DOI: 10.1002/ejoc.200700142

Tariquidar Analogues: Synthesis by Cu^{I}-Catalysed N/O–Aryl Coupling and Inhibitory Activity against the ABCB1 Transporter

Michael Egger,[a] Xuqin Li,[a] Christine Müller,[b] Günther Bernhardt,[b] Armin Buschauer,*[b] and Burkhard König*[a]

In memory of Dr. Thomas Suhs

Keywords: Cross-coupling / Medicinal chemistry / Lipophilicity

Less lipophilic and better water soluble tariquidar analogues were synthesised from one central anthranilic acid derived building block by Cu^{I}-catalysed N/O–arylation reactions. The compounds were tested for their inhibitory activity against the ABCB1 transporter in a flow cytometric calcein-AM efflux assay. 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.

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 as a result of the (over)expression of ABC transporters such as ABCB1 is a major limitation in cancer chemotherapy (classical multidrug resistance, MDR, of tumour 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), which prevents 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 tumours to chemotherapy.[7]

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 relative to the treatment with paclitaxel alone.[8] Moreover, in nude mice bearing orthotopically growing human glioblastoma the combination therapy led to a decrease in tumour volume by 90%, whereas application of paclitaxel alone was ineffective.[8] The antitumour 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 relative to the control, so that systemic paclitaxel toxicity became dose-limiting with valspodar, owing to modulation of ABCB1 in liver, kidneys and bone marrow. By using the more potent ABCB1 inhibitor tariquidar (**1**) (Figure 1), a higher brain/plasma ratio of paclitaxel was detected in mice.[9] However, despite the high tariquidar concentrations in the brain, paclitaxel brain levels did not increase relative to the valspodar group.[9] The latter result might be explained by the high lipophilicity of tariquidar, which results 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 soluble tariquidar analogues with more favourable pharmacokinetic properties.

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-catalysed N– or

[a] Institut für Organische Chemie, Universität Regensburg, Universitätsstrasse 31, 93040 Regensburg, Germany
Fax: +49-941-943-1717
E-mail: burkhard.koenig@chemie.uni-regensburg.de
[b] Institut für Pharmazie, Universität Regensburg, Universitätsstrasse 31, 93040 Regensburg, Germany

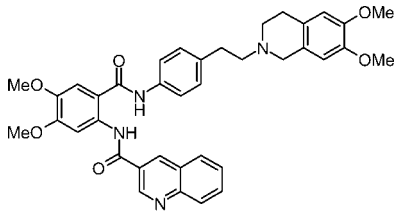


Figure 1. Structure of tariquidar (**1**).

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 optimised 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-catalysed *N*-arylation conditions for their application on tariquidar.

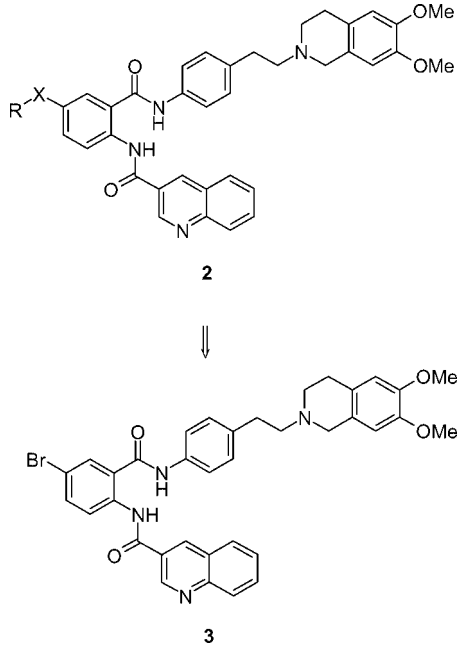
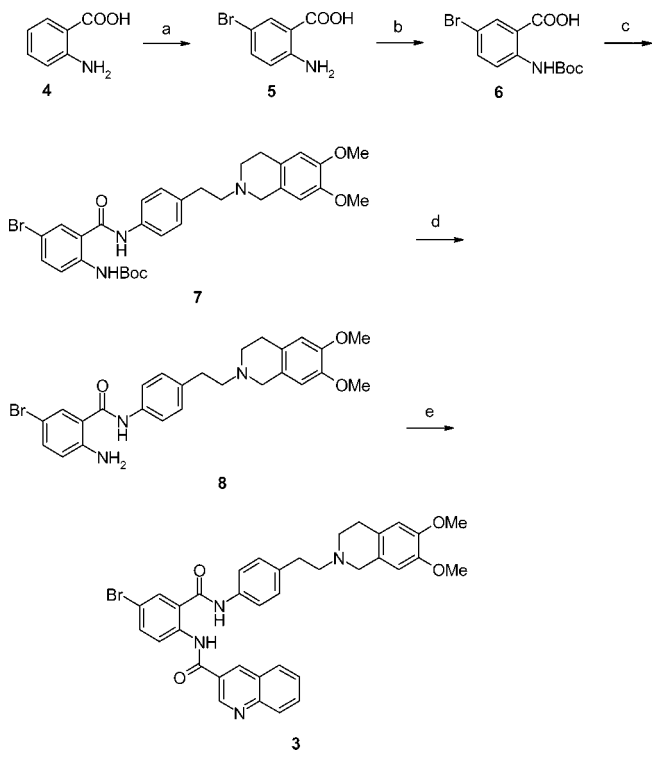


Figure 2. Synthesis of tariquidar derivatives **2** by N– or O–aryl coupling; X = O, N; R = alkyl.

Results and Discussion

Synthesis

The preparation of bromo tariquidar analogue **3** followed the parent synthesis[14] in coupling the upper aniline part and the lower quinolone 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 the literature in good yield.[15] Boc-protection highly improved the solubility of intermediate **7** and was crucial for the efficiency of the subsequent coupling reaction. After deprotection and acylation with quinoline-2-carbonyl chloride, 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 to obtain tariquidar analogues with increased water solubility and decreased lipophilicity (Figure 3).

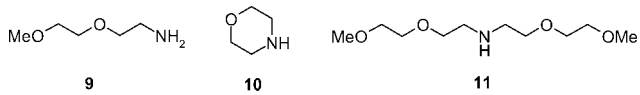
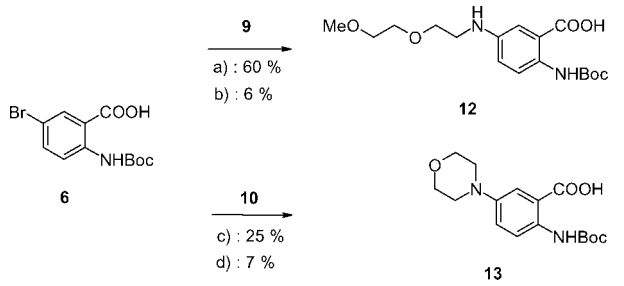


Figure 3. Amines for N–aryl coupling reactions with compound **3**.

To compare different catalyst systems, bromo tariquidar precursor **6** was allowed to react with morpholine (**10**) and primary amine **9** (Scheme 2) with the use of copper and

palladium salts and the ligand systems given in Figure 4. The ligands were adopted from procedures described in the literature for the coupling of aryl bromides with primary or secondary aliphatic amines.[12,16–18]



Scheme 2. Model reactions with aryl bromide **6**. Reagents and conditions: a) CuBr × DMS (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*t*Bu, 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.

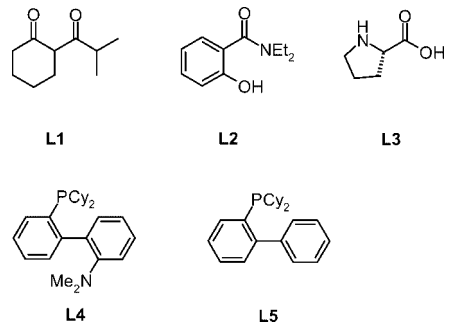


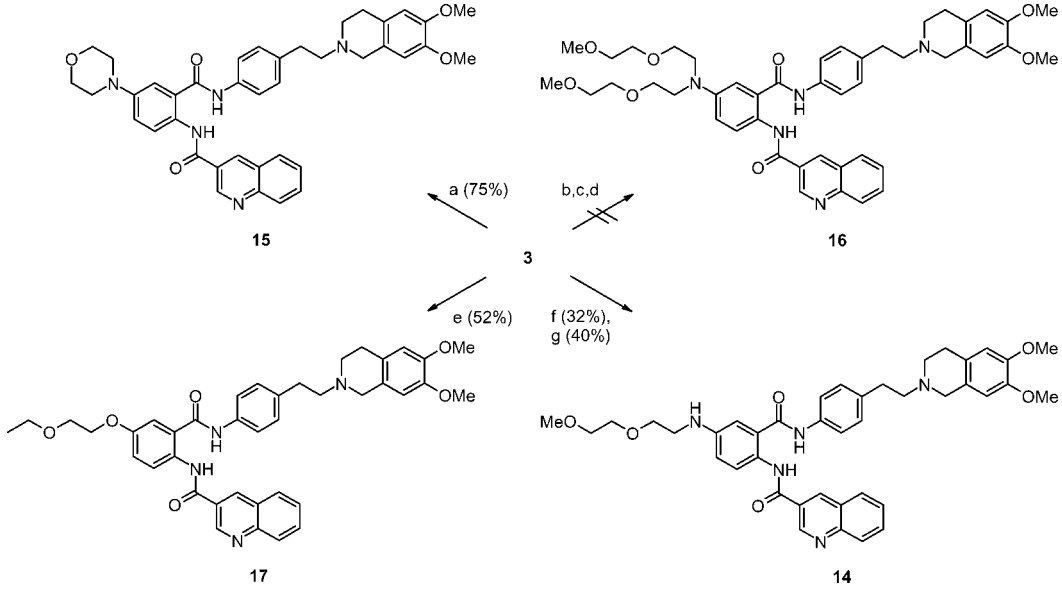
Figure 4. Ligands used for N–aryl coupling reactions.

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

Compound **3** was coupled with primary amine **9** by using CuI and *N*,*N*-diethylsalicylamide (**L2**) or 2-isobutyrylcyclohexanone (**L1**) as the ligand[16,20] to yield tariquidar analogue **14**, which shows improved solubility, in moderate yields. Morpholine (**10**) was introduced as a substituent into the tariquidar skeleton in good yields with L-proline (**L3**) as the 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-catalysed O–aryl coupling was demonstrated in the reaction with alcohol **19** in an analogous coupling reaction.

Flow Cytometric Calcein-AM Efflux Assay (ABCB1 Assay)

In Kb-V1 cells, calcein-AM is extruded by ABCB1 before nonspecific esterases can cleave the ester bonds, and calcein is not accumulated.[21] Therefore, ABCB1 inhibitors can easily be recognised 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



Scheme 3. Ullmann-type coupling reactions of **3** with amines **9**–**11**. Reagents and conditions: a) CuBr × DMS (20 mol-%), **L3** (40 mol-%), K_3PO_4, DMSO, 90 °C; b) CuBr × DMS (20 mol-%), **L3** (40 mol-%), K_3PO_4, DMSO, 90 °C; c) CuI (40 mol-%), **L1** (80 mol-%), K_3PO_4, DMF, 90 °C; d) $Pd_2(dba)_3$ (1.5 mol-%), **L4** (1.5 mol-%), NaO*t*Bu, toluene, 90 °C; e) (i) **19**, Na; (ii) CuCl (20 mol-%), DMF, 90 °C (46% of **10** recovered); f) CuBr × DMS (20 mol-%), **L2** (40 mol-%), K_3PO_4, DMF, 90 °C (66% of **10** recovered); g) CuI (40 mol-%), **L1** (75 mol-%), K_3PO_4, DMF, 90 °C (41% of **10** recovered).

measured. The assay was performed as recently described by Müller et al.[21]

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. Relative to tariquidar, both central building block **3** and aryl ether analogue **17** showed improved activities in the calcein-AM efflux assay, whereas arylamine analogues **15** and **14** showed a decreased potency relative to 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 the 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 to this hypothesis, under the assumption that the newly introduced side chains only affect physicochemical parameters.

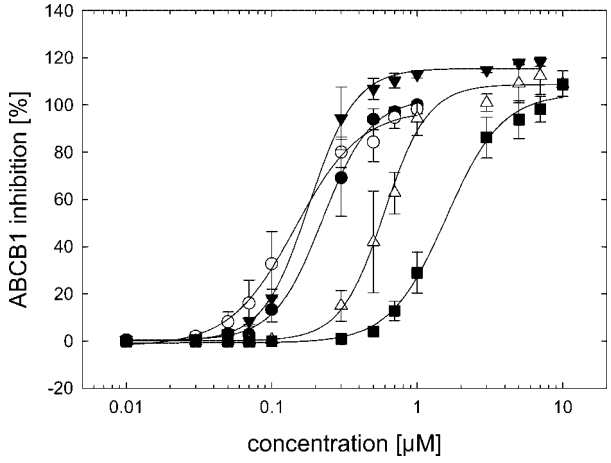


Figure 5. ABCB1 inhibition by tariquidar and the new inhibitors in dependence of their concentrations. Open circle **3**, filled triangle **17**, filled circle tariquidar, open triangle **15**, filled square **14**.

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

Conclusions

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-catalysed reaction each. The Ullmann-type coupling reactions using Cu^I seem to be more generally applicable for these substrate combinations than the Buchwald-Hartwig amination procedure 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 optimised 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 as 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.

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 apparatus 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) or 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, br. s = broad singlet, dd = double of doublets. The relative numbers of protons is determined by integration. Error of reported values: chemical shift 0.01 ppm (^{1}H NMR), 0.1 ppm (^{13}C NMR), coupling constant 0.1 Hz. The used solvent for each spectrum is reported. Mass spectra were recorded with Finnigan MAT TSQ 7000 (ESI) or Finnigan MAT 90 (HRMS) instruments, 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 synthesised under the conditions described above, and the experimental procedures were identical to 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 a nitrogen atmosphere and then heated at reflux for 24 h. The mixture was washed with water, 1 M citric acid and brine. The organic phase was dried with Na_2SO_4, evaporated under reduced pressure and separated by column chromatography on silica gel (hexane/acetone, 5:1, R_f = 0.35) to provide **6** as a white solid (3.8 g, 60%). M.p. 177–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 9 H, Boc), 7.62 (dd, ^3J = 9.1 Hz, ^4J = 2.5 Hz, 1 H, Ar-H), 8.10 (d, ^4J = 2.5 Hz, 1

H, Ar-H), 8.30 (d, ^3J = 9.1 Hz) ppm. ^{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}) ppm. IR (KBr): $\tilde{\nu}$ = 3330, 2978, 1729, 1682, 1578, 1516 cm^{-1}. UV/Vis (MeOH): λ (logε) = 308 (3.514), 253 (4.280) nm. MS (ESI–, DCM/MeOH + 10 mmol/L NH_4Ac): *m*/*z* (%) = 314 (100) [(M – H)$^+$]$^-$, 316 (98) [(M – H)$^+$]$^-$. $C_{12}H_{14}BrNO$ (316.15): calcd. C 45.59, H 4.46, N 4.43; found C 45.12, H 4.55, N 4.24.

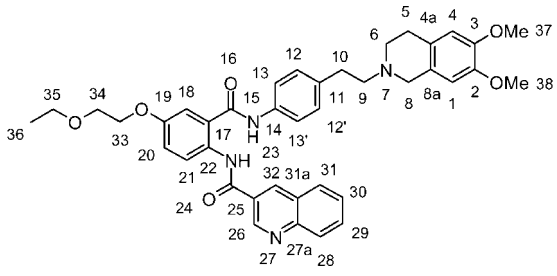
2-(*tert*-Butoxycarbonylamino)-5-[2-(2-methoxyethoxy)ethylamino]-benzoic Acid (12): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 9 H, Boc), 3.29–3.33 (m, 2 H, CH_2), 3.41 (s, 3 H, OCH_3), 3.56–3.60 (m, 2 H, CH_2), 3.64–3.68 (m, 2 H, CH_2), 3.70–3.74 (m, 2 H, CH_2), 6.93 (dd, ^3J = 9.1 Hz, ^4J = 3.0 Hz, 1 H, Ar-H), 7.35 (d, ^4J = 3.3 Hz, 1 H, Ar-H), 8.24 (d, ^3J = 9.1 Hz, 1 H, Ar-H), 9.65 (br. s, 1 H, CONH) ppm. ^{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}) ppm. MS (CI, NH_3): *m*/*z* (%) = 355 (100) [M + H]$^+$.

2-(*tert*-Butoxycarbonylamino)-5-morpholinobenzoic Acid (13): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.53 (s, 9 H, Boc), 3.11–3.14 (m, 4 H, 2 CH_2), 3.86–3.89 (m, 4 H, 2 CH_2), 7.19 (dd, ^3J = 9.3 Hz, ^4J = 3.3 Hz, 1 H, Ar-H), 7.58 (d, ^4J = 3.0 Hz, 1 H, Ar-H), 8.36 (d, ^3J = 9.3 Hz, 1 H, Ar-H), 9.79 (br. s, 1 H, CONH) ppm. ^{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}) ppm. MS (CI, NH_3): *m*/*z* (%) = 323 (83) [M + H]$^+$, 266 (100) [M + H – 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 warmed to room temperature and stirred for 24 h. The mixture was then diluted with CH_2Cl_2, and the organic phase was washed with water, a saturated aqueous solution of $NaHCO_3$ (3 ×) and dried with $MgSO_4$. After evaporation of the solvent, the crude product was purified by flash chromatography on silica gel (EtOAc/NEt_3, 99:1, R_f = 0.27) to obtain a pale yellow solid (1.3 g, 85%). M.p. 196 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.49 (s, 9 H, Boc), 2.75–2.95 (m, 8 H, 4 CH_2), 3.66 (s, 2 H, NCH_2), 3.84 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.54 (s, 1 H, Ar-H), 6.61 (s, 1 H, Ar-H), 7.26–7.29 (m, 2 H, Ar-H, *AA*′BB′), 7.46–7.49 (m, 2 H, Ar-H, AA′*BB*′), 7.56 (dd, ^4J = 2.5 Hz, ^3J = 9.1 Hz, 1 H, Ar-H), 7.68 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 7.74 (br. s, 1 H, CONH), 8.31 (d, ^3J = 9.1 Hz, 1 H, Ar-H), 9.72 (br. s, 1 H, CONH) ppm. ^{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}) ppm. IR (KBr): $\tilde{\nu}$ = 3321, 2974, 2933, 1728, 1512, 1156 cm^{-1}. UV/Vis (MeOH): λ (logε) = 281 (4.107), 256 (4.300) nm. MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m*/*z* (%) = 610 (100) [M + H]$^+$, 612 (97) [M + H]$^+$.

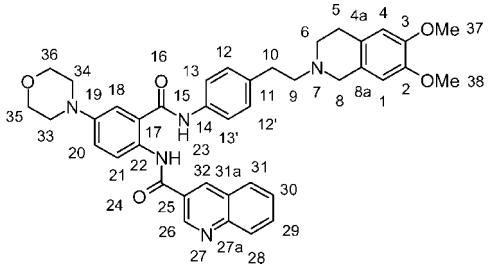
2-Amino-5-bromo-*N*-(4-{-[6,7-dimethoxy-1,2,3,4-tetrahydroiso-quinolin-2-yl]ethyl}-phenyl)benzamide (8): Compound **7** (1.3 g, 2.2 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled in an ice-bath. After the addition of HCl/Et_2O, the mixture was stirred overnight, concentrated in vacuo and the precipitate was suspended in CH_2Cl_2 and washed with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried with $MgSO_4$, and the solvent was removed to obtain the pure product as a white solid (1.0 g, 95%). M.p. 157 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.74–2.94 (m, 8 H, 4 CH_2), 3.65 (s, 2 H, NCH_2), 3.84 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 5.51 (br. s, 2 H, NH_2), 6.54 (s, 1 H, Ar-H), 6.61 (s, 1 H, Ar-H), 6.61 (d, ^3J = 8.8 Hz, 1 H, Ar-H), 7.23–7.26 (m, 2 H, Ar-H, *AA*′BB′), 7.32 (dd, ^4J = 2.2 Hz, ^3J = 8.8 Hz, 1 H, Ar-H), 7.46–7.49 (m, 2 H, Ar-H, AA′*BB*′), 7.56 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 7.65 (br. s, 1 H, CONH) ppm. ^{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 ppm. IR (KBr): $\tilde{\nu}$ = 3470, 3373, 3302, 1638, 1596, 1520, 818 cm^{-1}. UV/Vis (CH_2Cl_2): λ (logε) = 346 (3.366), 264 (3.808), 229 (4.067) nm. MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): *m*/*z* (%) = 510 (100) [M + H]$^+$, 512 (98) [M + H]$^+$.

***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 a saturated aqueous solution of $NaHCO_3$ and dried with $MgSO_4$. Evaporation of the solvent and purification by flash chromatography on silica gel (acetone/hexanes, 1:1, 1% NEt_3, R_f = 0.27) yielded the product as a pale yellow solid (761 mg, 82%). M.p. 204–207 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.76–2.97 (m, 8 H, 4 CH_2), 3.67 (s, 2 H, NCH_2), 3.84 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.54 (s, 1 H, Ar-H), 6.61 (s, 1 H, Ar-H), 7.31 (d, ^3J = 8.5 Hz, 2 H, Ar-H, *AA*′BB′), 7.57 (dd, ^4J = 2.2 Hz, ^3J = 8.8 Hz, 1 H, Ar-H), 7.61–7.67 (m, 1 H, Ar-H), 7.63 (d, ^3J = 8.5 Hz, 2 H, Ar-H, AA′*BB*′), 7.78 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 7.81–7.87 (m, 1 H, Ar-H), 7.99–8.02 (m, 1 H, Ar-H), 8.16–8.19 (m, 1 H, Ar-H), 8.47 (br. s, 1 H, CONH), 8.62 (d, ^3J = 8.8 Hz, 1 H, Ar-H), 8.78 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 9.52 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 12.07 (br. s, 1 H, CONH) ppm. ^{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}) ppm. IR (KBr): $\tilde{\nu}$ = 2932, 1677, 1597, 1512, 1464, 829 cm^{-1}. UV/Vis (CH_2Cl_2): λ (logε) = 287 (4.333), 238 (4.637) nm. $C_{36}H_{33}BrN_4O_4$ (665.59): calcd. C 64.96, H 5.00, N 8.42; found C 64.37, H 4.99, N 8.11. HRMS: calcd. for $C_{36}H_{33}BrN_4O_4$ [M]$^+$ 664.1685; found 664.1692.



***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-necked 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 an atmosphere of 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 a saturated aqueous solution of $NaHCO_3$ (3×), and the organic phase was dried with $MgSO_4$. The solvent was evaporated, and the remaining solid was purified by flash chromatography on silica gel (acetone/hexanes, 1:1, R_f = 0.31) to obtain a white solid (64 mg, 52%). M.p. 124–126 °C. ^{1}H NMR (400 MHz, CD_2Cl_2; HSQC, HMBC, COSY, ROESY): δ = 1.18 (t, ^3J = 7.2 Hz, 3 H, CH_3, 36-H), 2.76–2.84 (m, 6 H, 3 CH_2, 4-H, 5-H, 9-H), 2.90–2.93 (m, 2 H, CH_2, 10-H), 3.51 (q, ^3J = 7.2 Hz, 2 H, OCH_2, 35-H), 3.62 (s, 2 H, NCH_2, 8-H), 3.64 (t, ^3J = 4.8 Hz, 2 H, OCH_2, 34-H), 3.78 (s, 3 H, OCH_3, 38-H), 3.78 (s, 3 H, OCH_3, 37-H), 4.07 (t, ^3J = 4.8 Hz, 2 H, OCH_2, 33-H), 6.54 (s, 1 H, 1-H_{Ar}), 6.60 (s, 1 H, 4-H_{Ar}), 7.10 (dd, ^3J = 9.2 Hz, ^4J = 2.7 Hz, 1 H, 20-H_{Ar}), 7.28 (d, ^4J = 2.7 Hz, 1 H, 18-H_{Ar}), 7.31 (d, ^3J = 8.3 Hz, 2 H, 12-H_{Ar}, 12′-H_{Ar}), 7.62 (d, ^3J = 8.3 Hz, 2 H, 13-H_{Ar}, 13′-H_{Ar}), 7.62–7.66 (m, 1 H, 30-H_{Ar}), 7.80–7.84 (m, 1 H, 29-H_{Ar}), 7.99–8.02 (m, 1 H, 31-H_{Ar}), 8.13–8.15 (m, 1 H, 28-H_{Ar}), 8.38 (br. s, 1 H, 15-H), 8.66 (d, ^3J = 9.2 Hz, 1 H, 21-H_{Ar}), 8.75 (d, ^4J = 2.3 Hz, 1 H, 32-H_{Ar}), 9.43 (d, ^4J = 2.3 Hz, 1 H, 26-H_{Ar}), 11.80 (br. s, 1 H, 23-H) ppm. ^{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 (+, C-18), 118.3 (+, C-20), 121.3 (+, C-13, C-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, C-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) ppm. IR (KBr): $\tilde{\nu}$ = 2931, 1633, 1597, 1517, 1408, 1127 cm^{-1}. UV/Vis (CH_2Cl_2): λ (logε) = 282 (4.176), 238 (4.550) nm. HRMS: calcd. for $C_{40}H_{42}N_4O_6$ $[M]^+$ 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 to 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 an atmosphere of 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 a saturated aqueous solution of $NaHCO_3$ and dried with $MgSO_4$. After evaporation of the solvent, the remaining solid was purified by flash chromatography on silica gel (EtOAc/EtOH, 4:1, R_f = 0.29) to give a yellow solid (116 mg, 75%). M.p. 238 °C. ^{1}H NMR (600 MHz, CD_2Cl_2; HSQC, HMBC, COSY, ROESY): δ = 2.87–3.00 (m, 8 H, 4 CH_2, 5-H, 6-H, 9-H, 10-H), 3.15 (m, 4 H, 2 CH_2, 33-H, 34-H), 3.77 (s, 3 H, OCH_3, 38-H), 3.78 (s, 3 H, OCH_3, 37-H), 3.77–3.79 (m, 6 H, NCH_2, 8-H, 2 CH_2, 35-H, 36-H), 6.55 (s, 1 H, 1-H_{Ar}), 6.62 (s, 1 H, 4-H_{Ar}), 7.11 (dd, ^3J = 9.0 Hz, ^4J = 2.6 Hz, 1 H, 20-H_{Ar}), 7.26–7.30 (m, 3 H, 12-H_{Ar}, 12′-H_{Ar}, 18-H_{Ar}), 7.61–7.67 (m, 3 H, 13-H_{Ar}, 13′-H_{Ar}, 30-H_{Ar}), 7.81–7.85 (m, 1 H, 29-H_{Ar}), 8.00–8.03 (m, 1 H, 31-H_{Ar}), 8.11–8.14 (m, 1 H, 28-H_{Ar}), 8.51 (d, ^3J = 9.0 Hz, 1 H, 21-H_{Ar}), 8.75–8.77 (m, 1 H, 32-H_{Ar}), 9.30 (br. s, 1 H, 15-H), 9.38–9.40 (m, 1 H, 26-H_{Ar}), 11.70 (br. s, 1 H, 23-H) ppm. ^{13}C NMR (150 MHz, CD_2Cl_2): δ = 27.5 (C-5), 32.5 (C-10), 49.8 (C-33, C-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, C-36), 110.1 (C-1), 111.9 (C-4), 115.0 (C-18), 119.9 (C-20), 121.8 (C-13, C-13′), 123.4 (C-21), 123.8 (C-17), 124.5 (C-8a), 125.6 (C-4a), 127.4 (C-31a), 127.9 (C-25), 127.9 (C-30), 129.2 (C-28), 129.5 (C-31), 129.6 (C-12, C-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) ppm. IR (KBr): $\tilde{\nu}$ = 2933, 1655, 1598, 1516, 1228, 1123 cm^{-1}. UV/Vis (CH_2Cl_2): λ (logε) = 283 (4.245), 236 (4.562) nm. HRMS: calcd. for $C_{40}H_{41}N_5O_5$ $[M]^+$ 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 an atmosphere of 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 with $MgSO_4$. Evaporation of the solvent and purification of the crude product by flash chromatography on silica gel (EtOAc/MeOH, 4:1, 1% NEt_3, R_f = 0.25) gave **19** as a yellow solid (74 mg, 40%). M.p. 96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.82–3.00 (m, 8 H, 4 CH_2), 3.16 (t, ^3J = 5.1 Hz, 2 H, CH_2), 3.33 (s, 3 H, OCH_3), 3.44–3.51 (m, 6 H, 3 CH_2), 3.73 (s, 2 H, NCH_2), 3.84 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.54 (s, 1 H, Ar-H), 6.61 (s, 1 H, Ar-H), 6.72 (dd, ^3J = 9.1, ^4J = 2.7 Hz, 1 H, Ar-H), 6.92 (d, ^4J = 2.7 Hz, 1 H, Ar-H), 7.28 (d, ^3J = 8.5 Hz, 2 H, Ar-H, AA′BB′), 7.59–7.65 (m, 1 H, Ar-H), 7.67 (d, ^3J = 8.5 Hz, 2 H, Ar-H, AA′BB′), 7.78–7.83 (m, 1 H, Ar-H), 7.96–7.99 (m, 1 H, Ar-H), 8.14–8.17 (m, 1 H, Ar-H), 8.42 (d, ^3J = 9.1 Hz, 1 H, Ar-H), 8.69 (br. s, 1 H, CONH), 8.73 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 9.49 (d, ^4J = 2.2 Hz, 1 H, Ar-H), 11.60 (br. s, 1 H, CONH) ppm. ^{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 ppm. IR (KBr): $\tilde{\nu}$ = 2930, 1599, 1516, 1252, 1226 cm^{-1}. UV/Vis (MeOH): λ (logε) = 281 (4.150), 236 (4.519) nm. HRMS: calcd. for $C_{41}H_{45}N_5O_6$ $[M]^+$ 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-cm^2

culture flasks (NUNC, Wiesbaden, Germany) and serially passaged following trypsinisation by 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.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (GRK 760), the Asia Link programme of the European Union (training fellowship for X. L.) and the Fonds der Chemischen Industrie. M. E. thanks the Elitenetzwerk Bayern for a graduate fellowship.

[1] W. W. Johnson, *Methods Find. Exp. Clin. Pharmacol.* **2002**, *24*, 501–514.
[2] a) S. V. Ambudkar, C. Kimchi-Sarfaty, Z. E. Sauna, M. M. Gottesmann, *Oncogene* **2003**, *23*, 7468–7485; b) P. Labrie, S. P. Maddaford, J. Lacroix, C. Catalona, D. K. Lee, S. Rakhit, R. C. Gaudreault, *Bioorg. Med. Chem.* **2006**, *14*, 7972–7987.
[3] P. Ramakrishnan, *Einstein Quart. J. Biol. Med.* **2003**, *19*, 160–165.
[4] A. Schinkel, *Adv. Drug Deliv. Rev.* **1999**, *36*, 179–194.
[5] W. Löscher, H. Potschka, *Prog. Neurobiol.* **2005**, *76*, 22–76.
[6] J. Robert, C. Jarry, *J. Med. Chem.* **2003**, *46*, 4805–4817.
[7] A. Régina, M. Demeule, A. Laplante, J. Jodoin, C. Dagenais, F. Berthelet, A. Moghrabi, R. Béliveau, *Cancer Metastasis. Rev.* **2001**, *20*, 13–25.
[8] S. Fellner, B. Bauer, D. S. Miller, M. Schaffrik, M. Fankhänel, Th. Spruß, G. Bernhardt, C. Graeff, L. Färber, H. Gschaidmeier, A. Buschauer, G. Fricker, *J. Clin. Invest.* **2002**, *110*, 1309–1318.
[9] M. Fankhänel, S. Fellner, Th. Spruß, G. Bernhardt, A. Buschauer, *Turk. J. Cancer* **2005**, *35*, 41.
[10] F. Ullmann, *Ber. Dtsch. Chem. Ges.* **1903**, *36*, 2382–2384.
[11] For selected reviews, see:a) A. R. Muci, S. L. Buchwald, *Top. Curr. Chem.* **2002**, *219*, 131–209; b) S. V. Ley, A. W. Thomas, *Angew. Chem. Int. Ed.* **2003**, *42*, 5400–5449; c) D. Prim, J. Campagne, D. Joseph, B. Andrioletti, *Tetrahedron* **2002**, *58*, 2041–2075.
[12] J. P. Wolfe, H. Tomori, J. P. Sadighi, J. Yin, S. L. Buchwald, *J. Org. Chem.* **2000**, *65*, 1158–1174.
[13] J. F. Hartwig, M. Kawatsura, S. I. Hauck, K. H. Shaughnessy, L. M. Acazar-Roman, *J. Org. Chem.* **1999**, *64*, 5575–5580.
[14] M. Roe, A. Folkes, P. Ashworth, J. Brumwell, L. Chima, S. Hunjan, I. Pretswell, W. Dangerfield, H. Ryder, P. Charlton, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 595–600.
[15] Z. Le, Z. Chen, Y. Hu, Q. Zheng, *Synthesis* **2004**, *17*, 2809–2812.
[16] F. Y. Kwong, S. L. Buchwald, *Org. Lett.* **2003**, *5*, 793–796.
[17] D. W. Old, J. P. Wolfe, S. L. Buchwald, *J. Am. Chem. Soc.* **1998**, *120*, 9722–9723.
[18] H. Zhang, Q. Cai, D. Ma, *J. Org. Chem.* **2005**, *70*, 5164–5173.
[19] B. C. Hamann, J. F. Hartwig, *J. Am. Chem. Soc.* **1998**, *120*, 3694–3703.
[20] A. Shafir, S. L. Buchwald, *J. Am. Chem. Soc.* **2006**, *128*, 8742–8743.
[21] C. Müller, D. Gross, V. Sarli, M. Gartner, A. Giannis, G. Bernhardt, A. Buschauer, *Cancer Chemother. Pharmacol.* **2007**, *59*, 157–164.
[22] M. Wiese, I. K. Pajeva, *Curr. Med. Chem.* **2001**, *8*, 685–713.
[23] K. Kohno, J. Kikuchi, S. Sato, H. Takano, Y. Saburi, K. Asoh, M. Kuwano, *Jpn. J. Cancer Res.* **1988**, *79*, 1238–1246.

Received: February 17, 2007
Published Online: April 23, 2007

