiner, Frickenhausen, Germany) at a density of ca. 15 cells/microscopic field (magnification 320-fold). After 2-3 days the culture medium was removed by suction and replaced by fresh medium (200 µL/well) containing varying drug concentrations or vehicle. Drugs were added as 1000-fold concentrated feed solutions. On every plate 16 wells served as controls and 16 wells were used per drug concentration. After various times of incubation the cells were fixed with glutardialdehyde (Merck, Darmstadt, Germany) and stored in a refrigerator. At the end of the experiment all plates were processed simultaneously (staining with 0.02% aqueous crystal violet (SERVA, Heidelberg, Germany) solution (100 µL/well)). Excess dye was removed by rinsing the trays with water for 20 min. The stain bound by the cells was redissolved in 70% ethanol (180 µL/well) while shaking the microplates for about 3 hours. Absorbance (a parameter proportional to cell mass) was measured at 578 nm using a BIOTEK 309 Autoreader (TECNOMARA, Fernwald, Germany).

Drug effects were expressed as corrected T/C-values for each group according to

$$T/C_{corr} = \frac{T - C_0}{C - C_0} \cdot 100\ [\%]$$

where T is the mean absorbance of the treated cells, C the mean absorbance of the controls and C_0 the mean absorbance of the cells at the time (t = 0) when drug was added. When the absorbance of treated cells T is less than that of the culture at t = 0 (C_0), the extent of cell killing was calculated as

$$\text{cytocidal effect } [\%] = \frac{C_0 - T}{C_0} \cdot 100$$

For assays performed on quiescent U-373 MG cells, a high sowings density was chosen in order to observe as much as possible the effects of the compounds against resting cells. To detect maximum cytocidal effect rotenone, an ubiquinon reductase inhibitor, blocking ATP synthesis, served as positive control.

2.5. References

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3. Synthesis and Cannabinoid Receptor Activity of Ketoalkenes from *Echinacea pallida* and Non-natural Analogues [i]

Despite its popularity and widespread use, the efficacy of *Echinacea* products remains unclear and controversial. Among the various compounds isolated from *Echinacea*, ketoalkenes and ketoalkenynes exclusively found in the pale purple coneflower (*E. pallida*) are major components of the extracts which in contrast to *E. purpurea* alkamides have not been studied for immunostimulatory effects. The *E. pallida* ketoalkenes with skipped conjugation were synthesized[ii] along with some conjugated non-natural analogues[iii] and their effects on the human cannabinoid receptors CB_1 and CB_2 were investigated.[iv] While no significant activity of the natural products was observed at either receptor the new analogues showed micromolar potency at both cannabinoid receptors.

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[ii] The sequence from compound **12** to compound **15** was carried out in collaboration with Stephanie Graetz.
[iii] The conjugated compounds **36**-**42** were synthesized by Patrina Pellett.
[iv] The steady-state GTPase assays were carried out by Kathrin Nickl and Sarah Geiger at the Institute of Pharmacy, University of Regensburg.

3.1. Introduction

Echinacea extracts are one of the top selling herbal remedies in the USA and Europe to treat upper respiratory tract infections.[1] Some studies show that *Echinacea* is an effective immunostimulant,[2,3] while others do not,[4] and much controversy on the efficacy of these extracts exists.[5] The extracts consist of a complex mixture of caffeic acid derivatives, glycoproteins, polysaccharides and alkamides,[6] from 3 species of *Echinacea*: *E. purpura*, *E. pallida* and *E. angustifolia*.[7] The major components of *E. purpura* and *E. angustifolia* extracts are alkamides, while *E. pallida* extracts consist of mainly ketoalkenes and ketoalkenynes.[8]

Echinacea products on the market may contain only one of the above mentioned species or mixtures of all 3 species, and are not required to guarantee content, quality, variability, or contamination of the supplement.[9] Studies have shown that *Echinacea* extracts are wide-spectrum immunomodulators, but the exact phytochemical responsible for the active nature of the extracts has not been identified.[10] Determining the activity of *Echinacea* extracts as an effective herbal remedy is of high interest and intense research on the subject is being conducted, but further studies to elucidate the mode of action are required.[1-3, 7, 10]

Alkamides have been tested for biological activity and have been shown to bind with high affinity to the human cannabinoid 2 receptor (CB_2R), and may be considered a molecular mode of action explaining the immunomodulatory and anti-inflammatory effects.[7,11,12] In order to investigate the biological activity of ketoalkenes and ketoalkenynes found in many *Echinacea* extracts, we report a versatile synthetic route to the ketones found in *E. pallida* (Figure 1), and present the results from functional GTPase assays of these newly synthesized compounds at the CB_1R and the CB_2R. To determine any structure activity relationship responsible for receptor activation, we describe the synthesis and testing results of structurally similar ketone containing analogues.

Figure 1. *Echinacea pallida*.

3.2. Results and Discussion

Synthesis. In contrast to other *Echinacea* species, only *E. pallida* contains ketoalkenes and ketoalkenynes (Figure 2).

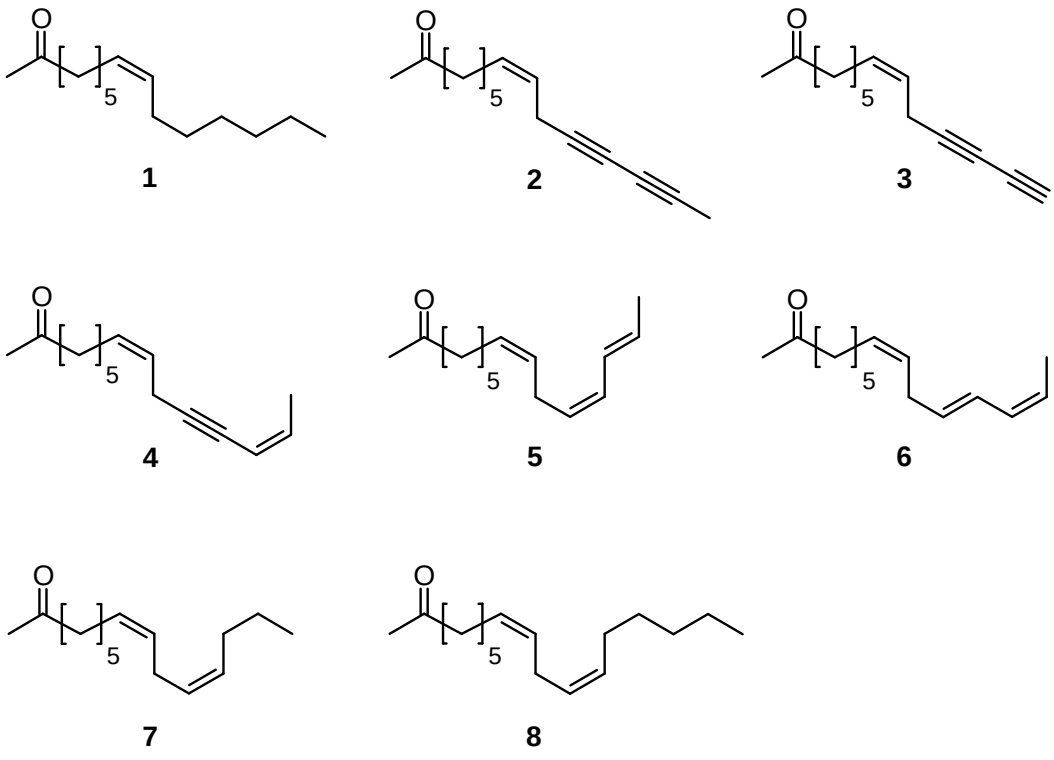
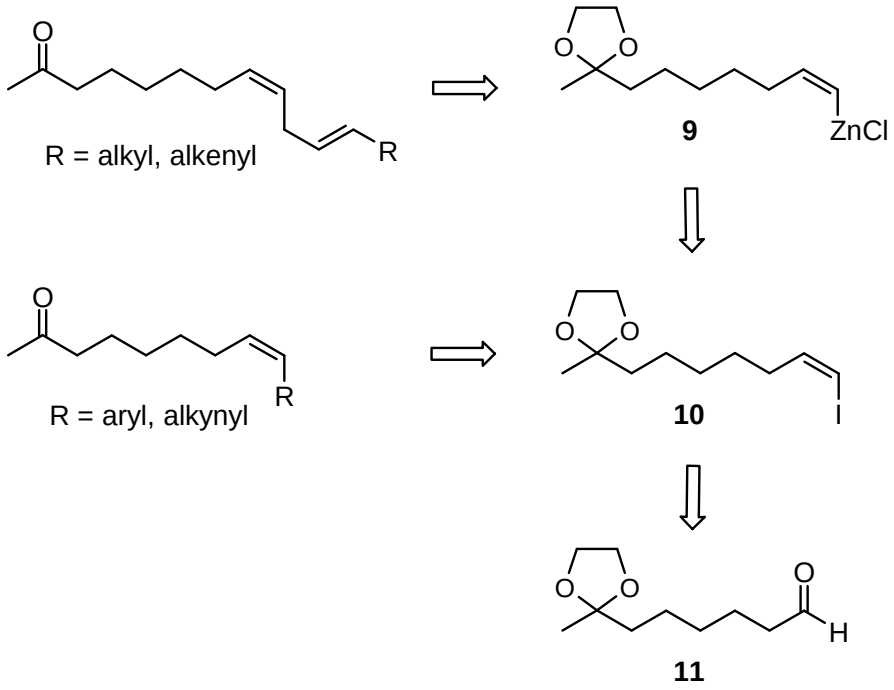


Figure 2. Ketoalkenes and ketoalkenynes isolated from *E. pallida*.

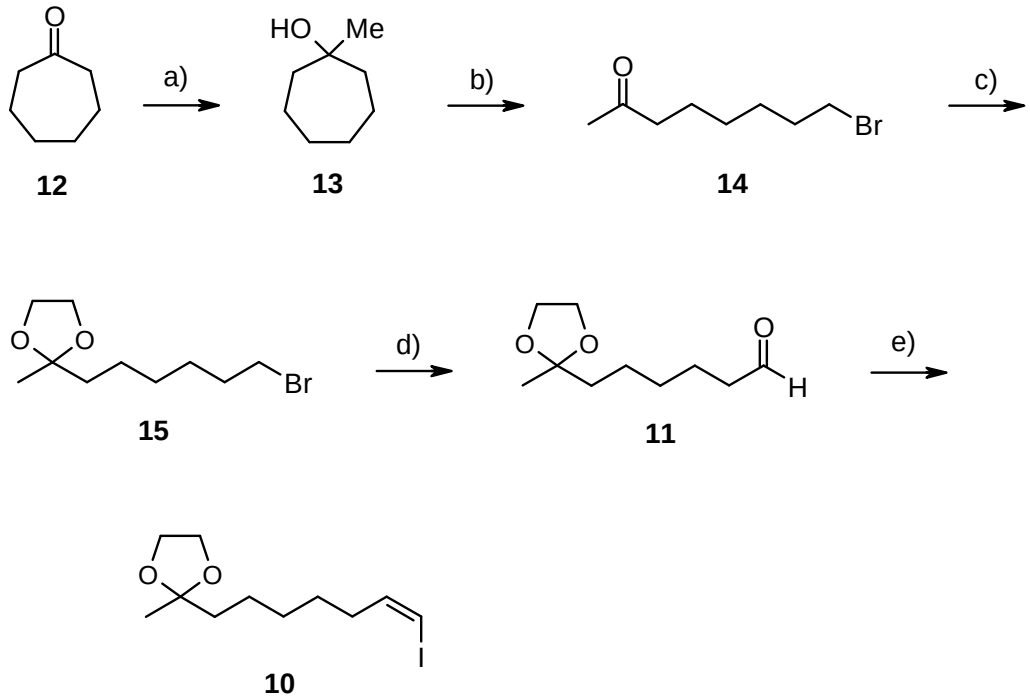
The syntheses of monoalkene **1** and bisacetylenes **2** and **3** have recently been reported by Dickschat et al[13] and Kraus et al.[14,15] Despite the large interest in obtaining synthetic samples of the remaining unsaturated compounds for biological evaluation the other ketoalkenes have not been prepared so far. We therefore planned to synthesize the polyenes **5**-**8** and test their activity at the human cannabinoid receptors in a functional GTPase assay. Our synthetic strategy was to obtain the 1,4-diene motifs via Pd-catalyzed cross coupling reactions of appropriate allyl acetates with vinyl zinc halides that were derived from viyl zinc chloride **10**. This route not only enabled us to prepare all compounds via one common precursor but also allowed for access to non-natural conjugated analogues via Sonogashira and Suzuki cross coupling reactions of the vinyl iodide intermediate **10** (Scheme 1).



Scheme 1. Retrosynthetic analysis of the natural products found in *E. pallida* and their conjugated analogues.

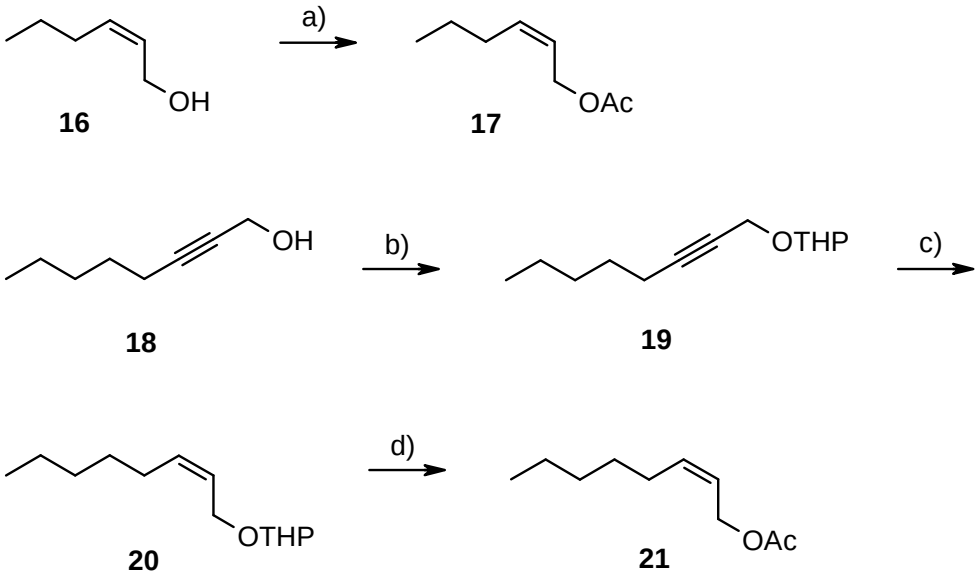
Aldehyde **11** was previously synthesized from methylacetoacetate in 8 steps with an overall yield of 38%.[16] Alternatively, we started from commercially available cycloheptanone which was converted into methylcycloheptanol in quantitative yield. Retro-Barbier fragmentation[17] and protection of the resulting keto group resulted in bromide **15**, which was then oxidized to aldehyde **11** (Scheme 2).[18] The synthesis of aldehyde **11** could thus be shortened by 4 steps and the overall yield increased to 59%.

Conversion to the vinyl iodide **10** was finally achieved by the method of Stork and Zhao in good yield and high stereoselectivity.[19]



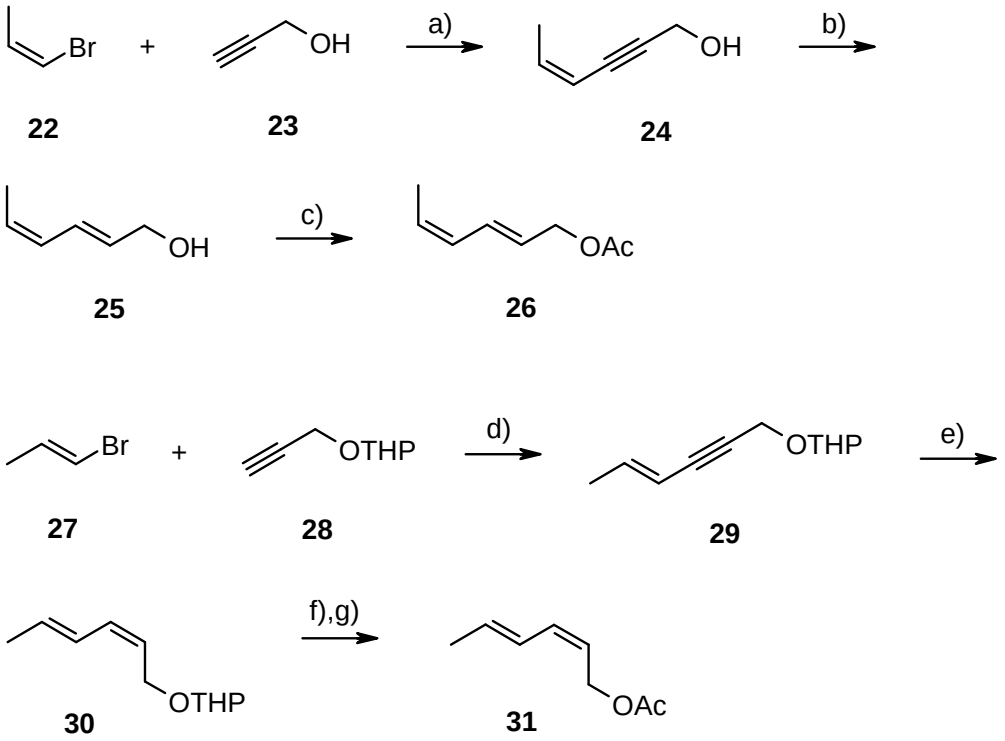
Scheme 2. Improved synthesis of aldehyde **11** and its conversion into the vinyl iodide intermediate **10**. Reagents and conditions: a) MeLi (1.1 eq), Et_2O, -78°C, 100%; b) Br_2, K_2CO_3, $CHCl_3$, 0°C, 93%; c) ethylene glycol, pTsOH, toluene, reflux, 92%; d) trimethylammonium-N-oxide, DMSO, RT, 69%; e) ICH_2PPh_3I, NaHMDS, rt; **11**, -78°C, 81%.

The synthesis of the 1,4-dienes required the selective preparation of *E*- and *Z*-allyl acetates. (*Z*)-Hex-2-enyl acetate **17** was directly obtained from the commercially available corresponding alcohol, whereas (*Z*)-oct-2-enyl acetate **21** was prepared by hydroboration of the THP protected propargyl alcohol **19**, followed by deprotection and acetylation. Reduction of the propargyl acetate was also attempted but turned out to be less selective and gave higher amounts of the undesired trans isomer. Direct conversion of the THP protected alcohol **20** into the acetate **21**[20] was inferior to the sequence of deprotection followed by acetylation due to lower yields and a more difficult separation (Scheme 3).



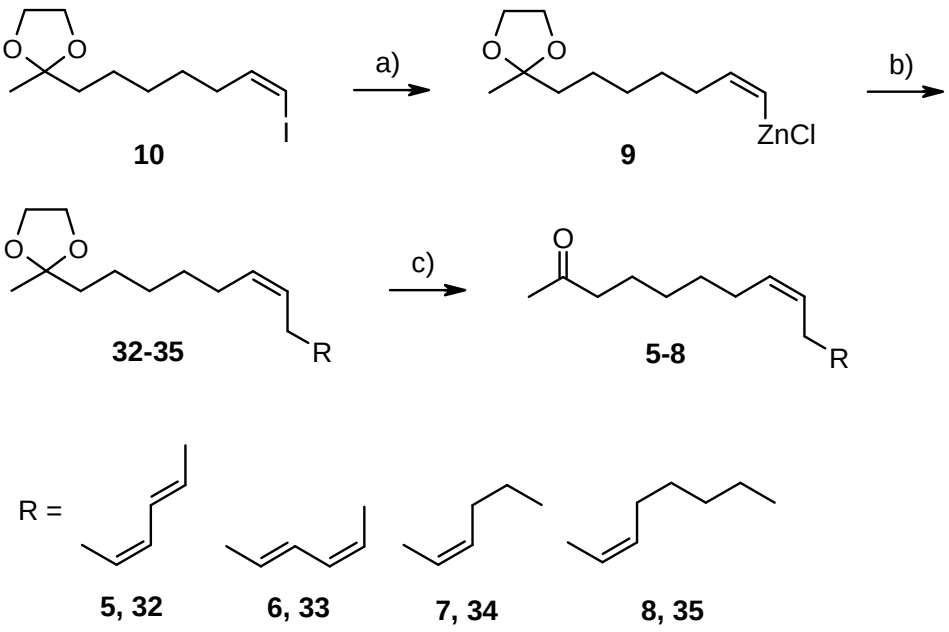
Scheme 3. Synthesis of allyl acetates **17** and **21**. Reagents and conditions: a) Ac_2O, DMAP, NEt_3, CH_2Cl_2, rt, 99%; b) DHP, pTsOH, CH_2Cl_2, rt, 96%; c) BH_3*Me_2S, cyclohexene, Et_2O, 0°C to rt; **19**, 0°C to rt; HOAc, 0°C to rt, 89%; d) 1.) pTsOH, MeOH, rt, 87%; 2.) Ac_2O, DMAP, NEt_3, CH_2Cl_2, rt, 99%.

Diene coupling partners **26** and **31** were prepared from 1,3-enynes **24** and **29**, which were synthesized by Sonogashira cross coupling of *cis*- or *trans*-1-bromo-1-propene and (THP-protected) propargyl alcohol. The *E*-allyl acetate **26** was then prepared via trans-hydroalumination while the *Z*-isomer **31** was again synthesized as described above (Scheme 4).



Scheme 4. Synthesis of the diene coupling partners. Reagents and conditions: a) $PdCl_2(PPh_3)_2$ (2 mol-%), CuI (4 mol-%), piperidine, rt, 91%; b) $LiAlH_4$, THF, -10°C to rt, 97%; c) Ac_2O, DMAP, NEt_3, CH_2Cl_2, rt, 99%; d) $PdCl_2(PPh_3)_2$ (2 mol-%), CuI (4 mol-%), piperidine, rt, 92%; e) BH_3*Me_2S, cyclohexene, Et_2O, 0°C to rt; **29**, 0°C to rt; HOAc, 0°C to rt, 91%; f) pTsOH, MeOH, rt, 84%; g) Ac_2O, DMAP, NEt_3, CH_2Cl_2, rt, 99%.

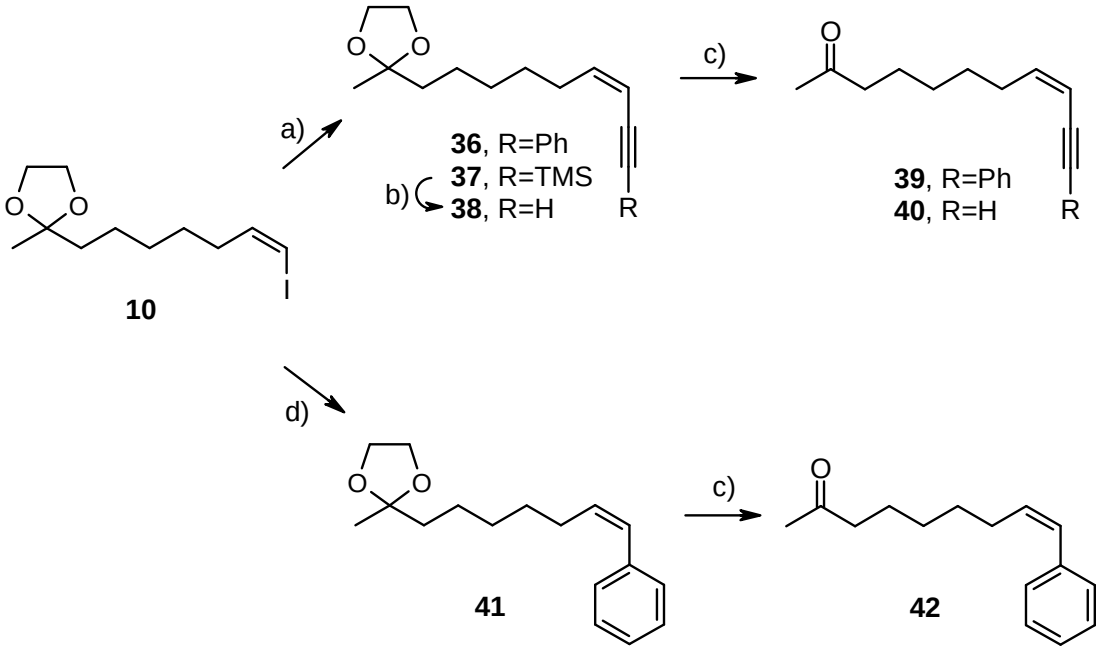
Pd-catalyzed cross coupling between the vinyl zinc chloride **9** and the corresponding allyl acetates gave the desired 1,4-dienes which were finally deprotected to yield the ketoalkenes **5-8** (Scheme 5).



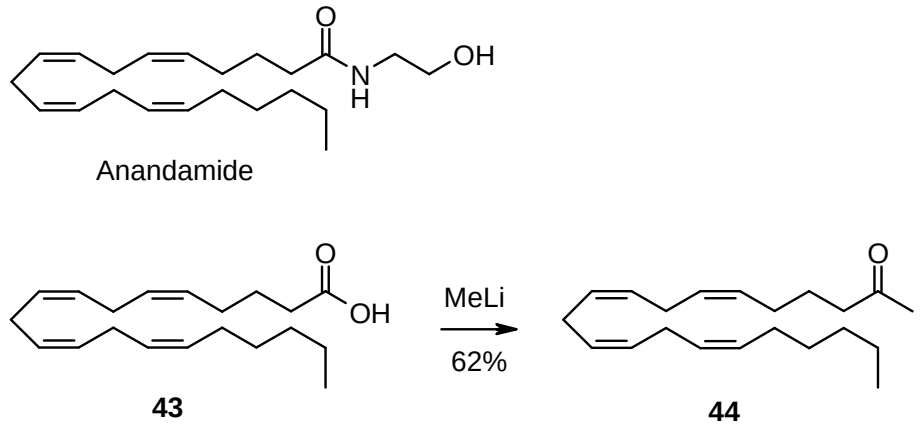
10 **9** **32-35** **5-8** **5, 32** **6, 33** **7, 34** **8, 35**

Scheme 5. Synthesis of the 1,4-dienes **5-8** via Pd-catalyzed cross couplings. Reagents and conditions: a) ^{t}BuLi, THF, -78°C, then $ZnCl_2$, -78°C to rt; b) $PdCl_2(PPh_3)_2$ (3 mol-%), ROAc, THF, rt, 20-27%; c) 1M HCl, THF, rt, quant.

The ketoalkenes of *E. pallida* share two common structural motifs: a skipped conjugation in the unsaturated part and the combination of a methyl ketone and a polyene moiety connected by a flexible alkyl chain. In order to explore whether the skip in conjugation plays an important role in a potential mechanism of action we prepared a series of conjugated analogues via Suzuki and Sonogashira cross coupling reactions of the vinyl iodide intermediate **10** (Scheme 6) and compared their activity at the CBRs to the natural products. We also investigated the influence of an amide versus a methylketone head group, which is the main difference between *E. purpurea* alkamides and *E. pallida* ketoalkenes. For that reason we replaced the amide group of the endogenous ligand anandamide by a simple methylketone, which was prepared by derivatization of arachidonic acid (Scheme 7).



Scheme 6. Preparation of non-natural conjugated analogues. Reagents and conditions: a) RCCH (1.2eq), $PdCl_2(PPh_3)_2$ (5 mol-%), CuI (5 mol-%), NEt_3, rt, 67-71%; b) Bu_4NF, THF, rt, 76%; c) 1M HCl, THF, rt, 80-96% d) $PhB(OH)_2$ (1.1eq), $Pd(OAc)_2$ (5 mol-%), NaOH (2.5eq), THF, reflux, 61%.



Scheme 7. Structure of anandamide and synthesis of its keto analogue **44**.

Pharmacology. Cannabinoid receptors couple to G_i/G_o-proteins and the nucleotide exchange can be monitored as an increase in steady-state GTPase activity.[21] All synthesized compounds were tested for agonistic activity at the CBRs in a steady-state GTPase assay (see Table 1). Compounds were considered to be CBR agonists when GTPase activation was more than 20% compared to basal GTPase activity [3% (v/v) DMSO]. The maximal stimulatory effect of the most abundant endogenous CBR agonist

2-arachidonoyl glycerol (2-AG) was set at 100% to determine E_{max} values of active compounds. The second endogenous CBR agonist, anandamide (ANA), was a full agonist at the CB_1R and a partial agonist at the CB_2R. Compounds **36** and **39** are analogues of the natural ketoalkenynes from *E. pallida* with a keto group at C2, a *cis*-double bond at C8 and a triple bond at C10. Introduction of a phenyl ring in compound **39** led to partial agonism at both CBRs with a potency in the micromolar range. Protection of the keto group at C2 (**36**) resulted in a complete loss of efficacy at the CB_1R and partial agonism at the CB_2R. The potency of compound **36** at the CB_2R was in the micromolar range. Compound **44**, an analogue of the endogenous agonist anandamide, was a full agonist at both CBRs. Replacement of the isobutylamide head group by a methyl ketone even led to a higher efficacy at the CB_2R compared to anandamide. However, the $logEC_{50}$ values of compound **44** were only in the micromolar range compared to anandamide or 2-AG. This finding suggests that substitution of the carboxyl group at C1 with a larger or an amine-containing group, e.g. ethanolamine (anandamide) or glycerol (2-AG) is necessary for a high potency at CBRs.

Table 1. Stimulation of GTPase activity, E_{max} values and $logEC_{50}$ values of 2-AG, anandamide (ANA) and synthesized compounds. GTPase activity was determined in Sf9 insect cell membranes expressing CBRs, $G\alpha_{i2}$, $G\beta_1\gamma_2$ and RGS4. Results are expressed as percentages of mean values ± S.D. compared to basal GTPase activation assessed by 3% (v/v) DMSO. E_{max} values represent the stimulation relative to the endogenous agonist 2-AG (defined as 100% stimulation). Data represents 3 independent experiments performed with different membrane preparations.

Cpd.	**CB_1R**			**CB_2R**		
	GTPase activation [%]	**E_{max} [%]**	**$logEC_{50}$**	**GTPase activation [%]**	**E_{max} [%]**	**$logEC_{50}$**
2-AG	69 ± 6	100	-6.60 ± 0.12	74 ± 25	100	-6.53 ± 0.51
ANA	80 ± 8	116 ± 19	-6.80 ± 0.10	49 ± 6	66 ± 15	-6.36 ± 0.44
36	<20	n.a.	n.a.	33 ± 3	45 ± 6	-5.23 ± 0.62
39	41 ± 20	59 ± 35	-5.28 ± 0.27	44 ± 11	59 ± 18	-5.84 ± 0.19
44	68 ± 6	99 ± 9	-5.71 ± 0.44	85 ± 7	114 ± 9	-5.46 ± 0.29

3.3. Conclusion

We have developed a versatile synthetic route for the preparation of *E. pallida* ketoalkenes and new non-natural conjugated analogues. A particular advantage of our synthetic strategy is the possibility of preparing different conjugated and non conjugated polyene motifs found in many natural products starting from only one common precursor and following the same reaction procedures. The simplicity of the reactions involved and the inexpensive commercially available starting materials are also advantageous.

Both classes of compounds were tested for their ability to activate the human cannabinoid receptors 1 and 2 (CB_1R and CB_2R). In contrast to the alkamides of *E. purpurea*, which have been shown to be CB_2R agonists,[7] no agonistic activity at either receptor was found in the functional GTPase assay for the ketoalkenes found in *E. pallida*. If the observed immunomodulatory activity of *Echinacea* products is mediated via the CBR system, *E. pallida* ketoalkenes will not contribute to this effect. However, a different mode of action for these compounds or synergic effects cannot be excluded and may be responsible for the observed physiological activity of *Echinacea* extracts.[2] Interestingly, the non-natural phenylacetylene containing analogue **39** as well as its keto-protected precursor **36** showed micromolar activity at both receptors. The same was true for the keto analogue **44** of anandamide, indicating that an amide or ester moiety is not necessarily important for activation but for high affinity at the CBRs.

3.4. Experimental Section

General: Commercial reagents and starting materials were purchased from Aldrich, Fluka or Acros and used without further purification. THF was distilled from sodium/benzophenone. Flash chromatography was performed on Acros silica gel 60 A (35-70 µm). TLC was performed on alumina plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm) and products were detected after staining with 15% phosphomolybdic acid in ethanol. 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 are: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet, dt = double triplet. The relative number 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 SSQ 710 A (CI) and Finnigan MAT 95 (HRMS), IR spectra with a Bio-Rad FT-IR Excalibur FTS 3000 MX spectrometer and UV/Vis spectra with a Cary BIO 50 UV/Vis/NIR spectrometer (Varian). The following compounds were prepared as described above and have been reported before: 1-methylcycloheptanol **13,**[22] 8-bromooctan-2-one (**14),**[17] 2-(6-bromohexyl)-2-methyl-1,3-dioxolane (**15),**[23] (iodomethyl)triphenyl-phosphonium iodide,[24] 6-(2-methyl-1,3-dioxolan-2-yl)hexanal (**11**),[16] (Z)-hex-2-enyl acetate (**17**),[25] 2-(oct-2-ynyloxy)-tetrahydro-2H-pyran (**19**),[26] (Z)-oct-2-en-1-ol,[27] (Z)-hex-4-en-2-yn-1-ol (**24**),[28] (2E,4Z)-hexa-2,4-dien-1-ol (**25**),[28] (E)-2-(hex-4-en-2-ynyloxy)-tetrahydro-2H-pyran (**29**),[29] (2Z,4E)-hexa-2,4-dien-1-ol.[30]

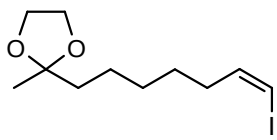
General procedure A for the semi-reduction of alkynes to the corresponding Z-alkenes: Borane-dimethylsulfide complex (1.1 eq) was added to dry Et_2O (~1 M) and cooled to 0 °C. Cyclohexene (2.2 eq) was slowly added by syringe and the mixture was stirred at 0 °C for 15 min before it was warmed to rt and stirred for 1 h. The white suspension was again cooled to 0 °C and the alkyne (1 eq) was slowly added via syringe. The mixture was stirred for 1 hr at 0 °C and 1.5 h at rt until the white precipitate completely dissolved and the reaction mixture was again cooled to 0 °C and acetic acid (~ 9 eq) was slowly added. The ice bath was removed and the solution was stirred at rt for 2 h. Et_2O was added and the organic phase was washed with water (4x), dried over $MgSO_4$, concentrated and purified by flash chromatography on silica gel (petroleum ether/EtOAc).

General procedure B for the acetylation of allyl alcohols: To a 0.3 M solution of the alcohol in dry DCM, triethylamine (1.3 eq), freshly distilled acetic anhydride (1.1 eq) and catalytic amounts of DMAP was added. The mixture was stirred at rt until complete consumption of the alcohol (~ 4 h), diluted with DCM and washed with water. The aqueous phase was extracted with DCM and the organic phases were dried over $MgSO_4$. The solvent was removed and the product purified by flash chromatography on silica gel (pentane/Et_2O).

General procedure C for the Sonogashira cross coupling with 1-bromo-1-propene and various acetylenes: To a 0.3 M solution of bromopropene (1.1 eq) in freshly distilled piperidine, $PdCl_2(PPh_3)_2$ (2 mol-%), the terminal acetylene (1 eq) and CuI (4 mol-%) was added. The mixture was stirred at rt until complete consumption of the acetylene, quenched with saturated solution of NH_4Cl and extracted with Et_2O. The combined organic phases were washed with brine, dried over $MgSO_4$, concentrated and purified by flash chromatography on silica gel (pentane/Et_2O).

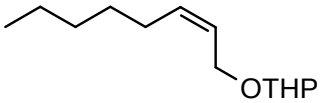
General procedure D for the Pd-catalyzed cross coupling of vinyl zinc chlorides and allyl acetates. Under an argon atmosphere a 0.2 M solution of vinyl iodide **11** in freshly distilled dry THF (Na/benzophenone) was cooled to -78 °C. ^tBuLi (1.6 M in pentane, 2.2 eq) was added via syringe and the mixture was stirred for 1 hr at -78 °C. Dry $ZnCl_2$ (1.1 eq) was added and the mixture was slowly warmed to rt. $PdCl_2(PPh_3)_2$ (5 mol%) and the allyl acetate were then added and the mixture was stirred at rt overnight, concentrated under a stream of nitrogen, quenched with saturated NH_4Cl and extracted with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$, concentrated and purified by flash chromatography on silica gel (petroleum ether/EtOAc).

General procedure E for the deprotection of 1,3-dioxolane-protected ketones: The dioxolane was dissolved in THF (5 mL) and 1M HCl (1 mL) was added. The mixture was stirred at rt overnight, diluted with Et_2O, washed with aqueous saturated solution of $NaHCO_3$ and dried over $MgSO_4$. The solvents were removed under reduced pressure and the products were purified by flash chromatography on silica gel (petroleum ether/EtOAc).

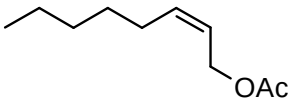


(Z)-2-(7-Iodohept-6-enyl)-2-methyl-1,3-dioxolane (10). To a suspension of (iodo-methyl)triphenylphosphonium iodide (5.1 g, 9.7 mmol) in dry THF (75 mL), 1 M sodium bis-(trimethylsilyl)amide in THF (11 mmol) was added. The orange suspension was cooled to -78 °C and DMPU (5.9 mL) was added. After 15 min aldehyde **11** (1.2 g, 6.4 mmol) was added via syringe and the mixture was slowly warmed to rt. The solvent

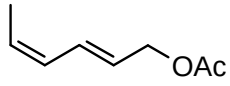
was removed under reduced pressure and the resulting brown oil was dissolved in DCM, washed with water and concentrated. Byproducts were removed by precipitation with petroleum ether, the mother liquor was concentrated and the product was purified by flash chromatography on silica gel to yield vinyl iodide **10** as a colourless oil (1.6 g, 81%). R_f = 0.31 (petroleum ether/EtOAc 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (s, 3H; CH_3), 1.33-1.46 (m, 6H; CH_2), 1.61-1.66 (m, 2H; CH_2), 2.10-2.17 (m, 2H; CH_2), 3.88-3.98 (m, 4H; OCH_2), 6.12-6.19 (m, 2H; CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.8 (+), 23.9 (-), 27.9 (-), 29.3 (-), 34.6 (-), 39.1 (-), 64.6 (-), 82.3 (+), 110.1 (C_{quat}), 141.3 (+); IR (neat): ν = 2981, 2937, 1375, 1069 cm^{-1}; MS (CI, NH_3): *m/z* (%) = 311 (100) [MH^+], 328 (12) [MNH_4^+].



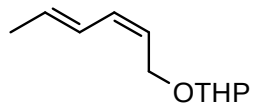
(Z)-2-(Oct-2-enyloxy)-tetrahydro-2H-pyran (20). The compound was prepared following general procedure A and isolated as colourless oil. Yield: 83%. R_f = 0.29 (petroleum ether/EtOAc 19:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, ^3J = 6.7 Hz, 3H; CH_3), 1.21-1.41 (m, 6H; CH_2), 1.50-1.88 (m, 6H; CH_2), 2.04-2.10 (m, 2H; CH_2), 3.47-3.55 (m, 1H), 3.85-3.92 (m, 1H), 4.04-4.10 (m, 1H), 4.23-4.29 (m, 1H), 4.64 (t, ^3J = 2.2 Hz, 1H), 5.50-5.63 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1, 19.6, 22.5, 25.5, 27.5, 29.3, 30.7, 31.4, 62.2, 62.8, 97.9, 125.7, 133.9; IR (neat): ν = 2925, 2856, 1455, 1025 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 102 (100) [DHP+NH_4^+], 230 (18) [MNH_4^+].



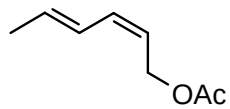
(Z)-Oct-2-enyl acetate (21). The compound was prepared following general procedure B and isolated as colourless oil. Yield: 99%. R_f = 0.31 (petroleum ether/EtOAc 19:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, ^3J = 7.0Hz, 3H; CH_3), 1.23-1.42 (m, 6H; CH_2), 2.03-2.12 (m, 5H; CH_2/CH_3), 4.61 (d, ^3J = 6.6 Hz, 2H; OCH_2), 5.48-5.69 (m, 2H; CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0 (+), 21.0 (+), 22.5 (-), 27.5 (-), 29.1 (-), 31.4 (-), 60.4 (-), 123.2 (+), 135.6 (+), 171.0 (C_{quat}); IR (neat): ν = 3024, 2928, 1739, 1372, 1225 cm^{-1}; CI-MS (NH_3): *m/z* (%)=188 (100) [MNH_4^+].



(2E,4Z)-Hexa-2,4-dienyl acetate (26). The compound was prepared following general procedure B and isolated as colourless oil. Yield: 99%. R_f = 0.33 (petroleum ether/ EtOAc 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.77 (dd, ^3J = 7.1 Hz, ^4J = 1.6 Hz, 3H; CH_3), 2.07 (s, 3H; CH_3), 4.62 (d, ^3J = 6.6 Hz, 2H; CH_2), 5.57 (dq, ^3J = 7.3 Hz, ^3J = 11.0 Hz, 1H), 5.73 (dt, ^3J = 7.0 Hz, ^3J = 15.4 Hz, 1H), 6.01 (dt, ^4J = 1.4 Hz, ^3J = 11.0 Hz, 1H), 6.55-6.65 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.4 (+), 21.0 (+), 65.0 (-), 125.9 (+), 128.2 (+), 128.2 (+), 129.6 (+), 170.8 (C_{quat}); IR (neat): ν = 3023, 1738, 1373, 1222 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 158 (10) [MNH_4^+], 98 (100) [MNH_4^+-HOAc].

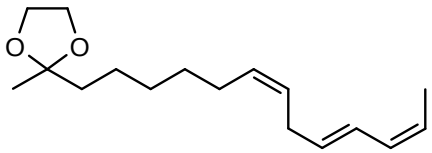


2-((2Z,4E)-Hexa-2,4-dienyloxy)-tetrahydro-2H-pyran (30). The compound was prepared following general procedure A and isolated as colourless oil. Yield: 91%. R_f = 0.36 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.50-1.88 (m, 6H; CH_2), 1.78 (d, ^3J = 6.3 Hz, 3H; CH_3), 3.49-3.56 (m, 1H), 3.86-3.93 (m, 1H), 4.16-4.38 (m, 2H), 4.64-4.66 (m, 1H), 5.44 (dt, ^3J = 7.1 Hz, ^3J = 11.0 Hz, 1H), 5.75 (dq, ^3J = 6.7 Hz, ^3J = 14.8 Hz, 1H), 6.11 (t, ^3J = 11.0 Hz, 1H), 6.29-6.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.3 (+), 19.5 (-), 25.5 (-), 30.7 (-), 62.3 (-), 62.8 (-), 97.8 (+), 124.4 (+), 126.6 (+), 131.6 (+), 131.8 (+); IR (neat): ν = 3024, 2940, 1117, 1021 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 102 (100) [DHP+NH_4^+], 200 (22) [MNH_4^+].

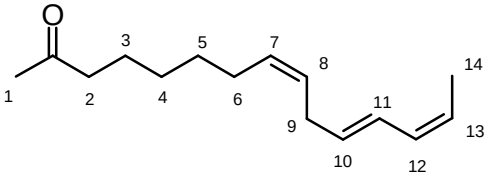


(2Z,4E)-Hexa-2,4-dienyl acetate (31). The compound was prepared following general procedure B and isolated as colourless oil. Yield: 99%. R_f = 0.39 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.80 (d, ^3J = 6.0 Hz, 3H; CH_3), 2.07 (s, 3H; CH_3), 4.72 (d, ^3J = 7.4 Hz, 2H; CH_2), 5.40 (dt, ^3J = 7.5 Hz, ^3J = 11.0 Hz, 1H), 5.81 (dq, ^3J = 7.0 Hz, ^3J = 14.8 Hz, 1H), 6.15 (t, ^3J = 11.1 Hz, 1H), 6.30-6.39 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4 (+), 21.0 (+), 60.5 (-), 121.5 (+), 126.0 (+), 132.9 (+), 133.2 (+), 171.0

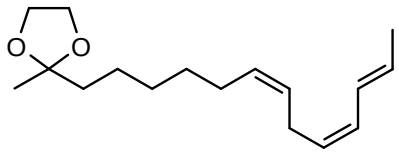
(C_{quat}); IR (neat): ν = 3031, 1735, 1372, 1223 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 158 (71) [MNH_4^+], 98 (100) [MNH_4^+-HOAc].



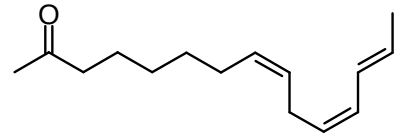
2-Methyl-2-((6Z,9E,11Z)-trideca-6,9,11-trienyl)-1,3-dioxolane (33). The compound was prepared following general procedure D and isolated as colourless oil. Yield: 27%. R_f = 0.36 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (s, 3H; CH_3), 1.31-1.43 (m, 6H; CH_2), 1.58-1.65 (m, 2H; CH_2), 1.73 (dd, ^3J = 6.9 Hz, ^4J = 1.6 Hz, 3H; CH_3), 2.01-2.07 (m, 2H, CH_2), 2.84 (t, ^3J = 6.4 Hz, 2H; CH_2), 3.88-3.98 (m, 4H; OCH_2), 5.34-5.49 (m, 3H), 5.63 (dt, ^3J = 7.0 Hz, ^3J = 15.1 Hz, 1H), 5.93-6.03 (m, 1H), 6.30-6.39 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.3 (+), 23.7 (+), 24.0 (-), 27.1 (-), 29.5 (-), 29.6 (-), 30.6 (-), 39.2 (-), 64.6 (-), 110.2 (C_{quat}), 124.4 (+), 125.6 (+), 126.9 (+), 129.4 (+), 131.0 (+), 132.3 (+); IR (neat): ν = 2981, 2935, 1375, 1064 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 265 (100) [MH^+], 282 (26) [MNH_4^+].



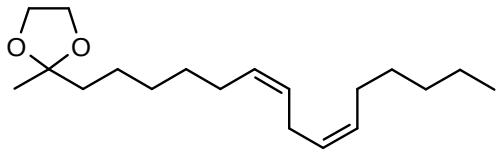
(8Z,11E,13Z)-Pentadeca-8,11,13-trien-2-one (6). The compound was prepared following general procedure E and isolated as colourless oil in quantitative yield. R_f = 0.33 (CH_2Cl_2); ^{1}H NMR (600 MHz, C_6D_6, COSY): δ = 1.09-1.14 (m, 2H, CH_2; H(4)), 1.23 (q, ^3J = 7.5 Hz, 2H, CH_2; H(5)), 1.43 (q, ^3J = 7.6 Hz, 2H, CH_2; H(3)), 1.62 (dd, ^3J = 7.2 Hz, ^4J = 1.8 Hz, 3H, CH_3; H(14)), 1.64 (s, 3H, CH_3; H(1)), 1.90 (t, ^3J = 7.3 Hz, 2H, CH_2; H(2)), 1.95-1.99 (m, 2H, CH_2; H(6)), 2.81 (t, ^3J = 6.7 Hz, 2H, CH_2; H(9)), 5.34 (dq, ^3J = 7.1 Hz, ^3J = 10.7 Hz, 1H, H(13)), 5.42-5.52 (m, 2H, H(7)/H(8)), 5.62 (dt, ^3J = 6.9 Hz, ^3J = 15.1 Hz, 1H, H(10)), 6.06-6.10 (m, 1H, H(12)), 6.44-6.48 (m, 1H, H(11)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.3 (+), 23.7 (-), 27.0 (-), 28.8 (-), 29.4 (-), 29.9 (+), 30.6 (-), 43.7 (-), 124.4 (+), 125.6 (+), 127.1 (+), 129.3 (+), 130.7 (+), 132.2 (+), 209.3 (C_{quat}); IR (neat): ν = 3014, 2932, 2856, 1716, 1358 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 238 (100) [MNH_4^+].



2-Methyl-2-((6Z,9Z,11E)-trideca-6,9,11-trienyl)-1,3-dioxolane (32). The compound was prepared following general procedure D and isolated as colourless oil. Yield: 21%. R_f = 0.27 (petroleum ether/EtOAc 24:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (s, 3H; CH_3), 1.29-1.40 (m, 6H; CH_2), 1.57-1.62 (m, 2H; CH_2), 1.69 (d, ^3J = 6.6 Hz, 3H; CH_3), 2.00 (quint, ^3J = 6.0 Hz, 2H; CH_2), 2.73-2.88 (m, 2H; CH_2), 3.84-3.94 (m, 4H; OCH_2), 5.28-6.03 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.0 (+), 23.7 (+), 24.0 (-), 27.1 (-), 29.5 (-), 29.6 (-), 30.3 (-), 39.2 (-), 64.6 (-), 110.2 (C_{quat}), 127.1 (+), 127.2 (+), 129.9 (+), 130.5 (+), 130.8 (+), 131.5 (+); CI-MS (NH_3): *m/z* (%) = 187 (100) [$(C_{11}H_{22}O_2)H^+$], 204 (61) [$(C_{11}H_{22}O_2)NH_4^+$].

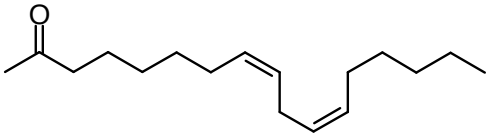


(8Z,11Z,13E)-Pentadeca-8,11,13-trien-2-one (5). The compound was prepared following general procedure E and isolated as colourless oil in quantitative yield. R_f = 0.47 (petroleum ether/EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.25-1.41 (m, 4H; CH_2), 1.52-1.62 (m, 2H; CH_2), 1.73 (d, ^3J = 6.9 Hz, 3H; CH_3), 2.04 (quint, ^3J = 6.3 Hz, 2H; CH_2), 2.13 (s, 3H; CH_3), 2.41 (t, ^3J = 7.7 Hz, 2H; CH_2), 2.77-2.92 (m, 2H; CH_2), 5.33-6.07 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.1 (+), 23.7 (-), 26.9 (-), 28.8 (-), 29.4 (-), 29.9 (+), 30.3 (-), 43.7 (-), 127.2 (+), 127.3 (+), 129.8 (+), 130.5 (+), 130.6 (+), 131.5 (+), 209.3 (C_{quat}); IR (neat): ν = 3013, 2927, 2855, 1716, 1359 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 238 (100) [MNH_4^+], 221 (4) [MH^+].

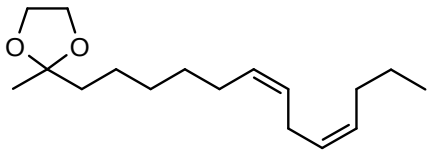


2-Methyl-2-((6Z,9Z)-pentadeca-6,9-dienyl)-1,3-dioxolane (35). The compound was prepared following general procedure D and isolated as colourless oil. Yield: 20%. R_f = 0.42 (petroleum ether/EtOAc 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, ^3J = 7.0 Hz, 3H; CH_3), 1.28-1.46 (m, 15H; CH_2/CH_3), 1.60-1.67 (m, 2H; CH_2), 1.96-2.08 (m,

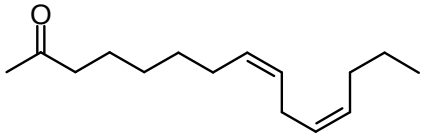
4H; CH_2), 2.74 (t, ^3J = 5.5 Hz, 2H; CH_2), 3.90-4.00 (m, 4H; OCH_2), 5.34-5.50 (m, 4H; CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ =14.1 (+), 22.6 (-), 23.7 (+), 24.0 (-), 27.1 (-), 29.2 (-), 29.5 (-), 29.6 (-), 30.5 (-), 31.4 (-), 32.6 (-), 39.2 (-), 64.6 (-), 110.2 (C_{quat}), 127.9 (+), 128.3 (+), 130.3 (+), 130.9 (+); IR (neat): ν = 2925, 2855, 1376, 1067 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 295 (100) [MH^+], 312 (16) [MNH_4^+].



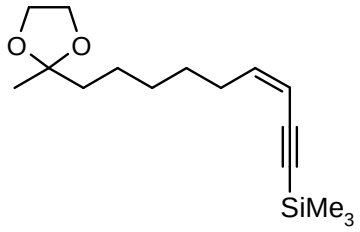
(8Z,11Z)-Heptadeca-8,11-dien-2-one (8). The compound was prepared following general procedure E and isolated as colourless oil in quantitative yield. R_f = 0.20 (petroleum ether/EtOAc 19:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, ^3J = 6.7 Hz, 3H; CH_3), 1.26-1.44 (m, 10H, CH_2), 1.55-1.65 (m, 2H, CH_2), 1.96-2.09 (m, 4H, CH_2), 2.15 (s, 3H, CH_3), 2.44 (t, ^3J = 7.5 Hz, 2H; CH_2), 2.74 (t, ^3J = 5.6 Hz, 2H; CH_2), 5.33-5.50 (m, 4H; CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (+), 22.6 (-), 23.7 (-), 26.9 (-), 28.8 (-), 29.2 (-), 29.4 (-), 29.9 (+), 30.5 (-),31.4 (-), 32.5 (-), 43.8 (-), 128.0 (+), 128.2 (+), 130.1 (+), 130.9 (+), 209.2 (C_{quat}); IR (neat): ν = 3008, 2925, 2855, 1717 cm^{-1}; HRMS (PI-EI) calcd. for $C_{17}H_{30}O$ [$M^{\cdot+}$]: 250.2297, found: 250.2295.



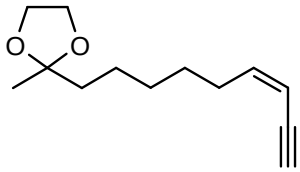
2-Methyl-2-((6Z,9Z)-trideca-6,9-dienyl)-1,3-dioxolane (34). The compound was prepared following general procedure D and isolated as colourless oil. R_f = 0.31 (pentane/Et_2O 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, ^3J = 8.0 Hz, 3H; CH3), 1.25-1.42 (m, 11H), 1.60-1.65 (m, 2H; CH_2), 1.98-2.09 (m, 4H; CH_2), 2.72 (t, ^3J = 5.2 Hz, 2H; CH_2), 3.88-3.98 (m, 4H; OCH_2), 5.32-5.42 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.7 (+), 22.7 (-), 23.7 (+), 24.0 (-), 27.1 (-), 29.5 (-), 29.6 (-), 30.5 (-), 34.7 (-), 39.2 (-), 64.6 (-), 110.2 (C_{quat}), 127.9 (+), 128.5 (+), 130.3 (+), 130.6 (+); IR (neat): ν = 2933, 2873, 1069, 627 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 267 (100) [MH^+], 284 (13) [MNH_4^+].



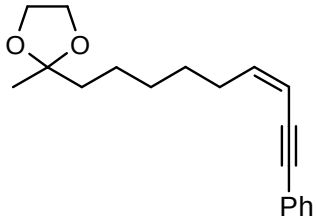
(8Z,11Z)-Pentadeca-8,11-dien-2-one (7). The compound was deprotected following general procedure E and obtained in quantitative yield. R_f = 0.29 (pentane/Et_2O 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (t, ^3J = 8.0 Hz, 3H; CH3), 1.22-1.40 (m, 6H; CH_2), 1.49-1.59 (m, 2H; CH_2), 1.89-2.03 (m, 4H; CH_2), 2.09 (s, 3H; CH3), 2.38 (t, ^3J = 8.0 Hz, 2H; CH_2), 2.68 (t, ^3J = 5.5 Hz, 2H; CH_2), 5.29-5.39 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.7 (+), 22.7 (-), 23.7 (-), 26.9 (-), 28.8 (-), 29.4 (-), 29.9 (+), 30.5 (-), 34.7 (-), 43.8 (-), 128.0 (+), 128.4 (+), 130.1 (+), 130.6 (+), 209.3 (C_{quat}); IR (neat): ν = 2936, 2867, 1711, 631 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 240 (100) [MNH_4^+].



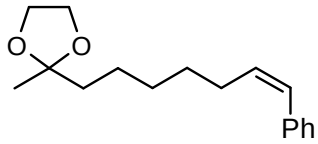
Trimethyl((Z)-9-(2-methyl-1,3-dioxolan-2-yl)non-3-en-1-ynyl)silane (37). To a dry flask **10** (0.23 g, 0.74 mmol), $PdCl_2(PPh_3)_2$ (10 mg, 0.032 mmol), CuI (5 mg, 0.026 mmol), and NEt_3 (3 mL) was added under an inert atmosphere. To the cloudy yellow solution TMS-acetylene (0.10 g, 1.03 mmol) was added. The reaction was stirred at rt for 4 hr, quenched with water (15 mL), extracted with ether (4 x 15 mL), washed with brine and purified by flash chromatography to afford 0.15 g (71%) of product. R_f = 0.40 (petroleum ether/EtOAc 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.20 (s, 9H; $SiMe_3$), 1.32 (s, 3H; CH_3), 1.35-1.47 (m, 6H; CH_2), 1.61-1.68 (m, 2H; CH_2), 2.27-2.37 (m, 2H; CH_2), 3.91-3.97 (m, 4H; OCH_2), 5.48 (dt, ^4J = 1.4 Hz, ^3J = 10.8 Hz, 1H), 5.90-5.99 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 0, 23.7, 23.9, 28.7, 29.4, 30.2, 39.1, 64.6, 102.1, 102.8, 109.1, 110.1, 145.5; IR (neat): *v* = 2941, 2878, 2146 cm^{-1}; HRMS (PI-EI) calcd. for $C_{16}H_{28}O_2Si$ [$M^{\cdot+}$]: 280.1859, found: 280.1855.



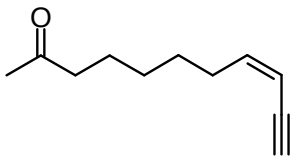
2-Methyl-2-((Z)-non-6-en-8-ynyl)-1,3-dioxolane (38). Compound **37** (0.14 g, 0.49 mmol) was dissolved in THF (25 mL) and Bu_4NF (0.16 g, 0.60 mmol) was added. The reaction mixture was allowed to stir at rt for 30 min, quenched with water (20 mL), extracted with petroleum ether (3×40 mL), washed with water and brine and purified by flash chromatography (EtOAc/hexane 1:9, R_f = 0.3) to afford 0.08 g (0.28 mmol, 76%) of product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (s, 3H), 1.31-1.44 (m, 6H), 1.59-1.63 (m, 2H), 2.23-2.34 (m, 2H), 3.05 (d, *J* = 1.9 Hz, 1H), 3.88-3.95 (m, 4H), 5.40-5.46 (dt, *J* = 1.2 Hz, *J* = 10.8 Hz, 1H), 5.93-6.03 (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 23.7, 23.9, 28.7, 29.3, 30.1, 39.1, 64.6, 80.5, 81.2, 108.1, 110.1, 146.1; IR (neat): *v* [cm^{-1}] = 3291, 2982, 2939, 2163, 2097; MS (CI, NH_3): m/z (%) = 209.2 [MH^+] (100), 226.2 [MNH_4^+] (14).



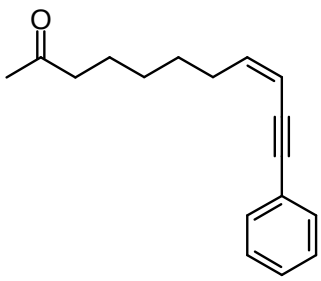
2-Methyl-2-((Z)-9-phenylnon-6-en-8-ynyl)-1,3-dioxolane (36). To a dry flask **10** (0.10 g, 0.32 mmol), $PdCl_2(PPh_3)_2$ (5 mg, 0.016 mmol), CuI (7 mg, 0.037 mmol), and NEt_3 (2.5 mL) was added under an nitrogen atmosphere. To the cloudy yellow solution phenyl acetylene (0.04 g, 0.42 mmol) was added. The reaction was stirred at rt for 4 h, quenched with water (15 mL), extracted with ether, washed with brine and purified by column chromatography (EtOAc/hexane 1:9, R_f = 0.3) to afford 0.06 g, (0.22 mmol, 67%) of product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (s, 3H), 1.34-1.51 (m, 6H), 1.60-1.67 (m, 2H), 2.35-2.44 (m, 2H), 3.88-3.95 (m, 4H), 5.64-5.70 (dt, *J* = 1.2 Hz, *J* = 10.7 Hz, 1H), 5.92-6.10 (m, 1H), 7.27-7.35 (m, 3H), 7.40-7.46 (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 23.7, 23.9, 28.8, 29.4, 30.3, 39.2, 64.6, 86.4, 93.5, 109.1, 110.2, 123.7, 128.0, 128.3, 131.4, 144.2; IR (neat): *v* [cm^{-1}] = 2981, 2983, 2861, 1983; HRMS (PI-EI) calcd. for $C_{19}H_{24}O$ [$M^{\cdot+}$]: 284.1776, found: 284.1770.



2-Methyl-2-((Z)-7-phenylhept-6-enyl)-1,3-dioxolane (41). To a flask containing **10** (78 mg, 0.25 mmol) in THF (2 mL), $Pd(OAc)_2$ (4 mg, 0.02 mmol), a solution of NaOH (29 mg, 0.72 mmol), THF (1 mL) and phenyl boronic acid (39 mg, 0.32 mmol) was added and the reaction mixture was refluxed overnight. The resulting brown solution was extracted with pentane, washed with brine and dried over $MgSO_4$. Flash chromatography (hexane/EtOAc 9:1, R_f = 0.25) afforded 40 mg of product (0.15 mmol, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (s, 3H), 1.32-1.51 (m, 4H), 1.54-1.64 (m, 2H), 2.33 (dq, *J* = 5.8 Hz, *J* = 1.6 Hz, 2H), 3.89-3.95 (m, 4H), 5.65 (m, 1H), 6.40 (m, 1H), 7.18-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$): δ = 23.8, 24.0, 28.6, 29.6, 30.0, 39.2, 64.7, 110.2, 125.9, 126.5, 128.1, 128.8, 133.1 137.8; IR (neat): *ν* [cm^{-1}] = 2981, 2939, 2178, 2004; MS (CI, NH_3): m/z (%) = 261.2 (100) [MH^+], 278.3 (33) [MNH_4^+].

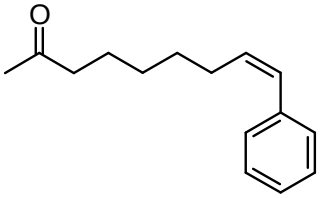


(Z)-Undec-8-en-10-yn-2-one (40). The compound was prepared following general procedure E and isolated as colourless oil in 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28-1.46 (m, 4H; CH_2), 1.54-1.65 (m, 2H; CH_2), 2.13 (s, 3H; CH_3), 2.30-2.36 (m, 2H; CH_2), 2.43 (t, ^3J = 7.3 Hz, 2H; CH_2), 3.07 (d, ^4J = 1.9 Hz, 1H), 5.41-5.48 (m, 1H), 5.93-6.03 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.6, 28.5, 28.6, 29.9, 30.0, 43.7, 80.5, 81.3, 108.3, 145.8, 209.2; IR (neat): *ν* = 3290, 3263, 2934, 2858, 2151, 2022, 1712 cm^{-1}; HRMS (PI-EI) calcd. for $C_{11}H_{15}O$ [MH^+]: 163.1123, found: 163.1123.

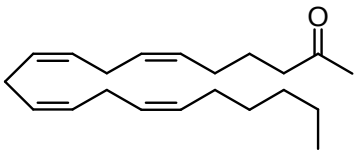


(Z)-11-Phenylundec-8-en-10-yn-2-one (39). The compound was prepared following general procedure E and isolated as colourless oil in 80% yield. ^{1}H NMR (300 MHz,

$CDCl_3$): δ = 1.30-1.67 (m, 6H; CH_2), 2.11 (s, 3H; CH_3), 2.38-2.46 (m, 4H; CH_2), 5.65-5.71 (m, 1H), 5.91-6.10 (m, 1H), 7.28-7.34 (m, 3H), 7.41-7.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.7, 28.6, 28.7, 29.9, 30.2, 43.7, 86.4, 109.3, 128.1, 128.3, 131.4, 143.9, 209.2; IR (neat): *ν* = 3019, 2929, 2855, 2197 cm^{-1}; HRMS (PI-EI) calcd. for $C_{17}H_{20}O$ [$M^{\cdot}$+]: 240.1514, found: 240.1517.



(Z)-9-Phenylnon-8-en-2-one (42). The compound was prepared following general procedure E and isolated as colourless oil in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.16-1.46 (m, 6H; CH_2), 2.07 (s, 3H; CH_3), 2.22-2.34 (m, 2H; CH_2), 2.36 (t, ^3J = 7.4 Hz, 2H; CH_2), 5.59 (m, 1H), 6.36 (m, 1H), 7.13-7.32 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.7, 28.4, 28.8, 29.7, 29.9, 43.7, 126.5, 128.1, 128.7, 128.9, 132.8, 137.7, 209.2; IR (neat): *ν* = 3006, 2929, 2855, 2206, 2006 cm^{-1}; HRMS (PI-EI) calcd. for $C_{15}H_{20}O$ [$M^{\cdot+}$]: 216.1514, found: 216.1513.



(6Z,9Z,12Z,15Z)-Henicosa-6,9,12,15-tetraen-2-one (44). Arachidonic acid **43** (0.25 g, 0.8 mmol) was dissolved in dry THF (2 mL). MeLi (1.6 M in Et_2O, 2.1 mL, 3.3 mmol) was added at 0°C resulting in a dark red solution. After 2 hr, freshly distilled TMS-chloride (0.6 mL, 4.8 mmol) was added and the mixture was allowed to warm to rt. The resulting colourless solution was treated with 2M HCl (2 mL), diluted with Et_2O, washed with water and brine, dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel gave **44** as colourless oil (150 mg, 62%). R_f = 0.40 (hexane/Et_2O 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, ^3J = 6.7 Hz, 3H; CH_3), 1.25-1.39 (m, 6H; CH_2), 1.64 (quint, ^3J = 7.8 Hz, 2H; CH_2), 2.02-2.11 (m, 4H; CH_2), 2.13 (s, 3H; CH_3), 2.43 (t, ^3J = 7.9 Hz, 2H; CH_2), 2.78-2.85 (m, 6H; CH_2), 5.28-5.44 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1, 22.6, 23.6, 25.7, 26.5, 27.2, 29.3, 29.9, 31.5, 43.0, 127.5, 127.9, 128.2, 128.2, 128.6, 128.8, 129.2, 130.5, 208.9; IR (neat): ν = 3011, 2925, 2857, 1715, 631 cm^{-1}; CI-MS (NH_3): *m/z* (%) = 320 (100) [MNH_4^+].

Pharmacological Materials. GTP, ATP, adenylyl imidodiphosphate, creatine kinase, creatine phosphate, bovine serum albumin and salts (highest purity available) were purchased either from Roche (Mannheim, Germany) or Sigma (St. Louis, MO, USA). Dimethyl sulfoxide was from Merck (Darmstadt, Germany). Tris base was purchased from USB (Cleveland, OH, USA). The cannabinoid receptor ligands 2-arachidonoyl glycerol and anandamide were purchased from Tocris Cookson (Ballwin, MO, USA). [γ-^{32}P]GTP was synthesized through enzymatic phosphorylation of GDP and [^{32}P]orthophosphoric acid (8,000 Ci/mmol, PerkinElmer Life Sciences, Boston, MA, USA) as described previously[31]. Mono(cyclohexyl)-ammoniumphosphoenolpyruvate, pyruvate kinase and myokinase were from Sigma (St. Louis, MO, USA). Stock solutions of 2-arachidonoyl glycerol, anandamide and synthesized compounds (10 mM each) were prepared in dimethyl sulfoxide. Dilutions of ligands were prepared in such a way that the dimethyl sulfoxide concentration was 30% (v/v) and that the final dimethyl sulfoxide concentration in the GTPase assay tubes was 3% (v/v).

Pharmacological Methods. The steady-state GTPase assay is an established approach to study G-protein coupling of GPCRs at a very proximal point of the signal transduction cascade. The GTPase assay was performed as described previously.[32] Recombinant baculoviruses encoding FLAG- and hexahistidine-tagged hCB_1R and hCB_2R, $G\alpha_{i2}$, $G\beta_1\gamma_2$ and RGS4 (Regulator of G-protein Signaling 4) were generated in Sf9 insect cells using the BaculoGOLD transfection kit (BD PharMingen, San Diego, CA, USA).[21, 32, 33] For transfection, cells were seeded (cell density 3.0 x 10^6 cells/mL) and infected with a 1:100 dilution of high-titer baculovirus stocks. Cells were cultured for 48 h, Sf9 membranes were prepared as described previously[33] and used in the steady-state GTPase assay. Assay tubes contained Sf9 membranes (15 μg of protein/tube), 1.0 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM ATP, 100 nM GTP, 0.1 mM adenylyl imidodiphosphate, 5 mM creatine phosphate, 40 μg of creatine kinase, 0.2% (w/v) bovine serum albumin in 50 mM Tris/HCl, pH 7.4, and synthesized or reference compounds at various concentrations. Reaction mixtures (80 μL) were incubated for 2 min at 25 °C before the addition of 20 μL of [γ-^{32}P]GTP (0.1 μCi/tube). All stock and work dilutions of [γ-^{32}P]GTP were prepared in 20 mM Tris/HCl, pH 7.4. Reactions were conducted for 20 min at 25 °C. Reactions were terminated by the addition of 900 μL of slurry consisting of 5% (w/v) activated charcoal and 50 mM NaH_2PO_4, pH 2.0. Charcoal absorbs nucleotides but not $^{32}P_i$. Charcoal-quenched reaction mixtures were

centrifuged for 7 min at room temperature at 15000 x g. 600 µL supernatant fluid of reaction mixtures was removed, and $^{32}P_i$ was determined by Čerenkov radiation in 3 mL water. Enzyme activities were corrected for spontaneous degradation of [γ-^{32}P]GTP. Spontaneous [γ-^{32}P]GTP degradation amounted to <1% of the total amount of radioactivity added. The experimental conditions chosen ensured that not more than 10% of the total amount of [γ-^{32}P]GTP added was converted to $^{32}P_i$. The GTPase activity was calculated and expressed as pmoles of P_i released per mg of membrane protein per min.

3.5. References

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4. Synthesis and Pharmacological Properties of New Tetracyclic Forskolin Analogues

New tetracyclic analogues of forskolin were prepared by derivatization of the natural product. Treatment of a primary obtained cyclic thionocarbonate with $HSnBu_3$/AIBN lead to 5-exo-trig cyclization with the double bond, whereas Corey-Winter conditions resulted in a rearrangement with formation of a cyclic carbonate.[i] Two of these new analogues were investigated for their ability to activate adenylyl cyclases 1, 2 and 5.[ii]

[i] Forskolin analogues **5** and **6** were first prepared by Prantik Maity and resynthesized for complete characterization.

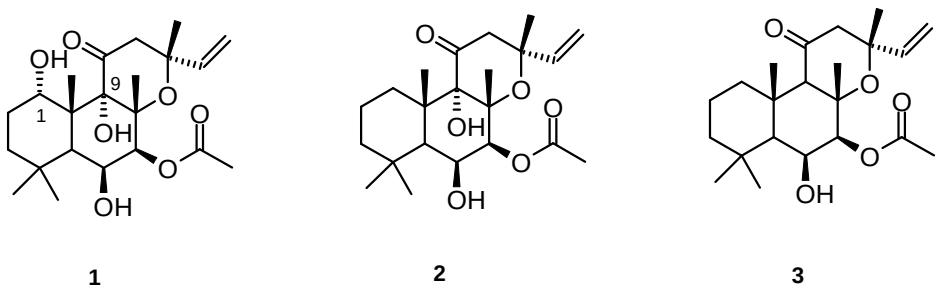
[ii] The adenylyl cyclase activity assay was carried out by Melanie Hübner at the Institute of Pharmacy, University of Regensburg.

4.1. Introduction

The roots of *Coleus forskohlii*, a member of the mint family that can be found in the subtropical areas of India, Pakistan, Sri Lanka and Brazil, have long been used for the treatment of asthma, glaucoma and cardiovascular diseases.[1] In 1977 Bhat et al. isolated the labdane diterpenoid forskolin as major component[2] which displayed antihypertensive and positive inotropic effects.[3] Forskolin was shown to directly activate the enzyme adenylyl cyclase (AC), thereby increasing the intracellular level of cAMP and leading to various physiological effects such as hypotension, cardiac inotropy, bronchodilation and reduction of intraocular pressure.[4] Nine membranous AC isoforms with distinct tissue distribution and regulatory properties are known to date.[5] Although AC 1-8 represents an interesting pharmaceutical target that is directly activated by forskolin, the lack of AC isoform selectivity resulting in numerous side effects has prevented a clinical use of forskolin so far. Recently we have shown that AC isoforms are not uniformly modulated by forskolin analogues which demonstrates the feasibility to develop highly desired isoform-selective AC activators and inhibitors.[6] Herein we report the synthesis and pharmacological characterization of new tetracyclic forskolin analogues.

4.2. Results and Discussion

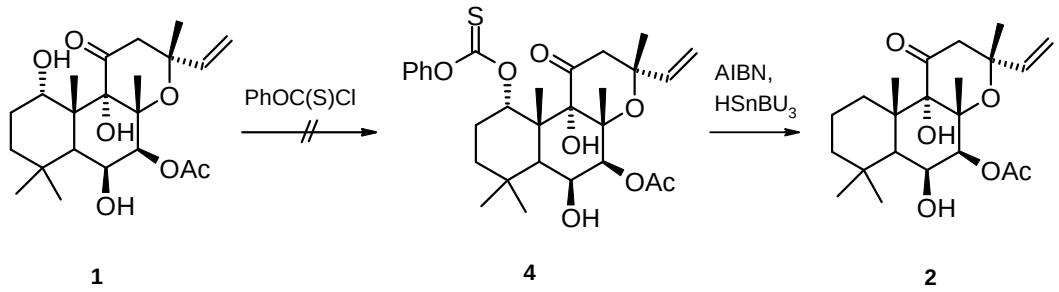
Synthesis. During our investigations on forskolin (**1**) analogues as isoform-selective adenylyl cyclase modulators we wanted to reassess the role of the 1-OH and 9-OH groups and therefore reinvestigate the activity profiles of 1-deoxyforskolin **2** and 1,9-dideoxyforskolin **3** (Figure 1). Both compounds were isolated as minor constituents from *Coleus forskohlii*.[2,7] 1,9-Dideoxyforskolin **3** has been synthesized starting from either E,E-farnesol[8] or the natural products larixol[9] and ptychantin A,[10] respectively and was itself used as starting material for the preparation of 1-deoxyforskolin **2**.[7]



1 **2** **3**

Figure 1. Structures of forskolin (**1**), 1-deoxyforskolin (**2**) and 1,9-dideoxyforskolin (**3**).

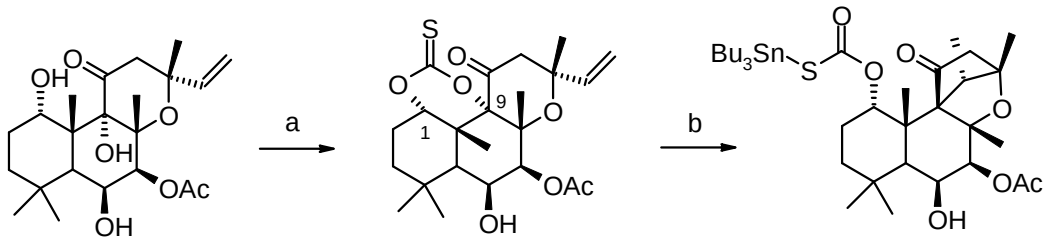
Because we had a reasonable amount of forskolin in hand we intended to prepare the deoxyforskolins **2** and **3** directly from forskolin which had not been described so far. From the many OH group functionalizations of forskolin it is known that apart from few exceptions 1-OH is generally more reactive than the sterically hindered 6-OH and especially 9-OH group.[4b,11] We therefore planned to selectively convert the 1-OH group into a suitable thiocarbonyl precursor which should then be used for a radical deoxygenation to provide 1-deoxyforskolin **2**. However, although this reaction has been described for a similar substrate,[10a] all attempts to form thionocarbonate **4** with O-phenylchlorothionoformate failed and only unreacted starting material was recovered (Scheme 1).



1 **4** **2**

Scheme 1. Attempted formation of **4** and its intended use in a radical deoxygenation.

When O-phenylchlorothionoformate was exchanged by thiocarbonyldiimidazole, quantitative conversion to the corresponding 1,9-disubstituted cyclic product **5** was observed. Treatment of **5** with $HSnBu_3$/AIBN resulted not only in the cleavage of the C9-O-bond but at the same time led to the formation of a new bicyclic ring system (Scheme 2).



1 **5** **6**

Scheme 2. Formation of the new forskolin analog **6** via the radical precursor **5**. Reagents and conditions: a) thiocarbonyldiimidazole, DMAP, CH_2Cl_2, rt, 97%; b) $HSnBu_3$, AIBN, toluene, reflux, 85%.

Compound **5**, in which both axial hydroxy groups are connected via a thionocarbonate bridge, crystallizes in the chiral space group P 21 21 21 (Figure 2).

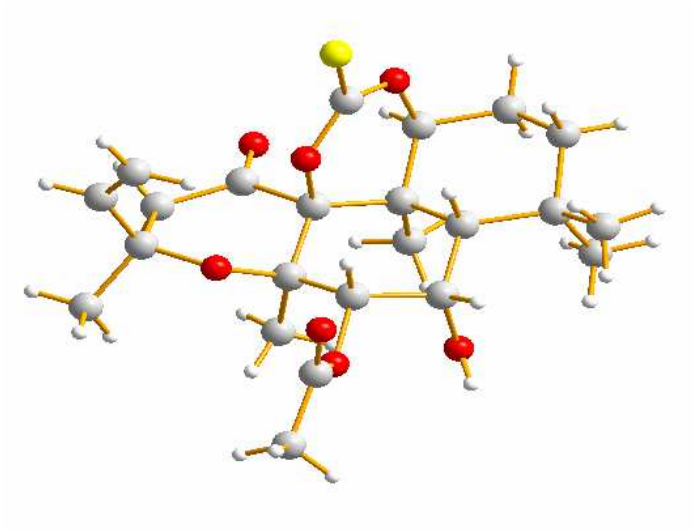
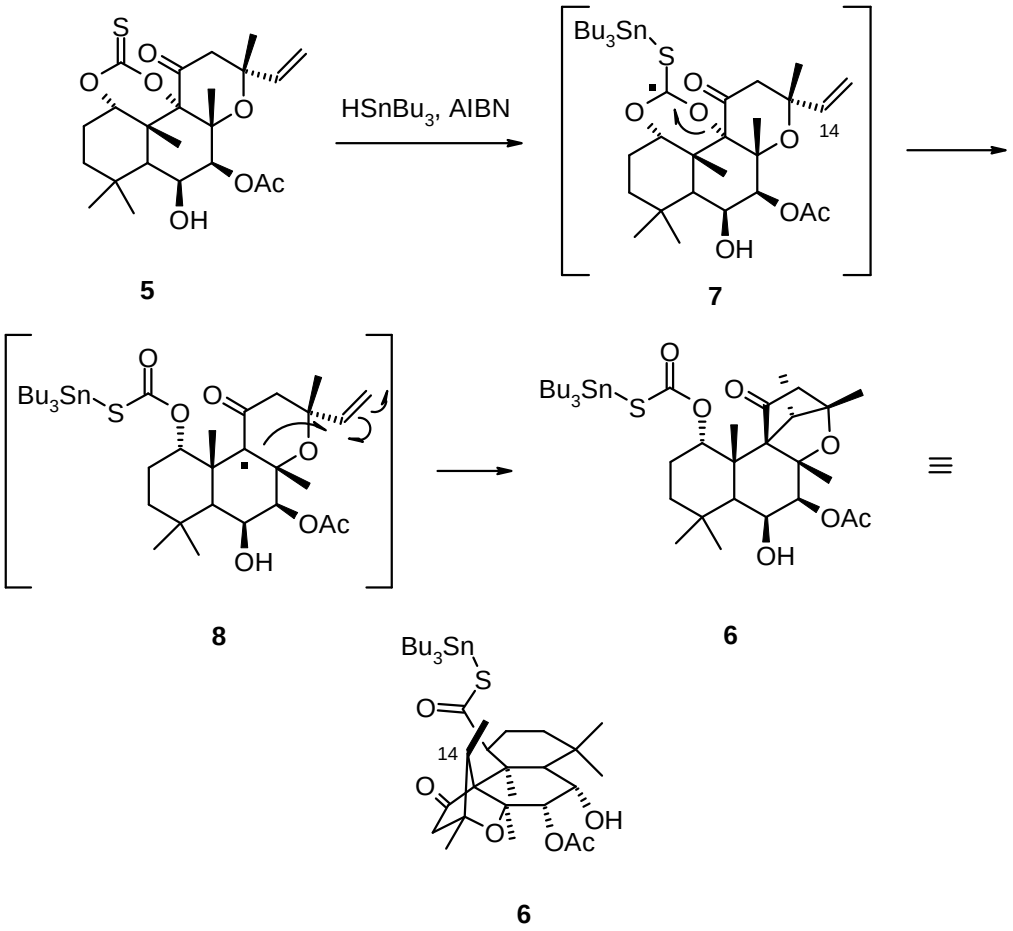


Figure 2. X-ray crystal structure of thionocarbonate **5**.

The proposed mechanism for the formation of **6** is shown in scheme 3. The primary radical **7** fragments in a way that the more stable tertiary radical at C9 is formed (compound **8**) as compared to the potential secondary C1 radical. This new radical intermediate then intramolecularly attacks the double bond at C14 in a 5-exo-trig cyclization to give after H-abstraction the new tetracyclic forskolin analogue **6**.



Scheme 3. Proposed mechanism for the formation of **6**.

The X-ray diffraction analysis of compound **6** revealed that the newly formed stereocenter at C14 is *S*-configured (figure 3).

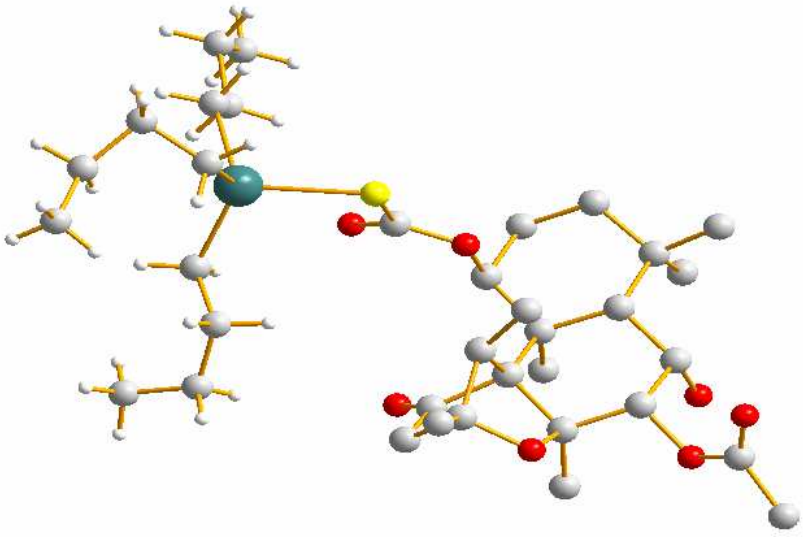
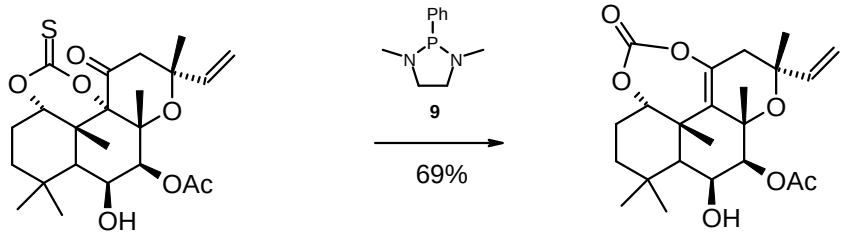


Figure 3. X-ray crystal structure of compound **6**. Hydrogen atoms of the forskolin skeleton are removed for more clarity.

In addition to this radical pathway **5** was also treated with 1,3-dimethyl-2-phenyl-1,3-diaza-phospholidine **9**, which is generally used for a mild variant of the Corey-Winter olefination of thionocarbonates derived from 1,2-diols,[12] to find out whether the thionocarbonate group in **5** could be removed. After stirring **5** with **9** at 50 °C, a new product had formed and X-ray structure analysis revealed that again a new ring was incorporated in the forskolin skeleton (compound **10**, Scheme 4, see Figure 4 for the X-ray crystal structure).



5 **10**

Scheme 4. Formation of the new forskolin analogue **10**.

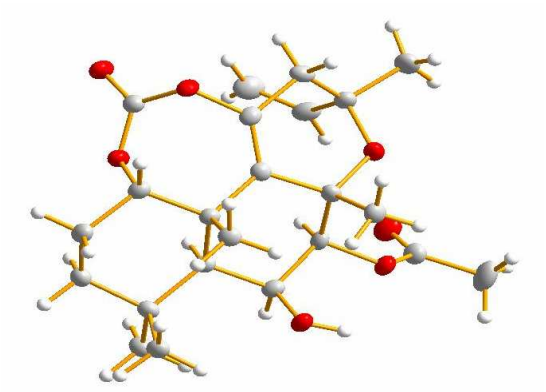
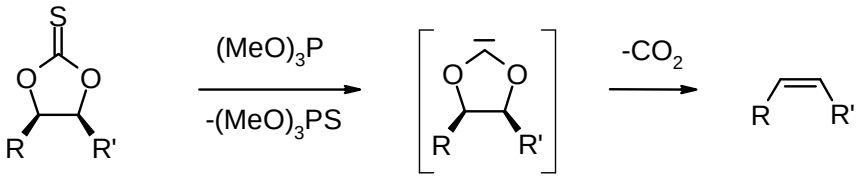


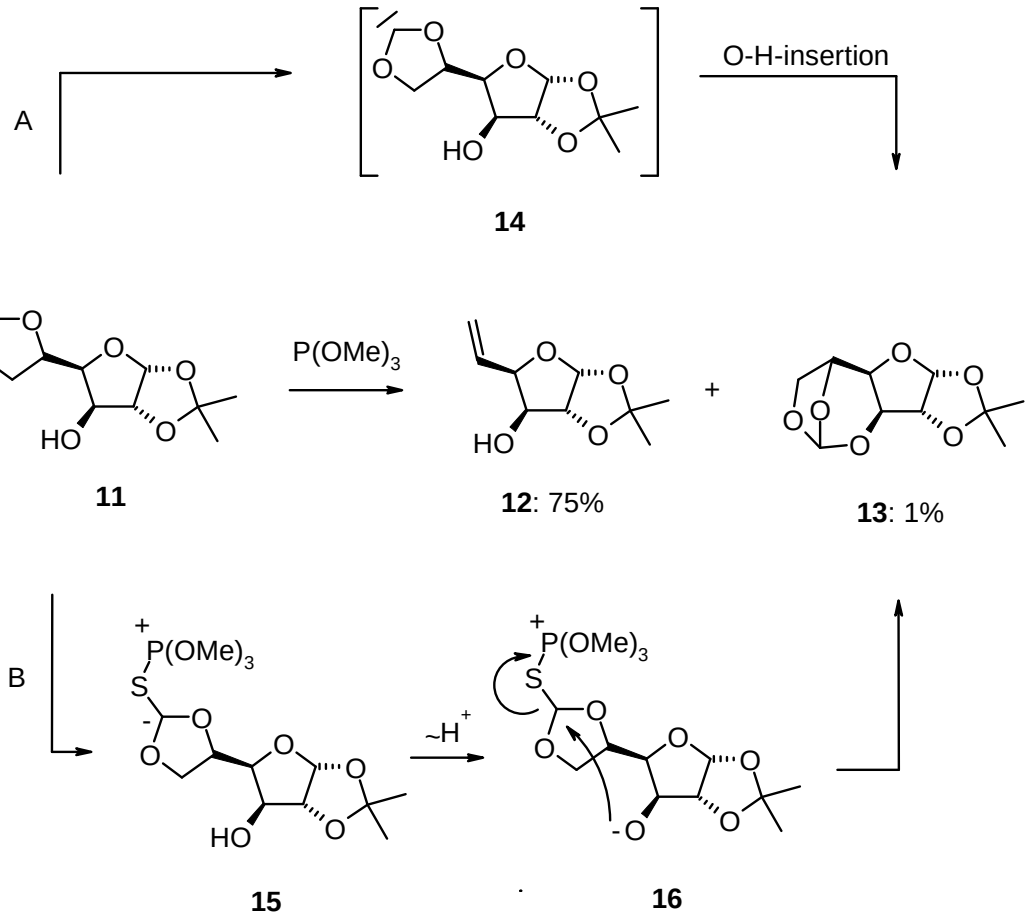
Figure 4. X-ray crystal structure of compound **10**.

The Corey-Winter olefination is postulated to proceed over carbene intermediates (scheme 5),[13] but the exact mechanism seems to be more complex and is not fully clarified.[14]



Scheme 5. Proposed mechanism of the Corey-Winter olefination.

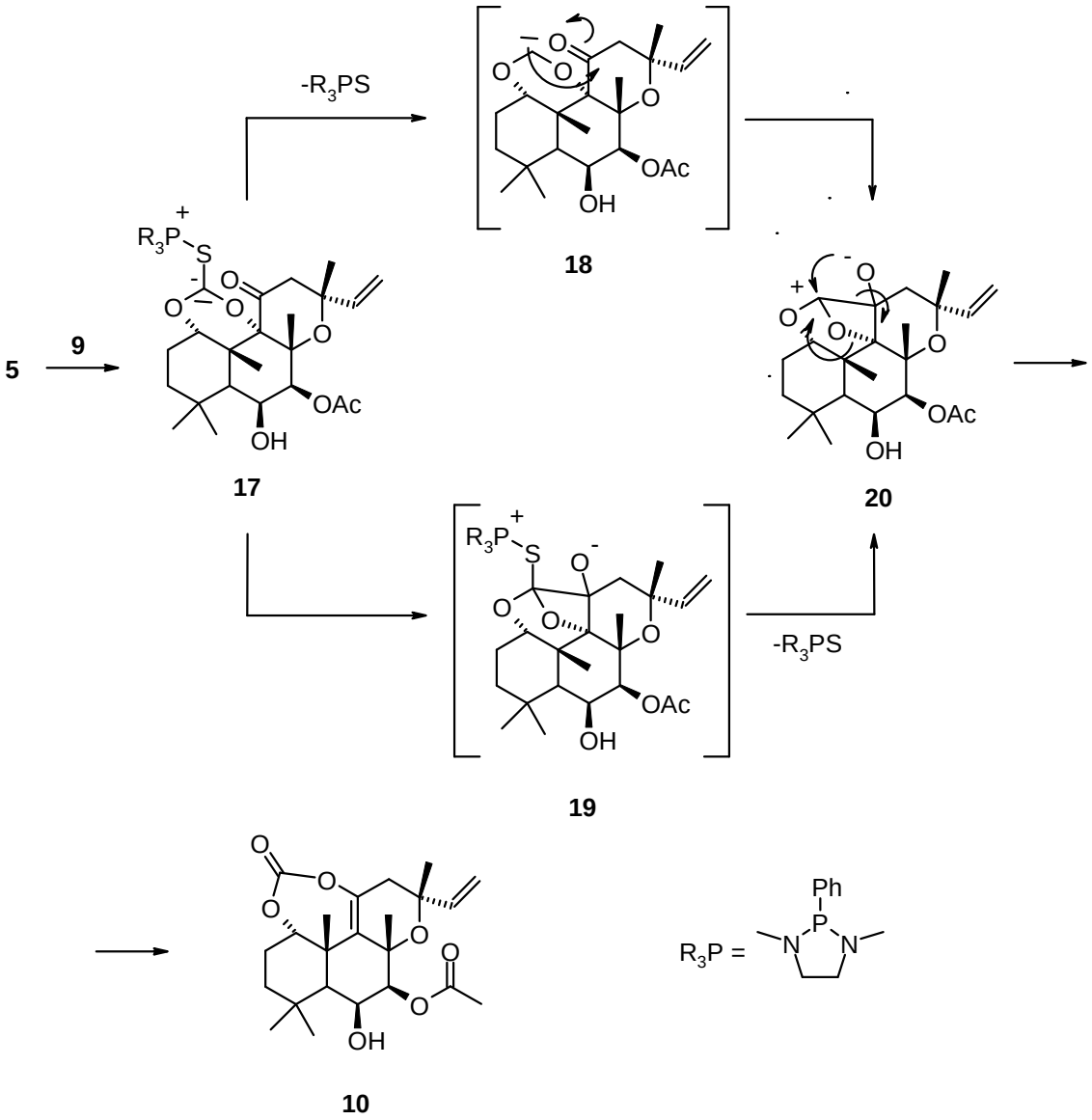
In the case of trithiocarbonates Corey and Märkl suggested the formation of phosphite ylide intermediates.[15] The results from Scherowsky and Weiland on the other hand point to the existence of 1,3-dipoles as reactive intermediates and argue against carbenes.[16] The existence of carbenes in the case of thionocarbonates is supported by the detection of a small amount of orthoester **13** in the reaction from **11** to **12**[17] (Scheme 6) which is generally cited as direct evidence for the intermediacy of carbenes.[14] However, this result may not only be explained by a carbene intermediate **14** (path A) which is claimed by the authors but also via a 1,3-dipole **15** that first deprotonates the free alcohol followed by nucleophilic attack of the alkoxide under elimination of thionophosphate (path B, Scheme 6).



Scheme 6. Possible reaction pathways that could explain the formation of side product **13**.

Based on these results a proposed mechanism for the formation of **10** is shown in Scheme 7. Thiophilic attack at 1,3-dimethyl-2-phenyl-1,3-diazaphospholidine **9** leads to

the 1,3-dipole **17** which then either looses diazaphospholidinesulfide to give a carbene intermediate **18** or directly attacks the C11 carbonyl group to form **19**. Elimination of diazaphospholidinesulfide from **19** would then lead to the same zwitterion **20** that is formed after nucleophilic attack of the carbene **18** at the carbonyl group. Such inter- and intramolecular reactions of dialkoxycarbenes with carbonyl compounds are well-known in literature.[18]



Scheme 7. Proposed mechanism for the formation of **10**.

Finally, in a concerted mechanism the alkoxide attacks the carbenium ion, the C11 – C(O)O-bond is broken and the new 7-membered ring is formed. An orthoester intermediate that would result from the attack of the alkoxide at the carbenium ion or in analogy to the formation of **13** from **16** (Scheme 6) can probably be ruled out due to the very high ring strain that would be generated in such a compound.

Adenylyl Cyclase Activity Assay. Due to the fact that forskolin is a direct activator of membranous AC-isoforms 1-8, it would be very interesting to identify new forskolin analogues as pharmaceuticals that show subtype-specificity and a higher potency in comparison to forskolin.[6] The aim of the pharmacological characterization of the new compounds was on the one hand to find out whether the tetracyclic analogues are still capable of binding to the forskolin binding site of different ACs and on the other hand to investigate their AC isoform selectivity. In our previous studies forskolin was found to have the highest potency on AC 1 compared to AC 2 and AC 5.[6] AC 1 is predominantly expressed in hippocampal areas of the brain and a decreased AC 1 activity is measured in Alzheimer's disease.[19] The identification of AC 1-specific activators could therefore be useful for the treatment of Alzheimer's disease. The effects of the new forskolin analogues **5** and **10** were investigated on ACs 1, 2 and 5, whereas the tin-containing analogue **6** was not used for pharmacological studies due to toxicological properties that are associated with organotin compounds.[20] For a better comparison of the molecular interactions of the compounds in recombinant membrane and tissue preparations we also studied the effect of the forskolin analogues in mouse cardiac membranes. Because it was shown in several studies that cardiac membranes are enriched in ACs 5 and 6, but have smaller quantities of ACs 1, 3, 4 and 7[5b, 21] we wished to find out whether an isoform-selectivity for AC 5 might be observed. The results of the AC activity assays are shown in Figure 5 and Table 1.

Table 1. Calculated potencies and efficacies of the new forskolin analogues **5** and **10**.

AC	compound **5**		compound **10**		forskolin	
	EC_{50}/µM	Efficacy/%	EC_{50}/µM	Efficacy/%	EC_{50}/µM	Efficacy/%
I	20.8 ± 0.7	17.1 ± 4.6	8.03 ± 1.08	45.3 ± 1.9	0.74 ± 0.07	100
II	228.6 ± 35	-18.7 ± 1.7	46.6 ± 11.1	19.7 ± 4.5	14.3 ± 3.0	100
V	65.2 ± 16.1	21.5 ± 1.7	7.3 ± 0.9	19.0 ± 2.5	4.65 ± 0.6	100
cardiac	100.8 ± 30	40.1 ± 4.2	41.7 ± 3.7	62 ± 3.9	4.75 ± 0.8	100

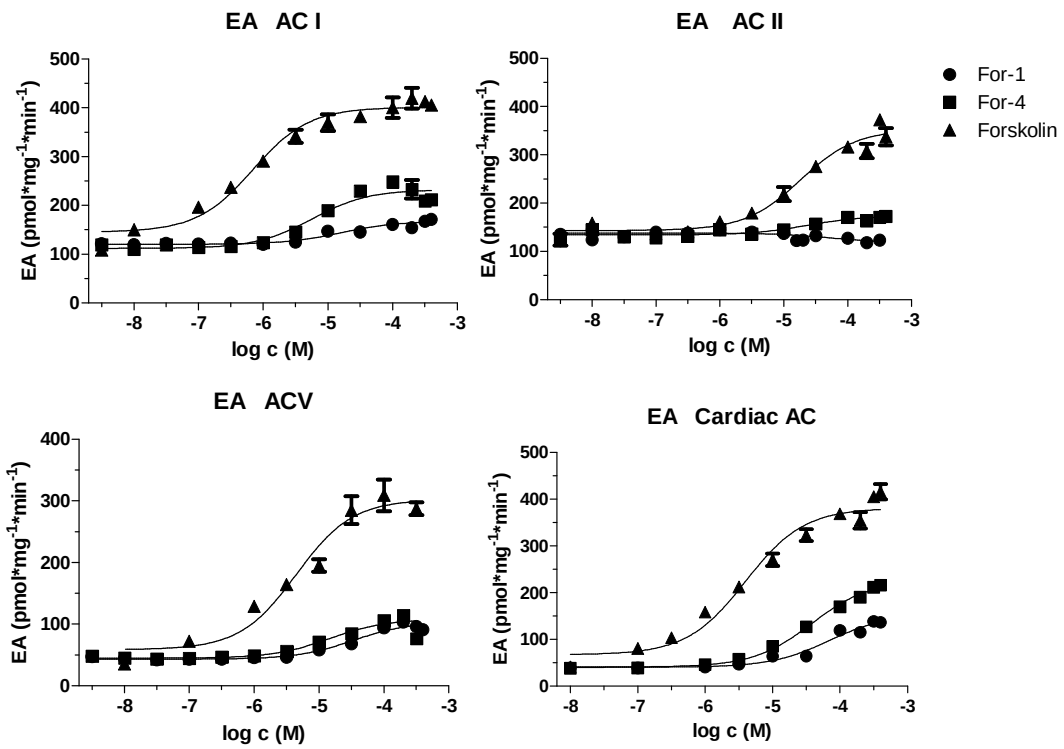


Figure 5. AC stimulation of recombinant AC isoforms 1, 2 and 5 as well as cardiac membranes by compounds **5** (filled circle) and **10** (filled squares) with 10 mM Mn^{2+} in comparison with forskolin (filled triangles).

Compared to forskolin, compounds **5** and **10** showed lower potency and efficacy for all isoforms examined. The cyclic thionocarbonate **5** is a weak partial agonist on AC 1 and AC 5 and even inhibits AC 2. Interestingly, **5** shows a stronger efficacy but lower potency on the cardiac membrane preparation. This effect could be due to an activation of other existing AC isoforms. The cyclic carbonate **10** has a higher potency on ACs 1, 2 and 5 compared to compound **5**, however, the EC_{50} values on ACs 1 and 5 are very similar and do not significantly differ from AC 2. Compound **5** has only a 3-fold higher potency on AC 1 than on AC 5. Both compounds do not show substantial selectivity for one of the investigated specific AC isoforms.

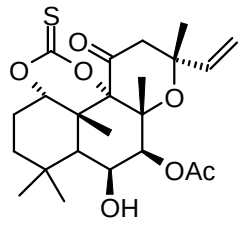
4.3. Conclusion

The introduction of another ring into the forskolin skeleton did not lead to a loss of binding affinity to AC. Although the new compounds are much more spacious they still seem to fit into the binding pocket located in close proximity to the catalytic domains C1 and C2 and lead to enzyme activation. However, both investigated compounds

stimulate cAMP production with a significantly lower efficacy and potency than forskolin does. Effective binding of the catalytic subunits seems to be prevented leading to less effective enzyme activation.

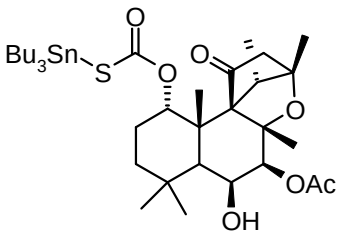
4.4. Experimental Section

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 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 after staining with 15% phosphomolybdic acid in ethanol. Melting points were determined with a Büchi SMP 20 and are uncorrected. NMR spectra were recorded with a Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) instrument. 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, dd = double doublet. The relative number 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 (ESI) were recorded with a Finnigan MAT TSQ 7000 spectrometer and IR spectra with a Bio-Rad FT-IR-FTS 155 spectrometer.



Compound **5**. An oven-dried schlenk tube was charged with forskolin (100 mg, 0.24 mmol) and dry DCM (2 mL). DMAP (60 mg, 0.49 mmol) and thiocarbonyldiimidazole (46 mg, 0.26 mmol) were added under nitrogen and the mixture was allowed to stir under nitrogen atmosphere at room temperature for 4 h. After consumption of all starting material as judged by TLC the solvent was removed and the crude product was purified by flash chromatography on silica gel (petrol ether/acetone 19:6, R_f = 0.27) to obtain the product as white crystalline solid (107 mg, 97%).

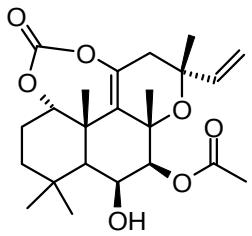
mp > 225°C – ^{1}H NMR (300 MHz) δ = 1.05 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.29-1.31 (m, 1H, CH), 1.41 (s, 3H, CH_3), 1.48-1.53 (m, 2H, CH/OH), 1.69 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 1.85-2.00 (m, 2H, CH), 2.08-2.16 (m, 1H, CH), 2.18 (s, 3H, C(O)CH_3), 2.52 (d, ^{2}J = 16.0 Hz, 1H, CH), 3.11 (d, ^{2}J = 16.0 Hz, 1H, CH), 4.49-4.52 (m, 1H), 4.85 (t, J = 2.7 Hz, 1H), 5.07 (dd, J_{cis} = 10.7 Hz, ^{1}J = 0.8 Hz, 1H), 5.33 (dd, J_{trans} = 17.0 Hz, ^{1}J = 0.8 Hz, 1H), 5.34 (d, ^{3}J = 4.1 Hz, 1H), 6.01 (dd, J_{trans} = 17.0, J_{cis} = 10.6 Hz, 1H) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.8 (+), 21.1 (+), 23.0 (+), 23.2 (+), 29.9 (+), 32.5 (+), 33.8 (C_{quat}), 36.2 (-), 36.6 (C_{quat}), 44.1 (+), 50.6 (-), 68.8 (+), 76.0 (+), 76.9 (C_{quat}), 80.3 (C_{quat}), 84.1 (+), 89.0 (C_{quat}), 111.9 (-), 144.7 (+), 169.3 (C_{quat}), 187.0 (C_{quat}), 200.1 (C_{quat}) – IR: ν = 3407, 2931, 1721, 1375, 1251 – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 453 (100) [MH^+], 470 (67) [MNH_4^+] – Elemental analysis calcd (%) for $C_{23}H_{32}O_7S$ (452.57): C 61.04, H 7.13, S 7.08; found C 61.12, H 7.00 S 7.29.



Compound **6**. A solution of thionocarbonate **5** (25 mg, 0.06 mmol), tributyltinhydride (0.47 mL, 0.17 mmol), and azoisobutyronitrile (1 mg, 6 µmol) in toluene (1.5 mL) under nitrogen atmosphere was heated at 100 °C for 4 h. After consumption of all starting material as jugded by TLC the reaction was quenched by adding petrol ether. Column chromatography (petrol ether → petrol ether/ether 1:4) afforded the crystalline compound **3** (35 mg, 85%).

mp > 225°C – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, ^{3}J = 7.7 Hz, 9H, 3 CH_3), 1.04, (s, 3H, CH_3), 1.13-1.19 (m, 2H), 1.21-1.39 (m, 17H), 1.51-1.63 (m, 13H), 1.67 (s, 3H, CH_3), 1.70-1.75 (m, 2H), 1.97-2.07 (m, 3H), 2.18 (s, 3H, C(O)CH_3), 2.29 (dd, J = 1.4 Hz, J = 17.8 Hz, 1H), 4.38-4.43 (m, 1H, CH), 5.08-5.12 (m, 1H, CH), 5.44 (d, ^{3}J = 3.8 Hz, 1H, CH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.7 (+), 15.0 (-), 15.4 (+), 17.5 (+), 19.6 (+), 21.3 (+), 22.9 (-), 25.4 (+), 25.8 (+), 27.0 (-), 27.9 (C_{quat}), 28.6 (-), 34.2 (+), 35.9 (-), 42.3 (C_{quat}), 45.7 (+), 51.5 (-), 53.3 (+), 69.4 (+), 71.5 (C_{quat}), 78.3 (+), 79.2 (+), 80.9 (C_{quat}), 81.4 (C_{quat}), 170.0 (C_{quat}), 171.3 (C_{quat}), 210.2 (C_{quat}) – IR: ν = 2863, 1741, 1369, 1238 – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 745 (31)

[MH^+] – despite several attempts satisfying combustion analysis or HRMS data could not be obtained for this compound.



Compound **10**. Thionocarbonate **5** (50 mg, 0.1 mmol) and 1,3-dimethyl-2-phenyl-1,3-diazaphospho-lidine **9** (107 mg, 0.5 mmol) were mixed in a small screw-capped glass vial and stirred at 50 °C for 12 h. The crude product was purified by flash chromatography on silica gel (petrol ether/acetone 4:1, R_f = 0.24) and isolated as white solid (29 mg, 69%). Crystallization for X-ray analysis was achieved from n-hexane.
mp = 198°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.00 (s, 3H, CH_3), 1.17-1.23 (m, 1H, CH_2), 1.22 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.48 (d, ^3J = 2.5 Hz, 1H, CH), 1.63 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.68-1.78 (m, 1H, CH_2), 1.82-1.90 (m, 1H, CH_2), 2.12-2.24 (m, 1H, CH_2), 2.19 (s, 3H, CH_3), 2.40-2.58 (m, 2H, CH_2), 4.37-4.39 (m, 1H, CH), 4.68-4.70 (m, 1H, CH), 4.98-5.05 (m, 3H, CH), 5.88-5.97 (m, 1H, CH) – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 21.3 (+), 23.7 (+), 23.9 (-), 24.7 (+), 27.5 (+), 29.6 (+), 32.4 (+), 34.1 (C_{quat}), 36.3 (-), 38.3 (-), 43.5 (C_{quat}), 46.7 (+), 69.5 (+), 74.3 (C_{quat}), 77.2 (C_{quat}), 77.6 (+), 83.3 (+), 112.9 (-), 127.8 (C_{quat}), 140.7 (C_{quat}), 142.6 (+), 152.6 (C_{quat}), 169.8 (C_{quat}) – IR: ν = 2950, 1762, 1739, 1224, 1036 – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 438 (100) [MNH_4^+], 421 (44) [MH^+] – Elemental analysis calcd (%) for $C_{23}H_{32}O_7$ (420.51): C 65.70, H 7.67; found C 65.48, H 7.80.

Materials. Baculoviruses for the expression of ACs 1, 2 and 5 were kindly provided by Drs. G. Gilman and R. K. Sunahara (University of Texas Southwestern Medical Center, Dallas, TX). Insect cells of *Spodoptera frugiperda* (Sf9) were from the American Type Cell Culture Collection (Rockville, MD). Guanosine-5´-[γ-thio] triphosphate (GTPγS), creatine kinase and adenosine triphosphate (ATP) were purchased from Roche Diagnostics (Mannheim, Germany). Isobutylmethylxanthine (IBMX) and cyclic adenosine monophosphate (cAMP) were purchased from Sigma Aldrich (Seelze, Germany) and forskolin was purchased from LC Laboratories (Woburn, MA). Forskolin and the forskolin analogues **5** and **10** were prepared in DMSO stock solutions (20 mM

each) and stored at -20 °C. The final DMSO concentration in the assay was 2% (v/v) DMSO. [α-^{32}P]-ATP (800 Ci/mmol) was from Perkin Elmer (Wellesley, MA) and neutral alumina (N Super I) was from MP Biomedicals (Eschwege, Germany). Highest quality $MnCl_2$ was purchased from Merck (Darmstadt, Germany). Protein concentration was determined using the DC protein assay kit (Bio-Rad, Hercules, CA).

AC expression and membrane preparation of Sf9 cells. Cell culture of Sf9 cells, expression of AC 1, 2 and 5 in Sf9 membranes and membrane preparation were performed as described previously.[22] The membrane preparations were stored in 1 mL aliquots at -80 °C. After thawing, the membranes were sedimented by a 15 min centrifugation at 4 °C, 13000 × g and resuspended in 75 mM TRIS buffer, pH 7.4, to obtain a concentration of 30 µg membrane protein/tube.

Preparation of cardiac AC. Female CD1 mice were housed in a controlled environment according to the German animal protection law. At the age of ten weeks, mice were sacrificed by cervical dislocation and hearts were removed, shock-frozen in liquid nitrogen and stored at -80 °C. Hearts were thawed and rinsed in ice-cold homogenization buffer containing 5 mM Tris·HCl, pH 7.4, and 5 mM EDTA. The ratio of buffer volume to heart tissue was 20-fold. Hearts were fragmented and homogenized in a glass-glass homogenizer (Braun, Melsungen, Germany) at 1500 rpm. After removal of organ debris (centrifugation at 500×g, 8 min) the supernatant was centrifuged at 40000 × g for 30 min. The suspended membranes were washed three times to remove residual endogenous ligands and nucleotides and then resuspended in assay buffer consisting of 50 mM triethanolamine, 10 mM $MnCl_2$ and 1 mM EGTA, pH 7.4. The membrane preparation was shock-frozen in liquid nitrogen and stored at -80 °C. The frozen cardiac membranes were thawn and centrifuged to reach a concentration of 20 µg cardiac membrane protein/tube.

AC- activity assay. AC-activity was determined as described before.[6] For the AC 1, 2 and 5 membrane preparation, the assay tubes contained 10 µL of forskolin analogue, 20 µL of membrane preparation (30 µg protein/tube) and 20 µL reaction mixture consisting of 2.7 mM phosphoenolpyruvate, 0.125 IU pyruvate kinase and 1 IU myokinase. The assay with cardiac ACs had the same composition, but the reaction mixture contained 0.4 mg/mL creatine kinase, 9 mM phosphocreatine and 100 µM IBMX. All tubes

additionally contained 10 mM Mn^{2+}, 10 μM GTPγS, 40 μM ATP, 100 μM cAMP and 0.2-1.0 μCi [α-^{32}P]ATP. Tubes were preincubated for 2 min at 30 °C. The reaction with the AC isoform preparation was initiated by the reaction mixture and the cardiac membrane preparation was initiated by the addition of 20 μL membrane preparation (20 μg protein/tube). The AC activity of AC 1, 2 and 5 was measured for a time interval of 20 minutes at 37 °C. To ensure linear reaction progress for the cardiac AC, those assays were incubated for only 10 min at 30 °C. The reactions were terminated by the addition of 20 μL 2.2 N HCl to denature the protein and by centrifugation at 12000×g for 2 minutes. [^{32}P]cAMP was separated from [α-^{32}P]ATP by transfer of the sample to a 1.4 g neutral alumina column and elution with 4 mL 0.1 M ammonium acetate, pH 7.0. After addition of 10 mL double distilled water, Čerenkov radiation was determined with a liquid scintillation counter Tri-Carb 2800 TR (Perkin Elmer, Wellesley, MA). The concentration-response curves and potency/efficacy values shown in Table 1 and Figure 1 were obtained by non-linear regression analysis with the Prism 5.01 software (Graphpad, San Diego, CA).

4.5. References

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5. Summary

The first part of this dissertation (chapters 1 and 2) deals with the inhibition of the ABC transporters ABCB1 (p-glycoprotein) and ABCG2 (breast cancer resistance protein). Less lipophilic and better water soluble analogues of the known ABCB1 inhibitor tariquidar were synthesized from one central building block via Cu(I)-catalyzed N/O-arylation reactions. These compounds were tested for their inhibitory activity against the ABCB1 transporter in a flow cytometric calcein-AM efflux assay and a correlation between their calculated log P values and their activities was observed. During this work it was discovered that slight structural modifications of the aromatic core resulted in potent ABCG2 inhibitors. Based on this finding a series of new ABCG2 inhibitors was prepared and found to be highly selective over ABCB1 and ABCC2. Such compounds, which are among the most potent and selective ABCG2 inhibitors known so far, might be useful for cancer treatment with respect to reversal of drug resistance, overcoming the blood brain barrier and targeting of tumor stem cells.

The second part of this work (chapter 3) describes the synthesis of ketoalkenes from the pale purple coneflower (*Echinacea pallida*). These compounds contain diene or triene motifs with skipped conjugation and were synthesized from one common vinyl zinc chloride precursor via Pd-catalyzed allylation reactions. The synthetic route developed at the same time allowed to prepare non-natural conjugated analogues via Suzuki and Sonogashira cross coupling reactions. Both the natural products and their analogues were tested for their ability to activate the human cannabinoid receptors 1 and 2 to find out whether such compounds might be responsible for the observed immunostimulatory effects of *Echinacea* extracts. While no significant activity of the natural products was observed at either receptor the new analogues showed micromolar potency at both cannabinoid receptors.

The last part (chapter 4) finally deals with the preparation of new tetracyclic forskolin analogues. Treatment of a forskolin-derived thionocarbonate with 1,3-dimethyl-2-phenyl-1,3-diaza-phospholidine resulted in an interesting rearrangement presumably via a carbene intermediate to yield a cyclic carbonate. Two new compounds were tested for their ability to activate adenylyl cyclase 1, 2 and 5 and found to be able to stimulate cAMP production at micromolar concentrations.

6. Zusammenfassung

Im ersten Teil dieser Arbeit (Kapitel 1und 2) wird die Hemmung der ABC-Transporter ABCB1 (p-Glycoprotein) und ABCG2 (breast cancer resistance protein) beschrieben. Ausgehend von einer zentralen Vorstufe wurden durch Cu(I)-katalysierte N- und O-Arylierungen weniger lipophile und besser wasserlösliche Analoga des ABCB1-Inhibitors Tariquidar synthetisiert. Die inhibitorische Aktivität dieser neuen Verbindungen gegen den ABCB1-Transporter wurde mittels eines durchflusszytometrischen Calcein-AM-Efflux-Assays bestimmt, wobei ein Zusammenhang zwischen den berechneten log P-Werten und den Aktivitäten der Verbindungen beobachtet wurde. Im Rahmen dieses Projekts wurde entdeckt, dass geringe strukturelle Modifikationen des aromatischen Grundgerüsts zu einem potenten ABCG2-Inhibitor führten. Aufgrund dieses überraschenden Ergebnisses wurde eine Reihe von ABCG2-Inhibitoren hergestellt, die sich als hoch selektiv gegenüber ABCB1 und ABCC2 herausstellten. Solche Verbindungen, die mit die potentesten und selektivsten bisher bekannten ABCG2-Inhibitoren sind, könnten im Hinblick auf eine Rückgängigmachung der Resistenz gegen Chemotherapeutika, Überwindung der Blut-Hirn-Schranke und Bekämpfung von Tumorstammzellen bedeutend für die Krebsbehandlung sein.

Der zweite Teil der Dissertation (Kapitel 3) beschreibt die Synthese von Ketoalkenen aus dem Blassen Sonnenhut (*Echinacea pallida*). Diese Verbindungen enthalten Dien- oder Trien-Motive mit unterbrochener Konjugation und wurden ausgehend von einer gemeinsamen Vinyl-Zinkchlorid-Vorstufe durch Pd-katalysierte Allylierungen hergestellt. Die entwickelte Syntheseroute ermöglichte darüber hinaus die Herstellung von konjugierten Analoga durch Suzuki- und Sonogashira-Kreuzkupplungen. Sowohl die Naturstoffe als auch ihre Analoga wurden hinsichtlich ihrer Fähigkeit untersucht, die humanen Cannabinoid-Rezeptoren 1 und 2 zu aktivieren, um herauszufinden, ob diese Verbindungen für den beobachteten immunostimulierenden Effekt von *Echinacea*-Extrakten verantwortlich sein könnten. Während für die Naturstoffe keine signifikante Aktivität beobachtet wurde, zeigten die Analoga mikromolare Potenz an beiden Rezeptoren.

Der letzte Teil der Arbeit (Kapitel 4) handelt schließlich von der Herstellung neuer tetrazyklischer Analoga des Naturstoffs Forskolin. Die Behandlung eines von Forskolin abgeleiteten Thionocarbonats mit 1,3-Dimethyl-2-phenyl-1,3-diaza-phospholidin führte zu einer interessanten, vermutlich über eine Carben-Zwischenstufe verlaufenden, Umlagerung zu einem zyklischen Carbonat. Zwei der neuen Analoga wurden auf ihre Fähigkeit untersucht, die Adenylylcyclase-Isoformen 1, 2 und 5 zu aktivieren. Trotz ihres deutlich höheren sterischen Anspruchs scheinen die untersuchten Verbindungen noch in die Bindungstasche des Enzyms zu passen und sind in der Lage, die cAMP-Bildung in mikromolaren Konzentrationen zu stimulieren.

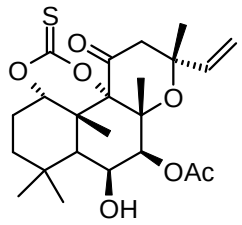
7. Abbreviations

ABC	ATP-binding cassette
ABCP	ABC transporter expressed in placenta
ABCB1	ABC transporter B1, p-gylocoprotein 170
ABCG2	ABC transporter G2, breast cancer resistance protein
ABCC2	ABC transporter C2, multidrug resistance related protein 2
AC	Adenylyl Cyclase
AIBN	Azoisobutyronitrile
ANA	Anandamide
2-AG	2-Arachidonoyl-glycerol
Aq	aqueous
ATP	Adenosine triphosphate
BBB	Blood-brain-barrier
BCRP	Breast cancer resistance protein
Bmim	1-butyl-3-methylimidazolium
Boc	*tert*-Butyloxycarbonyl
Calcd	Calculated
Calcein-AM	Calcein-acetoxymethylester
cAMP	Cyclic adenosine monophosphate
CB_1R	Human cannabinoid 1 receptor
CB_2R	Human cannabinoid 2 receptor
CMFDA	Chloromethylfluorescein-diacetate
CNS	Central nervous system
COSY	Correlated spectroscopy
DAD	Diode array detector
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	Dimethylsulfoxide
EC_{50}	Half maximal effective concentration
EDC	*N*-(3-Dimethylamino-propyl)-*N*'-ethylcarbo-diimide
EDTA	Ethylenediamine tetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
EI-MS	Electron-impact ionization mass spectrometry
ELSD	Evaporative light scattering detection
ES-MS	Electrospray ionization mass spectrometry

FACS	Fluorescence activated cell
FTC	Fumitremorgin C
FCS	Fetal calf serum
GTP	Guanosine-5'-triphosphate
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
HMBC	Heteronuclear multiple bond coherence
HOBt	Hydroxybenzotriazole
HPLC	High pressure liquid chromatography
HR-MS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IBMX	Isobutylmethylxanthine
IC_{50}	Half maximal inhibitory concentration
IR	Infrared
Log P	Partition coefficient between n-octanol and water
MCF-7/Topo	Topotecan resistant human breast cancer cells
MDR	Multidrug resistance
MDCK	Madin-Darby canine kidney cells
Mp	Melting point
MRP	Multidrug-related protein
MXR	Mitoxantrone resistance protein
NIR	Near infrared
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser enhancement spectroscopy
PBS	Phosphate buffered saline
P-gp	p-Glycoprotein
R_f	Retention factor
ROESY	Rotating frame NOE spectroscopy
RT	Room temperature
SEM	Standard error of the mean
TBAF	Tetrabutylammonium fluoride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TRIS	Tris(hydroxymethyl)-aminomethane
U-373 MG	Human glioblastoma cell line
UV	Ultraviolett
Vis	Visible

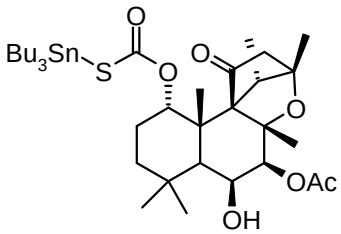
8. Appendix

Crystal data for the forskolin analogues



Forskolin analogue **5**

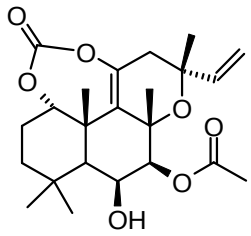
Empirical formula:	$C_{23}H_{32}O_7S$	
Formula weight:	452.56	
Crystal size:	0.220 x 0.180 x 0.120 mm	
Crystal description:	prism	
Crystal colour:	colourless	
Crystal system:	orthorhombic	
Space group:	P 21 21 21	
Unit cell dimensions:	a = 11.2098(10) A	$\alpha = 90°$
	b = 13.4111(10) A	$\beta = 90°$
	c = 15.5805(8) A	$\gamma = 90°$
Volume:	2342.3(2) A^3	
Z, Calculated density:	4, 1.283 Mg/m^3	
Absorption coefficient:	0.178 mm^{-1}	
F(000):	968	



Forskolin analogue **6**

Empirical formula:	$C_{35}H_{59}O_7SSn$
Formula weight:	742.60

Crystal size:	0.360 x 0.080 x 0.050 mm	
Crystal description:	rod	
Crystal colour:	colourless	
Crystal system:	orthorhombic	
Space group:	P 21 21 21	
Unit cell dimensions:	a = 8.6407(5) A	$\alpha = 90°$
	b = 16.7723(12) A	$\beta = 90°$
	c = 26.7336(17) A	$\gamma = 90°$
Volume:	3874.4(4) A^3	
Z, Calculated density:	4, 1.273 Mg/m^3	
Absorption coefficient:	0.755 mm^{-1}	
F(000):	1564	



Forskolin analogue **10**

Empirical formula:	$C_{23}H_{32}O_7$	
Formula weight:	420.49	
Crystal size:	0.110 x 0.100 x 0.090 mm	
Crystal description:	cube	
Crystal colour:	colourless	
Crystal system:	orthorhombic	
Space group:	P 21 21 21	
Unit cell dimensions:	a = 10.06440(10) A	$\alpha = 90°$
	b = 12.2719(2) A	$\beta = 90°$
	c = 17.7626(2) A	$\gamma = 90°$
Volume:	2193.85(5) A^3	
Z, Calculated density:	4, 1.273 Mg/m^3	
Absorption coefficient:	0.769 mm^{-1}	
F(000):	904	

Poster presentations

M. Egger, P. Pellett, K. Nickl, S. Geiger, J. Heilmann, B. König, *"Synthesis and Cannabinoid Receptor Activity of Ketoalkenes from Echinacea Pallida and Non-natural Analogues"*, Summer School Medicinal Chemistry, Regensburg 2008.

M. Egger, P. Pellett, K. Nickl, J. Heilmann, B. König, *"Synthesis and Functional Evaluation of Ketoalkenes and Ketoalkenynes Isolated from Echinacea Pallida"*, Frontiers in Medicinal Chemistry, Regensburg 2008.

M. Egger, C. Müller, X. Li, G. Bernhardt, A. Buschauer, B. König, *"Synthesis of Tariquidar Analogs as Inhibitors of the ABC Transporters ABCB1 and ABCG2"*, Frontiers in Medicinal Chemistry, Berlin 2007.

M. Egger, C. Müller, X. Li, G. Bernhardt, A. Buschauer, B. König, *"Synthesis of Tariquidar Analogs as Inhibitors of the ABC Transporters ABCB1 and ABCG2"*, Oral poster presentation, Summer School Medicinal Chemistry, Regensburg 2006.

M. Egger, G. Bernhardt, A. Buschauer, B. König, ***"****Reversal of Multidrug Resistance at the Blood Brain Barrier"*, Frontiers in Medicinal Chemistry, Frankfurt/Main 2006.

M. Egger, G. Bernhardt, A. Buschauer, B. König, ***"****Multidrug Resistance (MDR) Inhibitors with Selectivity at the Blood Brain Barrier"*, Summer School Medicinal Chemistry, Shanghai 2005.

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10/2005-02/2009	Dissertation: “Inhibition of ABC Transporters Associated with Multidrug Resistance”, University of Regensburg
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Publications

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