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Synthesis and Quantitative Structure–Activity Relationships of Selective BCRP Inhibitors

Federico Marighetti, Kerstin Steggemann, Markus Hanl, and Michael Wiese*[a]

The breast cancer resistance protein (BCRP/ABCG2) is a member of the ABC transporter superfamily. This protein has a number of physiological functions, including protection of the human body from xenobiotics. The overexpression of BCRP in certain tumor cell lines causes cross-resistance against various drugs used in chemotherapeutic treatment. In a previous work we showed that a new class of compounds derived from XR9576 (tariquidar) selectively inhibits BCRP. In this work we synthesized more members of this class, with modification on the second and third aromatic rings. The inhibitory activities against BCRP and P-gp were assayed using a Hoechst 33342 assay for BCRP and a calcein AM assay for P-gp. Finally, quantitative structure–activity relationships for both aromatic rings were established. The results obtained show the importance of the electron density on the third aromatic ring, influenced by substituents, pointing to interactions with aromatic residues of the protein binding site. In the second aromatic ring the activity of compounds is influenced by the steric volume of the substituents.

Introduction

A major problem in cancer chemotherapy is the development of multidrug resistance (MDR) mediated by ABC transporters. This class of membrane proteins uses the energy of ATP hydrolysis to actively pump metabolites or xenobiotics out of cells against a concentration gradient. If members of this family are overexpressed in chemotherapy-resistant tumor cells, they cause a remarkable decrease in cytoplasmic concentrations of chemotherapeutic agents. The ABC transporters that have been identified to play an important role in MDR are P-glycoprotein (P-gp, ABCB1), the breast cancer resistance protein (BCRP, ABCG2), and the multidrug-resistance-associated protein 1 (MRP1, ABCC1).[1]

In humans, 48 ABC transporters have been identified and classified into seven subfamilies (A–G).[2] P-gp belongs to the ABCB subfamily. It has a molecular weight of ~170 kDa and consists of 1280 amino acids. It has two transmembrane regions with two nucleotide binding domains. Each transmembrane region consists of six transmembrane segments.[3,4] BCRP is a member of the ABCG subfamily, and like all members of the ABCG subfamily is a half transporter. BCRP has a molecular weight of 72 kDa and is composed of 655 amino acids. While most ABC transporters contain an N-terminal transmembrane domain followed by the nucleotide binding domain, BCRP has a reverted structure with an N-terminal nucleotide binding domain followed by a transmembrane domain with six transmembrane helices. BCRP may function as a homodimer probably linked by a disulfide bond, or as a tetramer.[5,6] MRP1 belongs to the ABCC subfamily, and like many other transporters in this group, has an extra N-terminal transmembrane domain with five transmembrane helices.[7]

P-gp is able to transport a large variety of chemically diverse substrates. These are usually lipophilic and amphipathic compounds with molecular weights between 400 and 4000 Da.[8] Under physiological conditions BCRP transports conjugated and unconjugated steroids and primary bile acids.[9] Like P-gp, BCRP is also able to efflux a wide variety of chemotherapeutic substances such as mitoxantrone, anthracyclines, antifolates, and tyrosine kinase inhibitors.[10] MRP1 is involved in the transport of bile salts and other organic anions.[11]

A possible strategy to reverse ABC-transporter-mediated MDR is the inhibition of these proteins by inhibitors, also called modulators. To date, many inhibitors of P-gp are known, while for the other two ABC transporters the arsenal of inhibitors is relatively small. GF120918 (elacridar) and XR9576 (tariquidar) are potent third-generation P-gp inhibitors that also inhibit BCRP.[12] Tyrosine kinase inhibitors such as imatinib and gefitinib have been found to increase the accumulation of substrates like Hoechst 33342 in cells expressing BCRP and P-gp.[13–15]

Some natural products such as fumitremorgin C (FTC), tryprostatin A, and novobiocin are found to be selective inhibitors of BCRP. Ko143, a synthetic derivative of FTC, is a selective BCRP inhibitor with low cytotoxicity and is 10-fold more potent than its precursor.[16] Flavonoids, a large class of natural compounds, are also able to inhibit BCRP, but they also show inhibitory effects against P-gp and MRP1. We recently reported the discovery of a new class of specific BCRP inhibitors possessing a scaffold consisting of three aromatic rings connected by two amide bonds.[17] Herein we describe the further development of this class of BCRP inhibitors. Most of the synthe-

[a] F. Marighetti,[+] Dr. K. Steggemann,[+] M. Hanl, Prof. Dr. M. Wiese
Pharmaceutical Institute, University of Bonn
An der Immenburg 4, 53121 Bonn (Germany)
E-mail: mwiese@uni-bonn.de

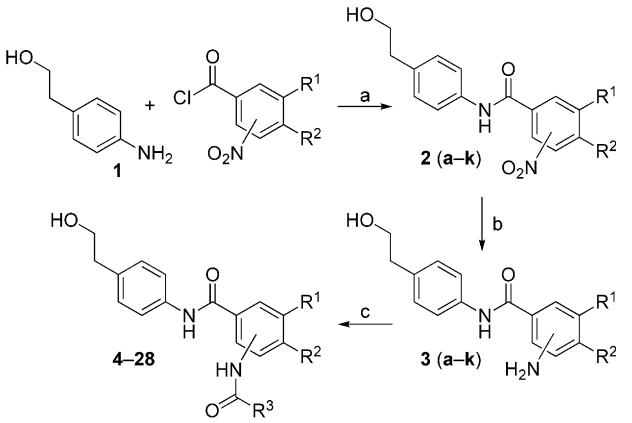
[+] These authors contributed equally to this work.

sized compounds are selective for BCRP, whereas some inhibit BCRP and P-gp. The results of biological testing were used to build a structure–activity relationship (SAR) model that is useful to understand the SAR for the second and third aromatic rings of this compound class.

Results and Discussion

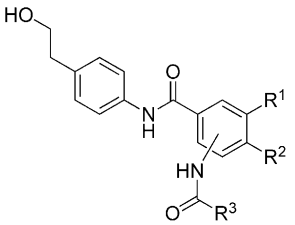
Chemistry

The basic scaffold of the investigated compounds consists of two aromatic rings connected by an amide linker. The first aromatic ring has a hydroxyethyl group at the *para* position with respect to the amide group. The second aromatic ring also bears an amide function with various substituents such as methyl, cyclohexyl, or substituted aromatic ring systems. The two amide groups on the second aromatic ring are at positions *ortho*, *meta*, or *para*. The studied compounds were prepared by following the general strategy shown in Scheme 1. In the first step 2-(4-aminophenyl)ethanol **1** was allowed to react with different substituted nitrobenzoyl chlorides, yielding amides **2a**–**k**. Afterward, the aromatic nitro group was reduced by catalytic hydrogenation with palladium/charcoal to get substituted anilines **3a**–**k**. The reaction of these anilines with an appropriate acid chloride yielded the desired compounds **4**–**28**.



Scheme 1. General strategy for the synthesis the new BCRP inhibitors. *Reagents and conditions:* a) THF, Et_3N, RT, 12 h; b) H_2, Pd/C, 2 bar, RT, 12 h; c) R-COCl, THF, Et_3N, RT, 12 h.

Table 1. Structures and inhibitory activities of the investigated compounds.



Compd	Subst.	R^1	R^2	R^3	IC_{50} [μM][a] BCRP	P-gp
4	*ortho*	H	H	4-Nitrophenyl	1.58 ± 0.36	NE
5	*meta*	H	H	4-Nitrophenyl	NE	NE
6	*para*	H	H	4-Nitrophenyl	NE	NE
7	*ortho*	OCH_3	OCH_3	4-Nitrophenyl	6.96 ± 2.20	NE
8	*ortho*	H	OCH_3	4-Nitrophenyl	3.93 ± 0.11	NE
9	*ortho*	OCH_3	H	4-Nitrophenyl	8.43 ± 1.63	NE
10	*ortho*	H	CH_3	4-Nitrophenyl	2.69 ± 0.86	NE
11	*ortho*	CH_3	H	4-Nitrophenyl	4.08 ± 0.48	NE
12	*ortho*	H	Cl	4-Nitrophenyl	2.59 ± 0.86	NE
13	*ortho*	Cl	H	4-Nitrophenyl	3.09 ± 0.22	NE
14	*ortho*	H	H	Cyclohexyl	NE	NE
15	*ortho*	H	H	Methyl	NE	NE
16	*ortho*	H	H	3-Nitrophenyl	1.31 ± 0.25	21.3 ± 2.87
17	*ortho*	H	H	3-Aminophenyl	8.65 ± 2.37	14.0 ± 4.29
18	*ortho*	H	H	4-Aminophenyl	8.12 ± 1.00	15.5 ± 2.05
19	*ortho*	H	H	4-Cyanophenyl	2.54 ± 0.19	NE
20	*ortho*	H	H	4-Chlorophenyl	3.08 ± 1.32	NE
21	*ortho*	H	H	3-Chlorophenyl	3.48 ± 0.54	NE
22	*ortho*	H	H	4-Trifluoromethylphenyl	2.20 ± 0.57	NE
23	*ortho*	H	H	4-Acetoxyphenyl	12.8 ± 2.10	NE
24	*ortho*	H	H	4-*tert*-Butylphenyl	2.95 ± 0.83	11.7 ± 3.87
25	*ortho*	H	H	4-Isopropoxyphenyl	1.15 ± 0.29	8.42 ± 2.45
26	*ortho*	H	H	4-Hydroxyphenyl	21.2 ± 3.67	NE
27	*ortho*	H	H	3,4-Dimethoxyphenyl	3.61 ± 0.97	7.75 ± 1.19
28	*ortho*	H	H	4-Methylphenyl	4.59 ± 0.57	NE

[a] Values are the mean ± SD from three independent experiments; NE: no effect up to a concentration of 10 μM.

Biological activity and QSAR

The inhibitory activity of the investigated substances against BCRP was determined in MCF-7 MX cells using the Hoechst 33342 assay, as described previously. The calcein AM assay was used to measure the inhibitory potency against P-gp in A2780 Adr cells. Table 1 lists the activity data for 25 compounds against BCRP and P-gp. Two of the inhibitors, compounds **4** and **28**, have been reported previously.[17] Furthermore, it was shown that six members of this new class of BCRP inhibitors are also able to block the transport of pheophorbide A in MDCK BCRP cells. As the pIC_{50} values from both assays were linearly correlated, we concluded that the inhibitory effect of the studied substances is not specific for Hoechst 33342 and could be extended to other BCRP substrates.

Two of the newly synthesized compounds, **5** and **6**, showed no activity against BCRP in the Hoechst 33342 accumulation

assay. These are the only compounds with *meta* or *para* substitution on the second ring. In contrast, the *ortho*-substituted compound **4** is a potent and selective BCRP inhibitor. Therefore, the relative position of the two amide groups seems to be crucial.

Owing to the different substitution patterns, the three compounds have a considerable difference in their spatial positioning of the aromatic rings, pointing to some steric requirements for BCRP inhibition. Furthermore, only *ortho*-substituted compounds can establish a hydrogen bond between the NH and the C=O of the amide groups on the second ring. This interaction may be important for the active conformation of this compound class.

In case of the second aromatic ring, the presence of any substituent decreases the inhibitory potency of the compounds relative to that of the reference inhibitor **4**. The substitution at position R^2 causes a slight decrease in activity (compounds **8**, **10**, and **12**), whereas substitution at R^1 leads to a more significant decrease in activity (compounds **9**, **11**, and **13**).

The presence of a methoxy group at position R^2 (compound **8**) results in a slight decrease in activity (IC_{50} = 3.93 μM), but at position R^1 the presence of the same substituent (in **9**) decreases the inhibitory activity by roughly fivefold (IC_{50} = 8.43 μM). Substitution at both positions (compound **7**) results in an inhibitory effect similar to that of compound **9** (IC_{50} = 6.96 μM). The activity of compound **9** could be explained by the predominance of the steric effect at position R^1 over the effect at position R^2.

With methyl groups as substituents the same trend is observed: A methyl group at position R^2 (compound **10**) decreases the activity to an IC_{50} value of 2.69 μM, whereas the same substitution at position R^1 (compound **11**) gives a less active compound, with IC_{50} = 4.08 μM. Compounds with chloro substituents at positions R^1 (**13**) or R^2 (**12**) on the second ring have similar inhibitory effects against BCRP (IC_{50} values of 3.09 and 2.59 μM, respectively).

As shown in Figure 1a the activity of the substituted compounds decreases linearly with increasing van der Waals volume of the substituted aromatic ring, as calculated with MOE.[18] This unfavorable steric effect is more evident at position R^1 than at position R^2. On the whole, the SAR of the second aromatic ring may be explained by a disadvantageous steric interaction of the substituents with the binding site of the protein.

To investigate the importance of the aromatic ring at position R^3, this ring was replaced with an aliphatic cyclohexyl group (in **14**) or a small methyl group (in **15**). The presence of either group resulted in loss of activity. Thus for BCRP inhibitory activity, position R^3 must be occupied by an aromatic ring.

For compounds bearing an aromatic ring at position R^3, activity was observed to be correlated with the electronic effect of the substituents present on this ring and with the lipophilicity of the aromatic system. The highest correlation was found with σ-Hammett and the hydrophobic surface area of the substituted ring [Eq. (1)]. Included in the correlation are all compounds with R^1, R^2 = H, and the σ values were selected with the amide group as reference position. Vsurf_S is the interaction field surface area of the substituted aromatic ring calculated by MOE. The relative importance of descriptors is 1 for σ and 0.963 for Vsurf_S. Figure 1b shows a plot of observed versus calculated pIC_{50} values.

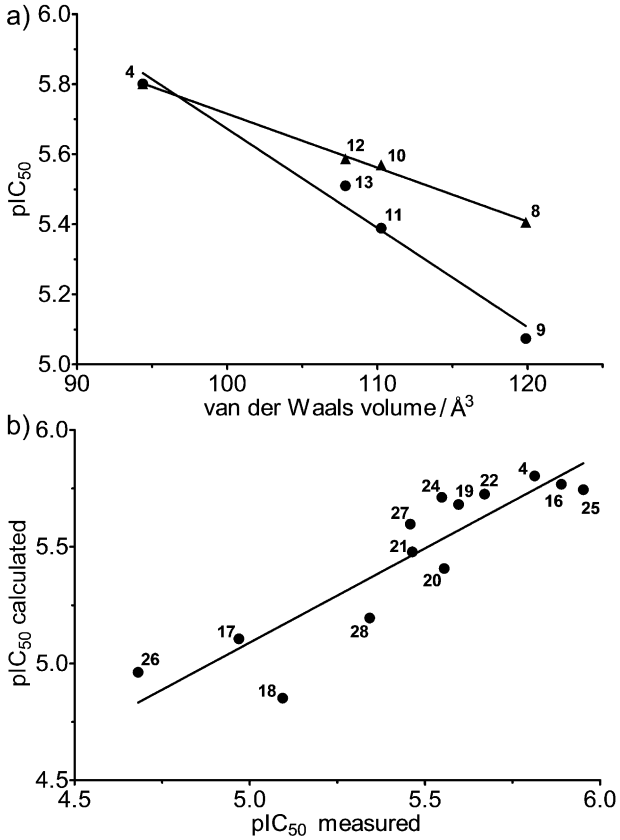


Figure 1. a) Plot of van der Waals volumes of the substituted second aromatic ring versus pIC_{50} values for compounds with a substituent on the second aromatic ring at position R^1 (●) and with substitution at position R^2 (▲). b) Plot of measured versus calculated potencies for inhibition of BCRP in MCF-7 MX cells according to Equation (1) for compounds with variations on the third aromatic ring.

$$pIC_{50} = 0.503 \times \sigma_{p+m} + 0.00932 \times \text{Vsurf\_S} + 3.010$$
$$n = 13,\ r^2 = 0.81,\ s = 0.156,\ F = 45.9 \quad (1)$$

A possible explanation for this trend could be the interaction of the ring system at position R^3 with aromatic residues of the protein binding site and the consequential π–π stacking. Furthermore, the presence of bulky substituents with a large contact surface area on this ring increases the affinity of the ligands and hence the inhibitory activity.

However, one compound (compound **23**) was found to be an outlier from the correlation, showing much less inhibition than calculated from the equation. A hypothesis suggested by the similar activity values of compounds **23** and **26** is a possible hydrolysis of the ester group to a phenolic hydroxy group under the assay conditions. To test this hypothesis, TLC was performed with compound **23** after various incubation times in assay buffer at 37 °C. After only 10 min of incubation, the

original spot disappeared and a new spot was observed with the same R_f value as that of compound **26**. Thus it can be concluded that compound **23** is not stable under the assay conditions.

We also tested whether lipophilicity could replace the surface area in the QSAR indicating that distribution into the lipophilic membrane would be important. However, the obtained equation possessed much lower statistical significance, and moreover, the descriptor used for lipophilicity, π, proved to be insignificant at the 95% level. This points to the importance of more specific interactions for the inhibitory activity of the compounds.

To evaluate the selectivity of this class of compounds, the activity against P-gp was investigated using the calcein AM assay. Most of the tested compounds did not show inhibition of P-gp at a fixed concentration of 10 μM. However, some unexpected exceptions were observed, as previously it was thought that the absence of the tetrahydroisoquinoline group is responsible for the selectivity of those compounds.[17] In this study we observed that the deletion of this group is necessary for selectivity, but not sufficient to assure that compounds are not P-gp inhibitors. Indeed, the highest inhibitory activity against P-gp is shown by compound **27**, which contains a dimethoxy substituent,in agreement with P-gp inhibitors from different structural classes, for which this substitution pattern was found to be advantageous.[19] The presence of a voluminous lipophilic group on the third aromatic ring, as present in compounds **24** and **25**, gives compounds with weak inhibitory activity against P-gp. Interestingly, the amino derivatives **17** and **18** show some inhibition of P-gp, while from the corresponding nitro compounds only the 3-nitrobenzene derivative showed some, albeit low inhibitory activity against P-gp.

To investigate the reversal of BCRP-mediated resistance by this new class of compounds, the toxicity of two cytotoxic BCRP substrates, mitoxantrone and SN-38 (the active metabolite of irinotecan), were determined in the presence and absence of two selected compounds. Cell viability was determined by MTT assay. The cytotoxicity of mitoxantrone and SN-38 were determined in parental MCF-7 and resistant MCF-7 MX cells, as well as in parental MDCK and resistant MDCK BCRP cells. MDCK BCRP cells were included because in this transfected cell line the resistance can be solely attributed to the presence of BCRP. EC_{50} values were determined for parental and resistant cells without the addition of inhibitor and in the presence of two different concentrations of inhibitor. For the MTT assays we used the potent and selective compounds **4** and **25** as inhibitors. Both studied compounds were able to reverse BCRP-mediated resistance. As the measured absorption values in the wells containing only compounds **4** or **25** remained constant for the sensitive cell lines, one can conclude that the compounds showed no toxicity up to 10 μM. Figure 2 shows the effect of compound **4** on the EC_{50} value of mitoxantrone in MDCK BCRP cells. In the presence of an inhibitor concentration of 5 μM, a significant decrease in resistance is observed, and in the presence of compound **4** at 10 μM the resistance is fully reversed and the cytotoxicity of mitoxantrone is restored, leading to a sensitivity of the MCF-7 MX cell line almost identical

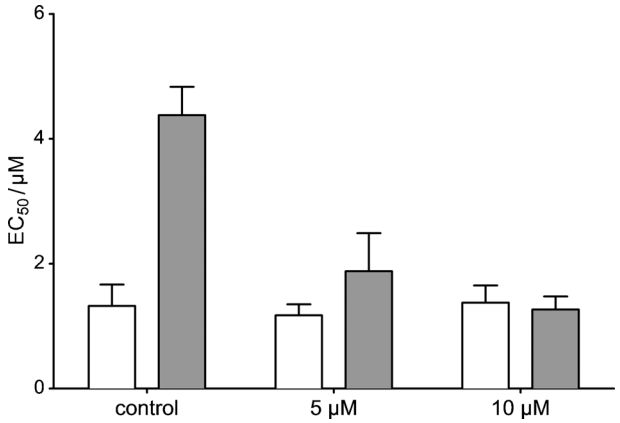


Figure 2. Effect of compound **4** on the EC_{50} value of mitoxantrone in MCF-7 (□) and MCF-7 MX (■) cells investigated at concentrations of 5 and 10 μM. Cells in the absence of inhibitor were used as control. Data are the average ± SD from three independent experiments.

to that of the parental MCF-7 cells. This result shows that this compound is able to reverse the resistance of MCF-7 MX cells against mitoxantrone completely. Figure 3 shows the dose–response curves of SN-38 in the presence and absence of compound **25**. The shift in the dose–response curves of MCF-7 MX

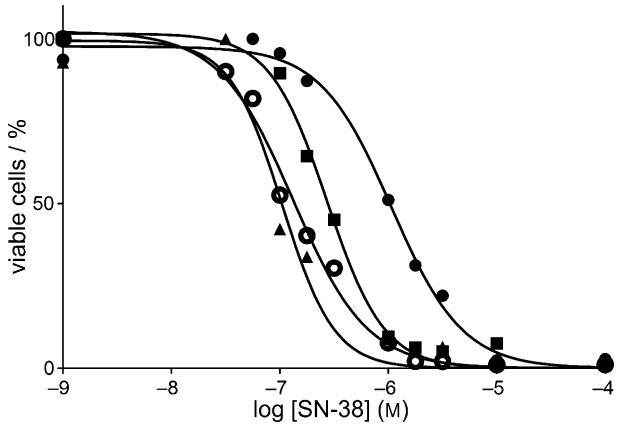


Figure 3. Shift of the dose–response curve of SN-38 caused by increasing inhibitor concentrations. Parental MDCK cells (○), resistant MDCK BCRP cells (●), MDCK BCRP cells + 5 μM compound **25** (■), MDCK BCRP cells + 10 μM compound **25** (▲).

cells caused by progressively higher inhibitor concentrations of compound **25** indicates that this compound also dose-dependently inhibits BCRP-mediated resistance to SN-38. In summary, these results prove the conclusions drawn from preliminary experiments reported in reference [17] that the inhibitory effect is not substrate dependent.

Conclusions

We have previously shown that these tariquidar analogues lacking the tetrahydroisoquinoline group are selective inhibitors of BCRP.[17] In this study we describe the synthesis of a larger series of compounds and their inhibitory potency

against BCRP and P-gp. The results obtained in the Hoechst 33342 assay in MCF-7 MX cells show that compounds **4**,[17] **16**, and **25** are the most potent inhibitors of BCRP in this class. However, whereas compound **4** is not a P-gp inhibitor, compounds **16** and **25** are also weak inhibitors of this transporter.

SAR analyses were performed to rationalize the activity differences within this class of compounds and to guide the synthesis of further derivatives. The results show that an electron-withdrawing substituent at position R^3 of the third aromatic ring is important for activity, probably because this part of the molecule interacts via a π–π stacking interaction with an aromatic residue in the BCRP binding site. At positions R^1 and R^2 all substituents led to a decrease in activity, pointing to steric hindrance in this region.

Experimental Section

General methods

Commercial reagents and starting materials were purchased from Sigma–Aldrich, Fluka, Acros, or Alfa Aesar and used without further purification. Reaction progress was monitored by TLC on alumina plates coated with silica gel (Merck silica gel 60 F_{254}) and visualized by UV light ($\lambda = 254$ nm). Spectral data were obtained on the following instruments: ^{1}H NMR, Bruker Advance 500 (500 MHz); ^{13}C NMR, Bruker Advance 500 (125.8 MHz). Chemical shifts (δ) are expressed in ppm, using solvent as an internal standard. The multiplicity of resonance peaks is indicated as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and multiplet (m). ^{13}C NMR signals are classified as primary (CH_3), secondary (CH_2), tertiary (CH or Ar-CH), or quaternary (Ar-C) carbon atoms. The J values are given in Hz, and the relative number of protons was determined by integration. The solvent used for each spectrum is reported. Elemental analyses were performed on a Vario EL instrument (Elementar). Found values were all within 0.4% of the theoretical values, except where indicated.

General procedure for the synthesis of acid chlorides (A)

The aromatic carboxylic acid (1 equiv) was dissolved/suspended in dry THF or CH_2Cl_2 and a catalytic amount of dry DMF. Oxalyl chloride (1.2 equiv) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. Finally, the solvent and excess oxalyl chloride were removed under reduced pressure to yield the desired acid chloride, which was immediately used for the synthesis of amides.

General procedure for the synthesis of the amides (B)

The aromatic amine (1 equiv) and Et_3N (3 equiv) were dissolved in dry THF and stirred at 0 °C. The acyl chloride (0.8 equiv) synthesized as per general method was suspended/dissolved in dry THF and added slowly. The solution was stirred for 12 h at room temperature. After completion of the reaction, the solvent was evaporated, and the residue was dissolved in H_2O and extracted three times with EtOAc. The organic phases were combined and washed with 1 N NaOH, 1 N HCl, and saturated brine, dried over $MgSO_4$, and concentrated. The product was precipitated with THF/petroleum ether (PE) and purified by recrystallization with CH_2Cl_2/PE (1:1) or EtOH or by column chromatography with EtOAc/PE (1:1) as eluent.

General procedure for the hydrogenation of an aromatic nitro group (C)

The nitro compound was dissolved in THF, and Pd/C was added. The suspension was stirred overnight at room temperature under H_2 (2 bar) in a Paar apparatus. The catalyst was filtered off, the solution was concentrated, and the product was precipitated with THF/PE.

***N*-[4-(2-Hydroxyethyl)phenyl]-4,5-dimethoxy-2-nitrobenzamide (2a)**. According to method B, 4,5-dimethoxy-2-nitrobenzoic acid (724 mg, 4 mmol) and 4-aminophenethyl alcohol (658 mg, 4.8 mmol) were reacted, and compound **2a** was obtained as a white powder (913 mg, 66%): $R_f = 0.58$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 10.41$ (s, 1H), 7.68 (s, 1H), 7.55 (d, $J =$ 8.47 Hz, 2H), 7.24 (s, 1H), 7.18 (d, $J = 8.48$ Hz, 2H), 4.61 (s, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.58 (t, $J = 7.06$ Hz, 2H), 2.69 ppm (t, $J =$ 7.10 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 164.03$, 153.13, 149.05, 138.89, 137.13, 135.06, 129.17 (2C), 127.59, 119.67 (2C), 111.23, 107.41, 62.41, 56.74, 56.46, 38.66 ppm; Anal. calcd for $C_{17}H_{18}N_2O_6 \cdot 0.33\,H_2O$: C 57.95, H 5.34, N 7.95, found: C 58.07, H 5.25, N 8.01.

***N*-[4-(2-Hydroxyethyl)phenyl]-5-methoxy-2-nitrobenzamide (2b)**. According to method B, 5-methoxy-2-nitrobenzoic acid (946 mg, 4.8 mmol) and 4-aminophenethyl alcohol (548 mg, 4 mmol) were reacted, and compound **2b** was obtained as a white powder (935 mg, 73%): $R_f = 0.54$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 10.52$ (s, 1H), 8.16 (d, $J = 9.77$ Hz, 1H), 7.55 (d, $J =$ 8.45 Hz, 2H), 7.22–7.17 (m, 4H), 4.64 (s, 1H), 3.93 (s, 3H), 3.58 (t, $J = 7.09$ Hz, 2H), 2.69 ppm (t, $J = 7.08$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 163.95$, 163.57, 138.91, 137.01, 135.88, 135.20, 129.22 (2C), 127.06, 119.72 (2C), 115.36, 114.35, 62.42, 56.67, 38.68 ppm; Anal. calcd for $C_{16}H_{16}N_2O_5 \cdot 0.33\,H_2O$: C 59.62, H 5.21, N 8.69, found: C 59.77, H 5.19, N 8.39.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-methoxy-2-nitrobenzamide (2c)**. According to method B, 4-methoxy-2-nitrobenzoic acid (986 mg, 5 mmol) and 4-aminophenethyl alcohol (685 mg, 6 mmol) were reacted, and compound **2c** was obtained as a yellow powder (573 mg, 36%): $R_f = 0.09$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 10.46$ (s, 1H), 7.70 (d, $J = 8.51$ Hz, 1H), 7.60 (d, $J =$ 2.55 Hz, 1H), 7.54 (d, $J = 8.41$ Hz, 2H), 7.37 (dd, $J = 8.53$, 2.54 Hz, 1H), 7.18 (d, $J = 8.42$ Hz, 2H), 4.59 (t, $J = 5.22$ Hz, 1H), 3.90 (s, 3H), 3.58 (dt, $J = 7.05$, 5.32 Hz, 2H), 2.69 ppm (t, $J = 7.08$, 7.08 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 163.64$, 160.59, 148.61, 137.02, 135.22, 130.71, 129.22 (2C), 124.80, 119.78 (2C), 118.92, 109.58, 62.42, 56.47, 38.67 ppm; Anal. calcd for $C_{16}H_{16}N_2O_5 \cdot 0.5\,H_2O$: C 59.07, H 5.27, N 8.61, found: C 60.08, H 5.05, N 8.62.

***N*-[4-(2-Hydroxyethyl)phenyl]-5-methyl-2-nitrobenzamide (2d)**. According to method B, 5-methyl-2-nitrobenzoic acid (906 mg, 5 mmol) and 4-aminophenethyl alcohol (823 mg, 6 mmol) were reacted, and compound **2d** was obtained as a yellow powder (1041 mg, 69%): $R_f = 0.71$ (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 10.48$ (s, 1H), 8.04 (d, $J = 8.3$, 1H), 7.54 (m, 4H), 7.18 (d, $J = 8.5$, 2H), 4.59 (t, $J = 5.2$, 1H), 3.58 (td, $J = 5.3$, 7.1, 2H), 2.69 (t, $J = 7.1$, 2H), 2.46 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 164.02$, 145.19, 143.96, 136.76, 135.07, 132.98, 130.87, 129.46, 129.06 (2C), 124.24, 119.54 (2C), 62.22, 38.49, 20.76 ppm; Anal. calcd for $C_{16}H_{16}N_2O_4$: C 63.99, H 5.37, N 9.33, found: C 64.23, H 5.43, N 9.16.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-methyl-2-nitrobenzamide (2e)**. According to method B, 4-methyl-2-nitrobenzoic acid (906 mg, 5 mmol) and 4-aminophenethyl alcohol (823 mg, 6 mmol) were reacted, and compound **2e** was obtained as a yellow powder

(972 mg, 65%): R_f = 0.64 (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.49 (s, 1H), 7.94 (s, 1H), 7.64 (m, 2H), 7.55 (d, J = 8.5, 2H), 7.18 (d, 8.5, 2H), 4.59 (t, J = 5.2, 1H), 3.58 (td, J = 5.3, 7.1, 2H), 2.69 (t, J = 7.1, 2H), 2.46 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.01, 146.87, 141.53, 136.95, 135.25, 134.27, 130.16, 129.22 (3C), 124.37, 119.75 (2C), 62.39, 38.66, 20.60 ppm; Anal. calcd for $C_{16}H_{16}N_2O_4 \cdot 0.16\,H_2O$: C 63.36, H 5.43, N 9.24, found: C 63.52, H 5.34, N 9.22.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-nitro-5-chlorobenzamide (2f)**. According to method B, 5-chloro-2-nitrobenzoic acid (1008 mg, 5 mmol) and 4-aminophenethyl alcohol (823 mg, 6 mmol) were reacted, and compound **2f** was obtained as a yellow powder (1269 mg, 79%): R_f = 0.52 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.60 (s, 1H), 8.16 (d, J = 8.72 Hz, 1H), 7.90 (d, J = 2.25 Hz, 1H), 7.82 (dd, J = 8.78, 2.27 Hz, 1H), 7.54 (d, J = 8.47 Hz, 2H), 7.20 (d, J = 8.50 Hz, 2H), 4.59 (t, J = 5.20 Hz, 1H), 3.59 (dt, J = 7.02, 5.27 Hz, 2H), 2.70 ppm (t, J = 7.07 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.47, 145.22, 138.82, 136.66, 135.57, 134.49, 130.82, 129.31 (2C), 129.27, 126.44, 119.83 (2C), 62.38, 38.67 ppm; Anal. calcd for $C_{15}H_{13}ClN_2O_4$: C 56.17, H 4.09, N 8.73, found: C 56.21, H 4.20, N 8.57.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-nitro-4-chlorobenzamide (2g)**. According to method B, 4-chloro-2-nitrobenzoic acid (1612 mg, 8 mmol) and 4-aminophenethyl alcohol (1316 mg, 9.6 mmol) were reacted, and compound **2g** was obtained as a yellow powder (2280 mg, 88%): R_f = 0.66 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.60 (s, 1H), 8.24 (d, J = 2.05 Hz, 1H), 7.95 (dd, J = 8.21, 2.11 Hz, 1H), 7.80 (d, J = 8.21 Hz, 1H), 7.53 (d, J = 8.47 Hz, 2H), 7.19 (d, J = 8.47 Hz, 2H), 4.59 (s, 1H), 3.61–3.55 (m, 2H), 2.69 ppm (t, J = 7.06 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.94, 147.59, 136.68, 135.55, 135.06, 133.77, 131.33, 131.04, 129.30 (2C), 124.32, 119.81 (2C), 62.37, 38.65 ppm; Anal. calcd for $C_{15}H_{13}ClN_2O_4$: C 56.17, H 4.09, N 8.73, found: C 55.92, H 4.28, N 8.41.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-nitrobenzamide (2h)**. According to method B, 2-nitrobenzoic acid (3.71 g, 20 mmol) and 4-aminophenethyl alcohol (2.74 g, 20 mmol) were reacted, and compound **2h** was obtained as a white powder (369 mg, 64%): R_f = 0.57 (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.55 (s, 1H), 8.13 (dd, J = 8.59, 1.06 Hz, 1H), 7.87–7.83 (dt, J = 15.01, 1.10 Hz, 1H), 7.76–7.72 (m, 2H), 7.55 (d, J = 8.47 Hz, 2H), 7.19 (d, J = 8.44 Hz, 2H), 4.59 (t, J = 5.23 Hz, 1H), 3.59 (dt, J = 7.03, 5.31 Hz, 2H), 2.70 ppm (t, J = 7.08 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.02, 146.00, 136.91, 135.35, 134.15, 132.92, 131.01, 129.40, 129.27 (2C), 124.35, 119.77 (2C), 62.40, 38.67 ppm; Anal. calcd for $C_{15}H_{14}N_2O_4 \cdot 0.25\,H_2O$: C 61.96, H 5.03, N 9.63, found: C 62.34, H 4.83, N 9.53.

***N*-[4-(2-Hydroxyethyl)phenyl]-3-nitrobenzamide (2i)**. According to method B, 3-nitrobenzoic acid (928 mg, 5 mmol) and 4-aminophenethyl alcohol (823 mg, 6 mmol) were reacted, and compound **2i** was obtained as a yellow powder (988 mg, 69%): R_f = 0.15 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.49 (s, 1H), 8.77 (t, J = 2.0, 1H), 8.44–8.37 (m, 2H), 7.83 (t, J = 8.0, 1H), 7.66 (d, J = 8.5, 2H), 7.21 (d, J = 8.5, 2H), 4.60 (t, J = 4.9, 1H), 3.60 (td, J = 4.8, 7.0, 2H), 2.70 ppm (t, J = 7.1, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.16, 147.77, 136.64, 136.42, 135.50, 134.19, 130.24, 129.10 (2C), 126.13, 122.44, 120.63 (2C), 62.28, 38.60 ppm; Anal. calcd for $C_{15}H_{14}N_2O_4 \cdot 0.167\,H_2O$: C 62.28, H 4.99, N 9.68, found: C 62.32, H 4.88, N 9.64.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-nitrobenzamide (2k)**. According to method B, 4-nitrobenzoic acid (329 mg, 2.4 mmol) and 4-aminophenethyl alcohol (371 mg, 2 mmol) were reacted, and compound **2k** was obtained as a yellow powder (440 mg, 76%): R_f = 0.68 (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.59 (s, 1H), 8.34 (d, J = 8.87 Hz, 2H), 8.19 (d, J = 8.90 Hz, 2H), 7.67 (d, J = 8.44 Hz, 2H), 7.20 (d, J = 8.50 Hz, 2H), 4.65 (s, 1H), 3.59 (t, J = 7.10 Hz, 2H), 2.70 ppm (t, J = 7.09 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.78, 149.23, 140.85, 136.79, 135.56, 129.32 (2C), 129.14 (2C), 123.61 (2C), 120.60 (2C), 62.33, 38.67 ppm; Anal. calcd for $C_{15}H_{14}N_2O_4 \cdot 1\,H_2O$: C 59.21, H 5.30, N 9.21, found: C 59.17, H 4.95, N 9.09.

2-Amino-*N*-[4-(2-hydroxyethyl)phenyl]-4,5-dimethoxybenzamide (3a). According to method C, compound **2a** (800 mg, 2.31 mmol) was reacted, and compound **3a** was obtained as a brown powder (607 mg, 83%): R_f = 0.21 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 9.64 (s, 1H), 7.54 (d, J = 8.49 Hz, 2H), 7.23 (s, 1H), 7.15 (d, J = 8.50 Hz, 2H), 6.36 (s, 1H), 6.27 (s, 2H$_2$), 4.59 (s, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.61–3.54 (m, 2H), 2.68 ppm (t, J = 7.14, 7.14 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 167.39, 153.43, 146.87, 139.18, 137.37, 134.45, 128.86 (2C), 120.98 (2C), 113.40, 105.57, 100.08, 62.45, 57.01, 55.32, 38.67 ppm; Anal. calcd for $C_{17}H_{20}N_2O_4 \cdot 0.33\,H_2O$: C 64.54, H 6.37, N 8.86, found: C 63.02, H 6.39, N 8.47.

2-Amino-*N*-[4-(2-hydroxyethyl)phenyl]-5-methoxybenzamide (3b). According to method C, compound **2b** (1.17 g, 3.7 mmol) was reacted, and compound **3b** was obtained as a brown powder (794 mg, 75%): R_f = 0.23 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 9.94 (s, 1H), 7.57 (d, J = 8.46 Hz, 2H), 7.18–7.14 (m, 3H), 6.89 (dd, J = 8.85, 2.88 Hz, 1H), 6.71 (d, J = 8.86 Hz, 1H), 5.83 (s, 2H), 4.65 (s, 1H), 3.72 (s, 3H), 3.60–3.56 (m, 2H), 2.68 ppm (t, J = 7.13 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 167.51, 149.53, 143.89, 137.17, 134.79, 128.93 (2C), 120.79 (2C), 119.75, 117.84, 116.05, 112.94, 62.43, 55.80, 38.68 ppm; Anal. calcd for $C_{16}H_{18}N_2O_3 \cdot 0.66\,H_2O$: C 64.41, H 6.53, N 9.39, found: 64.14, H 5.75, N 9.00.

2-Amino-*N*-[4-(2-hydroxyethyl)phenyl]-4-methoxybenzamide (3c). According to method C, compound **2c** (531 mg, 1.68 mmol) was reacted, and compound **3c** was obtained as a yellow powder (330 mg, 68%): R_f = 0.25 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 9.68 (s, 1H), 7.61 (d, J = 8.86 Hz, 1H), 7.56 (d, J = 8.48 Hz, 2H), 7.13 (d, J = 8.51 Hz, 2H), 6.53 (s, 2H$_2$), 6.27 (d, J = 2.54 Hz, 1H), 6.17 (dd, J = 8.81, 2.54 Hz, 1H), 4.58 (t, J = 5.23 Hz, 1H), 3.58 (dt, J = 7.14, 5.25 Hz, 2H), 2.68 ppm (t, J = 7.14 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 167.53, 162.51, 152.31, 137.45, 134.37, 130.46, 128.88 (2C), 120.67 (2C), 108.26, 102.51, 99.62, 62.46, 55.02, 38.67 ppm; Anal. calcd for $C_{16}H_{18}N_2O_3 \cdot 0.2\,H_2O$: C 66.28, H 6.40, N 9.66, found: C 66.53, H 6.402, N 9.624.

2-Amino-*N*-[4-(2-hydroxyethyl)phenyl]-5-methylbenzamide (3d). According to method C, compound **2d** (901 mg, 3 mmol) was reacted, and compound **3d** was obtained as a white powder (778 mg, 96%): R_f = 0.18 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 9.86 (s, 1H), 7.59 (d, J = 8.4, 2H), 7.41 (s, 1H), 7.15 (d, J = 8.4, 2H), 7.01 (dd, J = 1.9, 8.3, 1H), 6.66 (d, J = 8.3, 1H), 6.05 (s, 2H$_2$), 4.59 (s, 1H), 3.58 (t, J = 6.8, 2H), 2.69 (t, J = 7.1, 2H), 2.20 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 167.84, 147.47, 137.29, 134.61, 132.91, 128.92 (2C), 128.60, 123.26, 120.59 (2C), 116.66, 115.65, 62.43, 38.66, 20.16 ppm; Anal. calcd for $C_{16}H_{18}N_2O_2 \cdot 0.33\,H_2O$: C 69.54, H 6.81, N 10.14, found: C 69.30, H 6.58, N 9.82.

2-Amino-*N*-[4-(2-hydroxyethyl)phenyl]-4-methylbenzamide (3e). According to method C, compound **2e** (901 mg, 3 mmol) was reacted, and compound **3e** was obtained as a white powder (709 mg, 87%): R_f = 0.49 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 9.78 (s, 1H), 7.58 (d, J = 8.5, 2H), 7.54 (d, J = 8.1,

1H), 7.14 (d, $J=8.5$, 2H), 6.54 (d, $J=0.6$, 1H), 6.40 (dd, $J=1.4$, 8.2, 1H), 6.31 (s, $2H_2$), 4.59 (t, $J=5.0$, 1H), 3.58 (dd, $J=7.1$, 11.8, 2H), 2.68 (t, $J=7.1$, 2H), 2.19 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=167.75$, 150.08, 141.94, 137.33, 134.52, 128.89 (2C), 128.75, 120.66 (2C), 116.62, 116.02, 112.71, 62.43, 38.66, 21.24 ppm; Anal. calcd for $C_{16}H_{18}N_2O_2\cdot0.33\,H_2O$: C 69.54, H 6.81, N 10.14, found: C 69.37, H 6.41, N 9.97.

2-Amino-5-chloro-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (3 f). According to method C, compound **2 f** (1181 mg, 3.6 mmol) was reacted, and compound **3 f** was obtained as a white powder (945 mg, 90%): $R_f=0.40$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=10.00$ (s, 1H), 7.66 (d, $J=2.5$, 1H), 7.58 (d, $J=8.5$, 2H), 7.21 (dd, $J=2.5$, 8.8, 1H), 7.16 (d, $J=8.5$, 2H), 6.77 (d, $J=8.8$, 1H), 6.41 (s, 2H2), 4.23 (s, 1H), 3.59 (t, $J=7.1$, 2H), 2.69 ppm (t, $J=7.1$, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=166.52$, 148.66, 136.95, 134.98, 131.82, 128.96 (2C), 127.92, 120.75 (2C), 118.16, 118.02, 116.32, 62.39, 38.65 ppm; Anal. calcd for $C_{15}H_{13}ClN_2O_2$: C 61.97, H 5.20, N 9.64, found: C 61.95, H 5.30, N 9.25.

2-Amino-4-chloro-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (3 g). According to method C, compound **2 g** (1084 mg, 3.38 mmol) was reacted, and compound **3 g** was obtained as a yellow powder (914 mg, 93%): $R_f=0.66$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=10.13$ (s, 1H), 9.18 (s, 1H), 8.83 (d, $J=1.48$ Hz, 1H), 7.68 (d, $J=8.34$ Hz, 1H), 7.57 (d, $J=8.50$ Hz, 2H), 7.20 (d, $J=2.17$ Hz, 1H), 7.17 (d, $J=8.56$ Hz, 2H), 6.88 (dd, $J=8.31$, 2.19 Hz, 1H), 4.58 (t, $J=5.19$ Hz, 1H), 3.61–3.55 (m, 2H), 2.69 ppm (t, $J=7.10$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=166.10$, 153.16, 137.12, 136.73, 135.27, 130.26, 129.05 (2C), 120.76 (2C), 117.49, 116.11, 112.74, 62.38, 38.66 ppm.

2-Amino-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (3 h). According to method C, compound **2 h** (4.29 g, 15 mmol) was reacted, and compound **3 g** was obtained as a white powder (2.69 g, 70%): $R_f=0.70$ (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=9.88$ (s, 1H), 7.62–7.56 (m, 3H), 7.22–7.13 (m, 3H), 6.74 (dd, $J=8.28$, 1.02 Hz, 1H), 6.58 (ddd, $J=8.07$, 7.16, 1.14 Hz, 1H), 6.28 (s, 2H), 4.58 (t, $J=5.21$ Hz, 1H), 3.58 (dt, $J=7.10$, 5.22 Hz, 2H), 2.69 (t, $J=7.12$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=167.82$, 149.80, 137.26, 134.69, 132.10, 128.94 (2C), 128.74, 120.66 (2C), 116.48, 115.54, 114.82, 62.44, 38.67 ppm; Anal. calcd for $C_{15}H_{16}N_2O_2\cdot0.33\,H_2O$: C 68.68, H 6.40, N 10.68, found: C 68.96, H 6.15, N 10.60.

3-Amino-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (3 i). According to method C, compound **2 i** (2577 mg, 9 mmol) was reacted, and compound **3 i** was obtained as a gray powder (1779 mg, 77%): $R_f=0.48$ (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=9.94$ (s, 1H), 7.64 (d, $J=8.5$, 2H), 7.17–7.10 (m, 3H), 7.08–7.07 (m, 1H), 7.05–7.03 (m, 1H), 6.73 (ddd, $J=1.0$, 2.3, 7.9, 1H), 5.26 (s, 2H), 4.58 (t, $J=5.2$, 1H), 3.58 (td, $J=5.2$, 7.1, 1H), 2.68 ppm (t, $J=7.1$, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=166.28$, 148.83, 137.39, 136.14, 134.64, 128.95, 128.81, 120.29 (2C), 116.80, 114.82, 113.09, 62.41, 38.64 ppm.

4-Amino-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (3 k). According to method C, compound **2 k** (407 mg, 1.42 mmol) was reacted, and compound **3 k** was obtained as a white powder (299 mg, 82%): $R_f=0.46$ (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=9.65$ (s, 1H), 7.70 (d, $J=7.78$ Hz, 2H), 7.62 (d, $J=8.10$ Hz, 2H), 7.13 (d, $J=8.37$ Hz, 2H), 6.59 (d, $J=8.41$ Hz, 2H, Ar-CH), 5.68 (s, 2H), 4.58 (t, $J=5.12$ Hz, 1H), 3.58 (dd, $J=12.30$, 6.95 Hz, 2H), 2.67 ppm (t, $J=7.13$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=165.27$, 152.16, 137.81, 134.13, 129.40 (2C), 128.91 (2C), 121.41, 120.25 (2C), 112.70 (2C), 62.47, 38.67 ppm.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-(4-nitrobenzamido)benzamide (4)**. According to method B, compound **3 h** (153 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (92.8 mg, 0.5 mmol) were reacted, and compound **4** was obtained as a yellow powder (199 mg, 98%): $R_f=0.58$ (EtOAc/PE (1:1)); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=11.87$ (s, 1H), 10.50 (s, 1H), 8.42–8.35 (m, 3H), 8.15–8.11 (m, 2H), 7.94 (dd, $J=7.79$, 1.04 Hz, 1H), 7.65–7.57 (m, 3H), 7.32 (t, $J=7.57$ Hz, 1H), 7.19 (d, $J=8.41$ Hz, 2H), 4.60 (t, $J=5.19$ Hz, 1H), 3.58 (dt, $J=7.06$, 5.31 Hz, 2H), 2.69 ppm (t, $J=7.08$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=167.17$, 163.24, 149.53, 140.36, 138.19, 136.51, 135.71, 132.23, 129.15, 129.10 (2C), 128.77 (2C), 124.19 (2C), 124.05, 123.92, 121.98, 121.30 (2C), 62.37, 38.68 ppm; Anal. calcd for $C_{22}H_{19}N_3O_5\cdot0.5\,H_2O$: C 63.76, H 4.86, N 10.14, found: C 63.97, H 4.88, N 9.75.

***N*-[4-(2-Hydroxyethyl)phenyl]-3-(4-nitrobenzamido)benzamide (5)**. According to method B, compound **3 i** (123 mg, 0.48 mmol) and 4-nitrobenzoyl chloride (74 mg, 0.4 mmol) were reacted, and compound **5** was obtained as a yellow powder (31 mg, 19%): $R_f=0.39$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=10.84$ (s, 1H), 10.24 (s, 1H), 8.39–8.32 (m, 3H), 8.24 (d, $J=8.73$ Hz, 2H), 8.04 (d, $J=8.01$ Hz, 1H), 7.72 (d, $J=7.63$ Hz, 1H), 7.67 (d, $J=8.35$ Hz, 2H), 7.52 (t, $J=7.90$ Hz, 1H), 7.18 (d, $J=8.34$ Hz, 2H), 4.60 (s, 1H), 3.59 (t, $J=6.85$ Hz, 2H), 2.70 ppm (t, $J=7.05$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=165.32$, 164.12, 149.38, 140.46, 139.08, 137.19, 135.86, 135.01, 129.41 (2C), 129.05 (2C), 128.82, 123.69 (2C), 123.47, 123.17, 120.48 (2C), 120.26, 62.39, 38.66 ppm; Anal. calcd for $C_{22}H_{19}N_3O_5\cdot1.33\,H_2O$: C 61.53, H 5.09, N 9.79, found: C 61.17, H 5.11, N 9.66.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-(4-nitrobenzamido)benzamide (6)**. According to method B, compound **3 k** (154 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **6** was obtained as a white powder (210 mg, 100%): $R_f=0.63$ (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=10.89$ (s, 1H), 10.12 (s, 1H), 8.37 (d, $J=8.87$ Hz, 2H), 8.24 (d, $J=8.90$ Hz, 2H), 8.01 (d, $J=8.82$ Hz, 2H), 7.95 (d, $J=8.82$ Hz, 2H), 7.68 (d, $J=8.46$ Hz, 2H), 7.17 (d, $J=8.47$ Hz, 2H), 4.61 (t, $J=5.18$ Hz, 1H), 3.59 (dd, $J=12.31$, 7.07 Hz, 2H), 2.69 ppm (t, $J=7.11$, 7.11 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=164.78$, 164.34, 149.43, 141.78, 140.41, 137.30, 134.87, 130.33, 129.53 (2C), 129.02 (2C), 128.60 (2C), 123.67 (2C), 120.49 (2C), 119.81 (2C), 62.40, 38.67 ppm; HPLC (RP_8, H_2O/MeOH, 20:80) purity 99%.

***N*-[4-(2-Hydroxyethyl)phenyl]-4,5-dimethoxy-2-(4-nitrobenzamido)benzamide (7)**. According to method B, compound **3 a** (152 mg, 0.48 mmol) and 4-nitrobenzoyl chloride (74 mg, 0.4 mmol) were reacted, and compound **7** was obtained as a yellow powder (77 mg, 41%): $R_f=0.1$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=12.35$ (s, 1H), 10.33 (s, 1H), 8.38 (d, $J=8.68$ Hz, 2H), 8.26 (s, 1H), 8.12 (d, $J=8.69$ Hz, 2H), 7.54–7.50 (m, 3H), 7.20 (d, $J=8.29$ Hz, 2H), 4.60 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.62–3.57 (m, 1H), 2.70 ppm (t, $J=7.03$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=167.12$, 162.88, 151.71, 149.41, 144.43, 140.64, 136.29, 135.79, 134.26, 129.09 (2C), 128.55 (2C), 124.19 (2C), 121.89 (2C), 113.80, 112.21, 105.08, 62.35, 56.21, 55.75, 38.66 ppm; Anal. calcd for $C_{24}H_{23}N_3O_7\cdot0.4\,H_2O$: C 60.99, H 5.08, N 8.89, found: C 61.25, H 5.44, N 8.46.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-methoxy-2-(4-nitrobenzamido)-benzamide (8)**. According to method B, compound **3 c** (172 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **8** was obtained as a yellow powder (131 mg, 60%): $R_f=0.26$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=12.59$ (s, 1H), 10.32 (s, 1H), 8.40 (d, $J=8.86$ Hz, 2H),

8.25 (d, J=2.63 Hz, 1H), 8.12 (d, J=8.95 Hz, 2H), 8.00 (d, J=8.89 Hz, 1H), 7.56 (d, J=8.45 Hz, 2H), 7.20 (d, J=8.46 Hz, 2H), 6.87 (dd, J=8.84, 2.61 Hz, 1H), 4.59 (t, J=5.19 Hz, 1H), 3.87 (s, 3H), 3.59 (dt, J=7.07, 5.26 Hz, 2H), 2.70 ppm (t, J=7.07 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=167.40 (1C,CO), 163.14, 162.42, 149.59, 141.09, 140.22, 136.32, 135.76, 130.78, 129.09 (2C), 128.62 (2C), 124.30 (2C), 121.70 (2C), 113.84, 109.11, 106.10, 62.36, 55.68, 38.66 ppm; Anal. calcd for $C_{23}H_{21}N_3O_6$: C 63.44, H 4.86, N 9.65, found: C 63.42, H 4.88, N 9.28.

***N*-[4-(2-Hydroxyethyl)phenyl]-5-methoxy-2-(4-nitrobenzamido)-benzamide (9)**. According to method B, compound **3b** (171 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **9** was obtained as a yellow powder (91 mg, 56%): R_f=0.56 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=11.44 (s, 1H), 10.52 (s, 1H), 8.36 (d, J=8.74 Hz, 2H), 8.16 (d, J=8.95 Hz, 1H), 8.11 (d, J=8.89 Hz, 2H), 7.58 (d, J=8.72 Hz, 2H), 7.44 (d, J=3.27 Hz, 1H), 7.20–7.15 (m, 3H), 4.61 (s, 1H), 3.85 (s, 3H), 3.57 (t, J=7.18 Hz, 2H), 2.68 ppm (t, J=7.03 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=166.56, 163.12, 155.56, 149.35, 140.62, 136.61, 135.57, 130.87, 129.06 (2C), 128.74 (2C), 126.51, 124.38, 124.04 (2C), 121.17 (2C), 117.41, 114.13, 62.37, 55.77, 38.66 ppm; HPLC (RP$_8$, MeCN/H$_2$O, 80:20) purity 95.8%.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-methyl-2-(4-nitrobenzamido)-benzamide (10)**. According to method B, compound **3e** (162 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **10** was obtained as a yellow powder (71 mg, 31%): R_f=0.74 (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.10 (s, 1H), 10.39 (s, 1H), 8.38 (d, J=9.0, 2H), 8.33 (s, 1H), 8.11 (d, J=8.9, 2H), 7.87 (d, J=8.0, 1H), 7.57 (d, J=8.5, 2H), 7.19 (d, J=8.5, 2H), 7.13 (dd, J=1.0, 8.0, 1H), 4.59 (t, J=5.2, 1H), 3.59 (td, J=5.2, 7.1, 2H), 2.70 (t, J=7.1, 2H), 2.40 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=167.32, 162.99, 149.49, 142.67, 140.30, 138.66, 136.35, 135.73, 129.07 (2C), 128.99, 128.61 (2C), 124.44, 124.20 (2C), 121.78, 121.45 (2C), 119.96, 62.34, 38.64, 21.50 ppm; Anal. calcd for $C_{23}H_{21}N_3O_5$: C 65.86, H 5.05, N 10.02, found: C 65.58, H 5.09, N 9.98.

***N*-[4-(2-Hydroxyethyl)phenyl]-5-methyl-2-(4-nitrobenzamido)-benzamide (11)**. According to method B, compound **3d** (162 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **11** was obtained as a yellow powder (101 mg, 48%): R_f=0.76 (EtOAc); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=11.73 (s, 1H), 10.41 (s, 1H), 8.37 (d, J=8.6, 2H), 8.26 (d, J=8.3, 1H), 8.10 (d, J=8.6, 2H), 7.74 (s, 1H), 7.58 (d, J=8.2, 2H), 7.42 (d, J=8.1, 1H), 7.19 (d, J=8.3, 2H), 4.59 (t, J=5.1, 1H), 3.58 (dd, J=6.9, 12.3, 2H), 2.69 (t, J=7.0, 2H), 2.38 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=167.18, 162.98, 149.18, 140.24, 136.46, 135.65, 133.27, 132.61, 129.29, 129.07 (2C), 128.65 (2C), 124.13 (2C), 123.79, 121.92, 121.21 (2C), 62.34, 38.64, 20.58 ppm; Anal. calcd for $C_{23}H_{21}N_3O_5 \cdot 0.33\,H_2O$: C 64.93, H 5.13, N 9.88, found: C 64.54, H 5.12, N 9.73.

4-Chloro-*N*-[4-(2-hydroxyethyl)phenyl]-2-(4-nitrobenzamido)benzamide (12). According to method B, compound **3g** (140 mg, 0.84 mmol) and 4-nitrobenzoyl chloride (74 mg, 0.4 mmol) were reacted, and compound **12** was obtained as a yellow powder (36 mg, 19%): R_f=0.47 (EtOAc/PE (1:1)); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.03 (s, 1H), 10.54 (s, 1H), 8.52 (d, J=1.02 Hz, 1H), 8.39 (d, J=8.40 Hz, 2H), 8.12 (d, J=8.51 Hz, 2H), 7.98 (d, J=8.37 Hz, 1H), 7.56 (d, J=7.98 Hz, 2H), 7.42–7.36 (m, 1H), 7.19 (d, J=8.01 Hz, 2H), 4.59 (t, J=5.02 Hz, 1H), 3.58 (dd, J=12.22, 6.87 Hz, 2H), 2.69 ppm (t, J=6.86 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=166.33, 163.52–163.50, 163.57, 149.64, 139.92, 136.60, 136.29, 135.93, 130.87, 129.16 (2C), 128.84 (2C), 124.23 (2C), 123.70, 122.03, 121.37 (2C), 121.18, 62.35, 38.66 ppm; HPLC (RP$_8$, MeOH/H$_2$O, 80:20) purity 99.9%.

5-Chloro-*N*-[4-(2-hydroxyethyl)phenyl]-2-(4-nitrobenzamido)benzamide (13). According to method B, compound **3f** (174 mg, 0.6 mmol) and 4-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **13** was obtained as a yellow powder (84 mg, 48%): R_f=0.34 (silica gel, EtOAc/PE (1:1)); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=11.74 (s, 1H), 10.53 (s, 1H), 8.40–8.34 (m, 3H), 8.11 (d, J=8.85 Hz, 2H), 7.97 (d, J=2.44 Hz, 1H), 7.68 (dd, J=8.86, 2.46 Hz, 1H), 7.57 (d, J=8.41 Hz, 2H), 7.19 (d, J=8.44 Hz, 2H), 4.59 (t, J=5.18 Hz, 1H), 3.58 (dt, J=7.03, 5.29 Hz, 2H), 2.69 ppm (t, J=7.06 Hz, 2H, **7**); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=165.70, 163.32, 149.59, 140.02, 136.93, 136.29, 135.91, 131.82, 129.13 (2C), 128.82 (2C), 128.72, 128.01, 125.83, 124.18 (2C), 123.84, 121.26 (2C), 62.35, 38.66 ppm; Anal. calcd for $C_{22}H_{18}ClN_3O_5$: C 60.07, H 4.12, N 9.55, found: C 59.94, H 4.36, N 9.42.

2-(Cyclohexancarboxamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (14). According to method B, compound **3h** (154 mg, 0.6 mmol) and cyclohexanecarboxylic acid chloride (73 mg, 0.5 mmol) were reacted, and compound **14** was obtained as a white powder (150 mg, 82%): R_f=0.60 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.68 (s, 1H), 10.37 (s, 1H), 8.28 (d, J=8.18 Hz, 1H), 7.82 (dd, J=7.80, 1.26 Hz, 1H), 7.59 (d, J=8.39 Hz, 2H), 7.50 (t, J=8.59 Hz, 1H), 7.20–7.16 (m, 3H), 4.63 (t, J=5.19 Hz, 1H), 3.59 (dt, J=7.07, 5.41 Hz, 2H), 2.70 (t, J=7.10 Hz, 2H), 2.27 (tt, J=11.38, 3.53 Hz, 1H), 1.84 (dd, J=12.92, 2.54 Hz, 2H), 1.73–1.67 (m, 2H), 1.64–1.58 (m, 1H), 1.37 (ddd, J=23.93, 12.29, 2.89 Hz, 2H), 1.31–1.21 (m, 2H), 1.16 ppm (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=173.99, 167.12, 138.63, 136.63, 135.54, 131.90, 129.07 (2C), 128.89, 123.19, 122.89, 121.34, 121.09 (2C), 62.40, 45.56, 38.68, 29.19 (2C), 25.53, 25.23 ppm (2C); HPLC (RP$_8$, MeCN/H$_2$O, 80:20) purity 99.5%.

2-Acetamido-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (15). According to method B, compound **3h** (128,2 mg, 0.5 mmol) and acetyl chloride (31.0 mg, 0.4 mmol) were reacted, and compound **15** was obtained as a white powder (23 mg, 20%): R_f=0.44 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.43 (s, 1H), 10.33 (s, 1H), 8.15 (d, J=7.43 Hz, 1H), 7.74 (d, J=6.96 Hz, 1H), 7.59 (d, J=7.56 Hz, 2H), 7.49 (t, J=7.44 Hz, 1H), 7.22–7.17 (m, 3H), 4.59 (s, 1H), 3.58 (s, 2H), 2.69 (t, J=6.72 Hz, 2H), 2.05 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=168.38, 166.89, 138.05, 136.82, 135.39, 131.65, 129.06 (2C), 128.75, 124.36, 123.16, 121.79, 120.85 (2C), 62.43, 38.69, 24.56 ppm; HPLC (RP$_8$, MeCN/H$_2$O, 80:20) purity 98.9%.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-(3-nitrobenzamido)benzamide (16)**. According to method B, compound **3h** (154 mg, 0.6 mmol) and 3-nitrobenzoyl chloride (93 mg, 0.5 mmol) were reacted, and compound **16** was obtained as a yellow powder (105 mg, 52%): R_f=0.1 (EtOAc/cyclohexane, 1:2); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=11.81 (s, 1H), 10.44 (s, 1H), 8.69 (t, J=1.91 Hz, 1H), 8.43 (dd, J=8.20, 2.24 Hz, 1H), 8.33 (d, J=8.14 Hz, 1H), 8.30 (d, J=7.80 Hz, 1H), 7.91 (d, J=7.47 Hz, 1H), 7.86 (t, J=7.99 Hz, 1H), 7.64–7.59 (m, 3H), 7.32 (t, J=7.63 Hz, 1H), 7.19 (d, J=8.41 Hz, 2H), 4.59 (t, J=5.20 Hz, 1H), 3.59 (dt, J=7.06, 5.40 Hz, 1H), 2.70 ppm (t, J=7.07, 7.07 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=167.11, 162.79, 148.17, 137.98, 136.58, 136.17, 135.66, 133.25, 132.14, 130.82, 129.10 (2C), 129.05, 126.54, 124.42, 124.13, 122.26, 121.14 (2C), 62.37, 38.67 ppm; HPLC (RP$_8$, MeCN/H$_2$O 80:20) purity 97.1%.

2-(3-Aminobenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (17). According to method C, compound **16** (51.3 mg, 0.2 mmol)

was reacted, and compound **17** was obtained as a white powder (50 mg, 67%): $R_f = 0.89$ (MeOH); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 11.62 (s, 1H), 10.44 (s, 1H), 8.54 (dd, $J = 8.34$, 0.98 Hz, 1H), 8.07–7.75 (m, 1H), 7.61–7.56 (m, 3H), 7.25 (dt, $J = 7.67$, 1.15 Hz, 1H), 7.21 (d, $J = 8.49$ Hz, 2H), 7.17 (t, $J = 7.82$ Hz, 1H), 7.12 (t, $J = 1.99$ Hz, 1H), 6.99 (d, $J = 8.40$ Hz, 1H), 6.76 (ddd, $J = 7.90$, 2.22, 0.67 Hz, 1H), 5.37 (s, 2H), 4.60 (t, $J = 5.22$ Hz, 1H), 3.66–3.52 (m, 2H), 2.70 ppm (t, $J =$ 7.08, 7.08 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 167.51$, 165.42, 149.39, 139.21, 136.41, 135.80, 135.48, 132.39, 129.44, 129.17 (2C), 129.02, 123.00, 122.16, 121.37 (2C), 121.03, 117.41, 113.70, 112.61, 62.38, 38.69 ppm; Anal. calcd for $C_{22}H_{21}N_3O_3 \cdot 1.5\,H_2O$: C 68.41, H 5.79, N 10.88, found: C 68.04, H 5.67, N 10.44.

2-(4-Aminobenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (18). According to method C, compound **4** (324 mg, 0.8 mmol) was reacted, and compound **18** was obtained as a white powder (161 mg, 71%): $R_f = 0.55$ (silica gel, EtOAc/PE (1:1)); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 11.54$ (s, 1H), 10.44 (s, 1H), 8.56 (d, $J =$ 7.1, 8.4, 1H), 7.90 (d, $J = 7.1$, 7.9, 1H), 7.61 (dd, $J = 7.1$, 6.1, 8.4, 4H), 7.55 (t, $J = 7.1$, 7.8, 1H), 7.20 (dd, $J = 7.1$, 8.0, 15.0, 3H), 6.62 (d, $J =$ 7.1, 8.7, 2H), 5.83 (s, 2H), 4.61 (t, $J = 7.1$, 5.2, 1H), 3.59 (m, 2H), 2.71 ppm (t, $J = 7.1$, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 167.73$, 164.36, 152.68, 139.81, 136.39, 135.74, 132.30, 129.12 (2C), 128.96, 128.78 (2C), 122.28, 121.58, 121.36 (2C), 120.71, 120.65, 113.08 (2C), 62.34, 38.67 ppm; Anal. calcd for $C_{22}H_{21}N_3O_3 \cdot 0.33\,H_2O$: C 69.28, H 5.73, N 11.02, found: C 69.11, H 5.52, N 10.80.

2-(4-Cyanobenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (19). According to method B, compound **3h** (154 mg, 0.6 mmol) and 4-cyanobenzoyl chloride (83 mg, 0.5 mmol) were reacted, and compound **19** was obtained as a white powder (98 mg, 51%): $R_f =$ 0.23 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 11.83$ (s, 1H), 10.45 (s, 1H), 8.40 (dd, $J = 8.26$, 0.70 Hz, 1H), 8.06–8.02 (m, 4H), 7.92 (dd, $J = 7.85$, 1.27 Hz, 1H), 7.63–7.59 (m, 1H), 7.58 (d, $J =$ 8.44 Hz, 2H), 7.31 (dt, $J = 7.70$, 1.06 Hz, 1H), 7.20 (d, $J = 8.46$ Hz, 2H), 4.59 (t, $J = 5.20$ Hz, 1H), 3.59 (dt, $J = 7.10$, 5.26 Hz, 2H), 2.70 ppm (t, $J = 7.07$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 167.06, 163.22, 138.49, 138.10, 136.28, 135.60, 132.9 (2C), 132.11, 128.96 (2C), 127.86 (2C), 123.79, 123.46, 121.61, 121.15 (2C), 118.15, 114.22, 62.20, 38.50 ppm; Anal. calcd for $C_{23}H_{19}N_3O_3 \cdot 0.8\,H_2O$: C 69.09, H 15.19, N 10.51, found: C 68,95, H 4.79, N 10.47.

2-(4-Chlorobenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (20). According to method B, compound **3h** (154 mg, 0.6 mmol) and 4-chlorbenzoyl chloride (88 mg, 0.5 mmol) were reacted, and compound **20** was obtained as a white powder (166 mg, 84%): $R_f = 0.32$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 11.77$ (s, 1H), 10.45 (s, 1H), 8.45 (dd, $J = 8.35$, 0.82 Hz, 1H), 7.94–7.89 (m, 3H), 7.65–7.57 (m, 5H), 7.29 (dt, $J = 7.71$, 1.09 Hz, 1H),7.20 (d, $J =$ 8.48 Hz, 2H) 4.60 (t, $J = 5.21$ Hz, 1H), 3.61–3.57 (m, 2H), 2.70 ppm (t, $J = 7.07$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 167.37$, 163.70, 138.66, 137.01, 136.42, 135.79, 133.41, 132.32, 129.15, 129.13 (2C), 129.06 (2C), 123.57 (2C), 123.06, 121.50, 121.37 (2C), 121.26, 62.37, 38.67 ppm; Anal. calcd for $C_{22}H_{19}ClN_2O_3 \cdot 0.33\,H_2O$: C 65.92, H 4.95, N 6.99, found: C 65.97, H 4.69, N 6.83.

2-(3-Chlorobenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (21). According to method B, compound **3h** (154 mg, 0.6 mmol) and 3-chlorobenzoyl chloride (88 mg, 0.5 mmol) were reacted, and compound **21** was obtained as a white powder (1974 mg, 100%): $R_f = 0.21$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 11.72$ (s, 1H), 10.53 (s, 1H), 8.35 (dd, $J = 8.25$, 0.69 Hz, 1H), 7.96–7.91 (m, 2H), 7.86–7.83 (m, 1H), 7.67 (ddd, $J = 7.98$, 2.00, 0.89 Hz, 1H), 7.62–7.56 (m, 4H), 7.28 (t, $J = 7.50$ Hz, 1H), 7.19 (d, $J = 8.50$ Hz, 2H), 4.64 (m, 1H), 3.62–3.54 (m, 2H), 2.69 ppm (t, $J = 7.09$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 167.18$, 163.46, 136.83, 136.58, 135.64, 133.78, 132.13, 131.86, 130.99, 129.19, 129.08 (2C), 127.31, 125.70, 123.92, 123.78, 121.98, 121.22, 62.36, 38.68 ppm; Anal. calcd for $C_{22}H_{19}ClN_2O_3 \cdot 1.33\,H_2O$: C 63.08, H 5.21, N 6.69, found: C 63,49, H 5.11, N 6.92.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-[4-(trifluoromethyl)benzamido]-benzamide (22)**. According to method A, 4-trifluoromethylbenzoic acid (95 mg, 0.5 mmol) was reacted to 4-trifluoromethylbenzoyl chloride and then, according to method B, with compound **3h** (154 mg, 0.6 mmol) to obtain compound **22** as a white powder (74 mg, 34%): $R_f = 0.23$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 11.85$ (s, 1H), 10.46 (s, 1H), 8.43 (dd, $J = 8.24$, 0.79 Hz, 1H), 8.09 (d, $J = 8.09$ Hz, 2H), 7.96–7.93 (m, 3H), 7.64–7.60 (m, 1H), 7.59 (d, $J = 8.44$ Hz, 2H), 7.33–7.29 (m, 1H), 7.19 (d, $J =$ 8.47 Hz, 2H), 4.59 (t, $J = 5.20$ Hz, 1H), 3.58 (dt, $J = 7.05$, 5.23 Hz, 2H), 2.70 ppm (t, $J = 7.08$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 167.27, 163.63, 138.49, 138.41, 136.46, 135.75, 132.30, 132.20–131.39 (m,1C), 129.12 (2C), 128.12 (2C), 126.09 (2C), 123.96 (q, $J =$ 269.49 Hz, 1C), 123.84, 123.47, 121.71, 121.32 (2C), 62.37, 38.67 ppm; Anal. calcd for $C_{23}H_{19}F_3N_2O_3$: C 64.48, H 4.47, N 6.54, found: C 64.28, H 4.68, N 6.45.

4-{2-[4-(2-Hydroxyethyl)phenylcarbamoyl]phenylcarbamoyl}-phenyl acetate (23). According to method A, 4-acetoxybenzoic acid (216 mg, 1.2 mmol) was reacted to 4-acetoxybenzoyl chloride and then, according to method B, with compound **3h** (369 mg, 1.44 mmol) to obtain compound **23** as a white powder (60 mg, 12%): $R_f = 0.24$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 11.75 (s, 1H), 10.45 (s, 1H), 8.47 (d, $J = 7.4$, 1H), 7.94 (m, 3H), 7.60 (m, 3H), 7.33 (d, $J = 8.7$, 2H), 7.28 (td, $J = 1.2$, 7.7, 1H), 7.20 (d, $J =$ 8.5, 2H), 4.59 (t, $J = 5.2$, 1H), 3.59 (td, $J = 5.3$, 7.1, 2H), 2.70 (t, $J =$ 7.1, 2H), 2.30 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 168.99, 167.38, 163.97, 153.35, 138.81, 136.43, 135.74, 132.31, 132.31, 129.12 (2C), 129.05, 128.67 (2C), 123.42, 122.91, 122.49 (2C), 121.40, 121.33 (2C), 62.36, 38.68, 20.99 ppm; Anal. calcd for $C_{24}H_{22}N_2O_5 \cdot 0.66\,H_2O$: C 66.97, H 5.46, N 6.51, found: C 66.95, H 5.30, N 6.90.

2-(4-*tert*-Butylbenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (24). According to method A, 4-*tert*-butylbenzoic acid (98 mg, 0.4 mmol) was reacted to 4-*tert*-butylbenzoyl chloride and then, according to method B, with compound **3h** (123 mg, 0.48 mmol) to obtain compound **24** as a white powder (22 mg, 13%): $R_f = 0.44$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 11.82 (s, 1H), 10.49 (s, 1H), 8.54 (dd, $J = 8.29$, 0.80 Hz, 1H), 7.94 (dd, $J = 7.86$, 1.40 Hz, 1H), 7.86–7.83 (m, 2H), 7.58 (ddd, $J = 6.44$, 5.47, 1.98 Hz, 5H), 7.25 (t, $J = 7.61$ Hz, 1H), 7.20 (d, $J = 8.46$ Hz, 2H), 4.62 (s, 1H), 3.59 (t, $J = 7.12$ Hz, 2H), 2.70 (t, $J = 7.08$ Hz, 2H), 1.30 ppm (s, 9H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 167.49$, 164.63, 155.10, 136.75, 136.44, 135.76, 132.36, 132.00, 129.12 (2C), 129.08, 126.98 (2C), 125.87 (2C), 123.05, 122.29, 121.39 (2C), 121.07, 62.36, 38.68, 34.86, 31.01 ppm (3C); Anal. calcd for $C_{26}H_{28}N_2O_3 \cdot 1.4\,H_2O$: C 70.69, H 7.03, N 6.34, found: C 70.83, H 7.49, N 6.77.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-(4-isopropoxybenzamido)benzamide (25)**. According to method A, 4-isopropoxybenzoic acid (98 mg, 0.4 mmol) was reacted to 4-isopropoxybenzoyl chloride and then, according to method B, with compound **3h** (154 mg, 0.6 mmol) to obtain compound **25** as a white powder (132 mg, 63%): $R_f = 0.73$ (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 11.74 (s, 1H), 10.45 (s, 1H), 8.54 (m, 1H), 7.92 (dd, $J = 1.4$, 7.9, 1H), 7.85 (d, $J = 8.9$, 2H), 7.58 (m, 3H), 7.23 (m, 3H), 7.06 (d, $J = 8.9$, 2H), 4.75–4.67 (m, 1H), 4.60 (t, $J = 5.2$, 1H), 3.60 (td, $J = 5.3$, 7.1, 2H), 2.71 (t, $J = 7.1$, 2H), 1.28 ppm (d, $J = 6.0$, 6H); ^{13}C NMR (125 MHz,

[D_6]DMSO): δ = 167.59, 164.13, 160.71, 139.32, 136.37, 135.82, 132.38, 129.13 (2C), 129.05 (2C), 129.02, 126.25, 122.92, 122.10, 121.43 (2C), 120.99, 115.58 (2C), 69.75, 62.36, 38.67, 21.84 ppm; Anal. calcd for $C_{25}H_{26}N_2O_4 \cdot 0.25\,H_2O$: C 70.99, H 6.31, N 6.62, found: C 70.63, H 6.18, N 6.53.

2-(4-Hydroxybenzamido)-*N*-[4-(2-hydroxyethyl)phenyl]benzamide (26). Compound **23** was suspended in MeOH (10 mL) and NaOH (3.3 mmol). The reaction proceeded at 60 °C. Compound **26** was extracted with EtOAc into the organic phase and then precipitated as a white powder (102 mg, 54%) with THF/PE: R_f = 0.71 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 11.66 (s, 1H), 10.44 (s, 1H), 10.19 (s, 1H), 8.53 (dd, J = 1.0, 8.4, 1H), 7.91 (dd, J = 1.4, 7.9, 1H), 7.77 (d, J = 8.8, 2H), 7.58 (m, 3H), 7.23 (m, 3H), 6.90 (d, J = 8.8, 2H), 4.60 (s, 1H), 3.60 (t, J = 7.0, 2H), 2.71 ppm (t, J = 7.1, 2H); ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 167.61, 164.34, 161.11, 139.41, 136.39, 135.80, 132.36, 129.15 (4C), 129.02, 125.09, 122.81, 122.04, 121.42 (2C), 120.97, 115.62 (2C), 62.37, 38.69 ppm; Anal. calcd for $C_{22}H_{20}N_2O_4 \cdot 0.5\,H_2O$: C 68.56, H 5.49, N 7.27, found: C 68.18, H 5.38, N 7.11.

***N*-{2-[4-(2-hydroxyethyl)phenylcarbamoyl]phenyl}-3,4-dimethoxybenzamide (27)**. According to method A, 3.4-dimethoxybenzoic acid (94 mg, 0.5 mmol) was reacted to 3,4-dimethoxybenzoyl chloride and then, according to method B, with compound **3h** (154 mg, 0.6 mmol) to obtain compound **27** as a white powder (135 mg, 64%): R_f = 0.05 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 11.74 (s, 1H), 10.45 (s, 1H), 8.51 (d, J = 7.67 Hz, 1H), 7.92 (dd, J = 7.86, 1.27 Hz, 1H), 7.63–7.57 (m, 3H), 7.50 (dd, J = 6.53, 2.06 Hz, 2H), 7.25 (dt, J = 7.72, 1.05 Hz, 1H), 7.21 (d, J = 8.45 Hz, 2H), 7.13 (d, J = 8.97 Hz, 1H), 4.60 (t, J = 5.19 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.59 (dt, J = 7.08, 5.30 Hz, 2H), 2.70 ppm (t, J = 7.07 Hz, 2H); ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 167.53, 164.28, 152.11, 148.83, 139.17, 136.47, 135.78, 132.36, 129.12 (2C), 129.01, 126.90, 123.03, 122.43, 121.20 (2C), 121.03, 120.06, 111.58, 110.75, 62.36, 55.87, 55.63, 38.67 ppm; Anal. calcd for $C_{24}H_{24}N_2O_5 \cdot 0.33\,H_2O$: C 67.59, H 5.83, N 6.57, found: C 67.99, H 6.01, N 6.39.

***N*-[4-(2-Hydroxyethyl)phenyl]-2-(4-methylbenzamido)benzamide (28)**. According to method B, compound **3h** (154 mg, 0.6 mmol) and 4-methylbenzoyl chloride (77 mg, 0.5 mmol) were reacted, and compound **28** was obtained as a white powder (170 mg, 91%): R_f = 0.53 (EtOAc/PE, 1:1); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 11.80 (s, 1H), 10.50 (s, 1H), 8.51 (dd, J = 8.34, 0.98 Hz, 1H), 7.93 (dd, J = 7.85, 1.36 Hz, 1H), 7.81 (d, J = 8.20 Hz, 2H), 7.58 (dd, J = 11.99, 4.96 Hz, 3H), 7.36 (d, J = 7.92 Hz, 2H), 7.27–7.22 (m, 1H), 7.20 (d, J = 8.53 Hz, 2H), 4.64 (s, 1H), 3.59 (t, J = 7.10 Hz, 2H), 2.70 (t, J = 7.08 Hz, 2H), 2.37 ppm (s, 3H); ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 167.50, 164.68, 142.23, 139.33, 136.49, 135.75, 132.34, 132.01, 129.58 (2C), 129.15 (2C), 129.07, 127.16 (2C), 123.10, 122.52, 121.37 (2C), 121.24, 62.39, 38.69, 21.15 ppm; Anal. calcd for $C_{23}H_{22}N_2O_3 \cdot 0.33\,H_2O$: C 72.61, H 6.01, N 7.36, found: C 72.69, H 5.92, N 7.24.

Cell culture

The breast cancer resistant cell line MCF-7 MX and the parental cell line MCF-7 were kindly provided by Dr. E. Schneider (Wadsworth Center, Albany, NY, USA). Parental MDCK and MDCK BCRP cell lines were kindly provided by Dr. A. H. Schinkel (NCI, Amsterdam). The human ovarian carcinoma cell line A2780 and the corresponding P-gp-overexpressing cell line A2780 Adr were purchased from the European collection of animal cell cultures (ECACC, Nos. 93112519 [A2780] and 93112520 [A2780 Adr], UK). The cell lines were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (20% for MCF-7 cells), 50 μg mL^{-1} streptomycin, and 50 U mL^{-1} penicillin G. Cells were incubated in a humidified atmosphere containing 5% CO_2 at 37 °C.

Hoechst 33342 assay

For investigation of BCRP activity the Hoechst 33342 assay was used as described previously, applying a Hoechst 33342 concentration of 1 μM in the assay.[14,18] In brief, the breast cancer resistant cell line MCF-7 MX and the parental cell line MCF-7 were grown in RPMI-1640 medium supplemented with 20% fetal bovine serum, 50 μg mL^{-1} streptomycin, and 50 U mL^{-1} penicillin G, in a 5% CO_2 atmosphere at 37 °C. After reaching a confluence of 80–90%, cells were harvested with trypsin–EDTA (0.05% trypsin, 0.02% EDTA), transferred to a 50 mL tube, centrifuged (266 *g*, 4 °C, 4 min) and resuspended in fresh culture medium. The cell density was measured with a Casy I Modell TT cell counter device (Schaerfe System GmbH, Reutlingen, Germany). Cells were again centrifuged and resuspended in Krebs–HEPES buffer. Cells were seeded into black 96-well plates at a density of 27000 cells per well in a volume of 90 μL. Test compounds (10 μL) at various concentrations were added to give a total volume of 100 μL. The prepared plates were then incubated under 5% CO_2 atmosphere and 37 °C for 30 min. After this pre-incubation period, Hoechst 33342 solution (20 μL, 6 μM) was added to each well. Fluorescence was measured immediately at constant intervals of 60 s, at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, using a BMG POLARstar microplate reader held at 37 °C.

Calcein AM assay

The inhibitory activity of studied compounds against P-gp was tested in A2780 Adr cells using the calcein AM assay as described previously.[20] In brief, the cells were prepared as described for the Hoechst 33342 assay, but seeded into clear 96-well plates. To 90-μL cell suspensions each containing 27000 cells, test compounds (10 μL) at varying concentration were added, and the cells were pre-incubated for 30 min. After the pre-incubation period, calcein AM solution (33 μL, 1.25 μM) was added to each well. The fluorescence of each well was detected at constant intervals (60 s) up to 60 min using an excitation wavelength of 485 nm and an emission wavelength of 520 nm, applying a 37 °C tempered BMG POLARstar microplate reader. The slope of the initial linear portion of each fluorescence–time curve was calculated by linear regression. From the slopes, concentration–response curves were generated by nonlinear regression using the four-parameter logistic equation with variable Hill slope (GraphPad Prism 5.0 software, San Diego, CA, USA).

MTT cytotoxicity assay

The influence of the new BCRP inhibitors on the cytotoxicity of mitoxantrone and SN-38 was investigated in MCF-7 MX and MDCK BCRP cells by MTT cytotoxicity assay. Assays were performed as described previously, with minor modifications.[19] Cells were seeded into 96-well tissue-culture plates at a density of 10000 cells per well for MCF-7 MX cells, or 1250 in case of MDCK BCRP cells, in a total volume of 80 μL and kept at 37 °C under 5% CO_2 for 6 h. After cells had attached to the well bottom, 10 μL test compound and 10 μL mitoxantrone solution were added. Cells were incubated for 72 h, and then the MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 20 μL of a 5 mg mL^{-1} solution) was added to each well. After 1 h incubation with MTT, the supernatant

was removed, and cells were lysed with 100 μL DMSO per well. Viability of the cells was determined spectrophotometrically by measuring absorbance at 544 nm with background correction at 710 nm using a BMG POLARstar microplate reader.

Keywords: ABC transporter · antitumor agents · BCRP · drug design · inhibitors · QSAR

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