Structure—Activity Relationships, Ligand Efficiency, and Lipophilic Efficiency Profiles of Benzophenone-Type Inhibitors of the Multidrug Transporter P-Glycoprotein

Ishrat Jabeen, † Karin Pleban, † Uwe Rinner, ‡ Peter Chiba, § and Gerhard F. Ecker*†

†University of Vienna, Department of Medicinal Chemistry, Althanstrasse 14, 1090, Vienna, Austria
‡University of Vienna, Department of Organic Chemistry, Währingerstrasse 38, 1090, Vienna, Austria
§Medical University of Vienna, Institute of Medical Chemistry, Währingerstrasse 10, 1090, Vienna, Austria

ABSTRACT: The drug efflux pump P-glycoprotein (P-gp) has been shown to promote multidrug resistance (MDR) in tumors as well as to influence ADME properties of drug candidates. Here we synthesized and tested a series of benzophenone derivatives structurally analogous to propafenone-type inhibitors of P-gp. Some of the compounds showed ligand efficiency and lipophilic efficiency (LipE) values in the range of compounds which entered clinical trials as MDR modulators. Interestingly, although lipophilicity plays a dominant role for P-gp inhibitors, all compounds investigated showed LipE values below the threshold for promising drug candidates. Docking studies of selected analogues into a homology model of P-glycoprotein suggest that benzophenones show an interaction pattern similar to that previously identified for propafenone-type inhibitors.

INTRODUCTION

Membrane transporters are increasingly recognized for playing a key role in safety profiles of drug candidates, predominantly by their involvement in drug–drug interactions.1,2 One of the most intensively studied families in this context is the ATP-binding cassette (ABC) transporter superfamily.3−5 Several members of these ATP-driven transporters are expressed at tissue barriers and thus influence uptake and elimination of drugs and drug candidates.6 Originally they have been linked to development of multidrug resistance (MDR) in tumor therapy, as they transport a wide variety of natural product toxins such as anthracyclines, vincristine, and taxanes out of tumor cells.7,8 Thus, P-glycoprotein (P-gp/ABCB1), discovered in 1976 and considered the paradigm ABC transporter,9,10 shows a remarkably broad substrate pattern, transporting numerous structurally and functionally diverse compounds across cell membranes.3 P-gp is expressed at the blood−brain barrier (BBB), the blood−cerebrospinal fluid (B-CSF) barrier, and the intestinal barrier, thus modulating the absorption and excretion of xenobiotics across these barriers.6 P-gp and its ligands (substrates and inhibitors) are therefore extensively studied both with respect to reversing multidrug resistance in tumors and for modifying ADME-Tox properties of drug candidates,11 such as central nervous system (CNS) active agents.12,13 Within the past two decades, numerous modulators of P-gp mediated drug efflux have been identified14,15 and several entered clinical studies up to phase III. However, up to now no compound achieved approval, which is mainly due to severe side effects and lack of efficacy. This further emphasizes the physiological role of efflux transporters in general and P-gp in particular and stresses the need for a more detailed knowledge on the structure and function of these proteins and the molecular basis of their interaction with small molecules.17 The latter has been approached by numerous SAR and QSAR studies, which revealed that high lipophilicity seems to be a general prerequisite for high P-gp inhibitory potency, valid across different chemical scaffolds. This is also in line with recent structure-based studies, which indicate an entry pathway via the membrane bilayer.18,19

In recent years the concepts of “Binding energy of the ligand per atom” or ligand efficiency (LE)20−22 and lipophilic efficiency (LipE)23,24 which combines both “potency and lipophilicity,” have been shown to be useful tools in the lead optimization process.25,26 In the light of our extensive SAR and QSAR studies on propafenone analogues27,28 (Figure 1) and related compounds, we also utilized benzophenone-based probes, which contain a photoactive arylcarbonyl group as part of the pharmacophore. This led to the identification of key amino acid residues interacting with these ligands.29,30 Within this study, we extended the set of benzophenones in order to identify compounds with higher potency, utilizing also the concepts of LE and LipE. In addition, docking studies of selected compounds into a homology model of P-gp were performed to shed light on the potential binding mode of these compounds and to compare it with the binding hypothesis derived for analogous propafenones.17
of at least three independently performed experiments. Generally, interexperimental variation was below 20%.

**Structure—Activity Relationships.** Table 1 shows the P-gp inhibitory potency of compounds 6–24. The IC₅₀ values cover a broad range, spanning from 0.05 μM for the dimer 23 up to 13.37 μM for the morpholine analogue 15. Besides the ortho-benzophenone dimer 23, also the ortho analogues showing an arylpiperazine moiety (6, 9) are highly active. Interestingly, the heterodimer 24 is one of the least active compounds in the data set, together with the morpholine derivatives 15 and 16. With respect to substitution pattern at the central aromatic benzene moiety, the rank order for arylpiperazine substituted compounds generally is ortho > meta > para. An analogous trend has also been observed for propafenone analogues. However, for compounds bearing piperidine or morpholine moieties, this trend is partly reversed. In the case of piperidine derivatives, the para-derivative is slightly more active than the meta analogue (1.20 vs 3.55 vs 2.18). Interestingly, also for the morpholine analogues, the para-derivative is by a factor of 2 more active than ortho-derivative (P = 0.01). Thus, the influence of the substitution pattern at the central aromatic ring seems to be more pronounced if the vicinity of the nitrogen comprises large, lipophilic moieties. This is in line with our previous findings using hydrophobic moments as descriptors in QSAR studies.

To assess the role of lipophilicity as a general predictor for high potency, we also calculated logP values using the software Bio-Loom version 1.5 and correlated them with pIC₅₀ values (Figure 2). Boi-Loom, which calculates logP values by a fragment-based approach, was validated against experimental logP values by Sakuratani et al. The r² value of 0.56 indicates that also in the series of benzophenones biological activity increases with the lipophilicity of the compounds. This is in agreement with the notion that compounds most probably enter the binding cavity of P-gp directly from the membrane bilayer. This is additionally supported by the recent X-ray structure of mouse P-gp, which shows a large inner cavity accessible from the membrane via putative entry ports composed of transmembrane helices 4/6 on one side and 10/12 on the other side.

The 4-hydroxy-4-phenyl-piperidine analogue 19 is located above the clogP/pIC₅₀ correlation line (pIC₅₀, 5.76 calcld vs 6.51 obs), which further confirms our previous results on the
importance of the 4-hydroxy-4-phenyl-piperidine moiety for high biological activity of propafenone derivatives. These results were recently supported by extensive docking studies of propafenone analogues. It is also interesting to note that the homodimer is about one log unit more potent than predicted by the clogP/pIC50 plot (pIC50, 6.10 calcd vs 7.27 obs). A pairwise comparison of equilipophilic compounds (clogP, 4.27 vs 4.28; IC50, 0.05 vs 0.48 μM) and 19 vs 20 (clogP, 3.65 vs 3.64; IC50, 0.31 vs 1.21 μM) indicates that mutual activity differences might also be due to difference in molecular size. The dimer 23 (44 heavy atoms) is about 1 order of magnitude more active than 21 (35 heavy atoms). Similarly, 19 (32 heavy atoms) is about a factor of 4 more active than 20 (24 heavy atoms). This also points toward a commonly observed phenomenon in lead optimization programs, i.e., activity increases with the size of the molecules. Therefore, ligand efficiency (LE) and lipophilic efficiency (LipE) profiles of inhibitors/substrates of P-gp have been used to

Table 1. Chemical Structure, Ligand Efficiency (LE), Lipophilic Efficiency (LipE), and Pharmacological Activity of Compounds 6–24

| Comp | Position | R1 | R2 | IC50 (μM) | SEM | LE | LE_Scale | clogP | LipE |
|------|----------|----|----|----------|-----|----|----------|------|------|
| 6    | Ortho    | H  |    | 0.08 ± 0.01 | 0.30 | 0.30 | 5.52 | 1.58 |
| 7    | Meta     | H  |    | 0.17 ± 0.18 | 0.29 | 0.30 | 5.52 | 1.34 |
| 8    | Para     | H  |    | 0.65 ± 0.08 | 0.26 | 0.30 | 5.52 | 1.66 |
| 9    | Ortho    | H  |    | 0.15 ± 0.03 | 0.30 | 0.30 | 4.96 | 1.86 |
| 10   | Meta     | H  |    | 0.58 ± 0.02 | 0.28 | 0.30 | 4.96 | 1.27 |
| 11   | Para     | H  |    | 0.97 ± 0.02 | 0.27 | 0.30 | 4.96 | 1.04 |
| 12   | Ortho    | H  |    | 1.20 ± 0.36 | 0.33 | 0.36 | 3.88 | 2.04 |
| 13   | Meta     | H  |    | 3.55 ± 0.08 | 0.31 | 0.36 | 5.57 | 1.57 |
| 14   | Para     | H  |    | 2.18 ± 0.07 | 0.32 | 0.36 | 3.88 | 1.78 |
| 15   | Ortho    | H  |    | 13.37 ± 1.18 | 0.28 | 0.36 | 5.57 | 2.04 |
| 16   | Para     | H  |    | 5.32 ± 0.89 | 0.30 | 0.36 | 2.66 | 2.61 |
| 17   | Meta     | H  |    | 0.20 ± 0.01 | 0.30 | 0.30 | 5.07 | 1.62 |
| 18   | Para     | H  |    | 0.50 ± 0.08 | 0.28 | 0.30 | 5.07 | 1.23 |
| 19   | Ortho    | H  |    | 0.31 ± 0.00 | 0.29 | 0.30 | 3.65 | 2.86 |
| 20   | Ortho    | CH3|    | 1.21 ± 0.18 | 0.35 | 0.37 | 3.64 | 2.28 |
| 21   | Ortho    | H  |    | 0.48 ± 0.01 | 0.26 | 0.28 | 4.28 | 2.04 |
| 22   | Ortho    | H  |    | 0.38 ± 0.01 | 0.26 | 0.28 | 5.07 | 1.34 |
| 23   | Ortho    | H  |    | 0.05 ± 0.00 | 0.23 | 0.23 | 4.27 | 3.01 |
| 24   | Ortho    | H  |    | 9.48 ± 0.18 | 0.18 | 0.25 | 3.17 | 1.85 |

“Position of the side chain at central aromatic ring.

“Reagents and conditions: (i) NaOH, epichlorohydrine, reflux for 24 h; (iv) p-tolyl isocyanate, CH2Cl2, stirring 2 h (X = O), p-tolylisothiocyanate, CH2Cl2, stirring 2 h (X = S); (v) methanol, reflux 5 h.
identify the derivatives with the best activity/size (or logP) ratio, which should provide further insights for the design of new ligands.25,26

**Ligand Efficiency (LE).** LE, most commonly defined as the ratio of free energy of binding to the number of heavy atoms, is a simple metric for assessing whether a ligand derives its potency from optimal fit with the target protein or simply by virtue of making many contacts.38 To get more information on the most promising P-gp inhibitors and to compare them to well established P-gp inhibitors/substrates, we calculated ligand efficiency values of benzophenones 6−24, selected propafenone analogues (Figure 1), as well as P-gp inhibitors which entered clinical studies. Ligand efficiencies were calculated as described in the Materials and Methods section. For benzophenones, small ligands such as the N-propyl derivative 20 and the piperidine analogue 12 show higher efficiency values (0.35; 0.33) than the large dimers 23 and 24 (0.23; 0.18). For the whole data set, it can be observed that ligand efficiencies drop dramatically when the size of the ligands increases above 50 heavy atoms (Figure 3).

A similar trend has been observed in the literature, with LE showing generally a dependency on ligand size.21 As LE in principle is supposed to normalize for the size of the ligand, various proposals have been made to solve this problem.22,39 As the heavy atom count of the ligands in our data set varies from 24 to 86 (20; valsdpodar), LE values were subsequently scaled as described by Reynolds et al.21,22 to retrieve a size-independent ligand efficiency value (LE_Scale). This was achieved by fitting the top ligand efficiency versus heavy atom count to a simple exponential function, as outlined by Reynolds et al.21 (eq 1; Figure 3). Subsequently, the ratio of ligand efficiency over normalized ligand efficiency scale gives a scoring function called “Fit Quality” (FQ) (eq 2). According to Reynolds et al., fit quality scores close to 1.0 or above indicate near optimal ligand binding, while low fit quality scores are indicative of suboptimal binding.

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\text{LEScale} = 0.104 + 0.65e^{-0.037\times\text{HA}} \\
\text{FQ} = \frac{\text{LE}}{\text{LE}_{\text{Scale}}}
\]

Use of this criterion shows that most of the compounds under clinical investigation show FQ scores above 1, including zosuquidar, ONT093, elacridar, and tariquidar, along with benzophenones 6 and 23, as well as propafenone and its analogues GPV062 and GPVS76 (Figure 4; Table 2).

It is interesting to note that especially those compounds which were specifically designed as P-gp inhibitors (ONT093, zosuquidar, elacridar, tariquidar) show higher FQ values than those originating from drug repurposing attempts (verapamil, cyclosporine, and its analogue valsdpodar). With respect to propafenone analogues, GPVS76 is the hitherto most active analogue we synthesized showing a highly lipophilic but quite compact substituent at the nitrogen atom (4-tolypiperazine). Interestingly, the top ranked benzophenone analogue 6 also has a 4-tolypiperazine moiety. This might point toward the tolylpiperazine substituent for being a privileged substructure for P-gp inhibitors. GPV062 bears a 4-hydroxy-4-phenyl-piperidine moiety, which has been shown to influence biological activity independent of lipophilicity, resulting in an almost 10-fold increase of inhibitory potency when compared to compounds having other substituents at the nitrogen atom. This points toward a distinct additional interaction mediated by the 4-hydroxy group, most probably in the form of a hydrogen bond. Finally, propafenone itself shows a very good value, thus retrospectively demonstrating its validity as starting point for structural modifications. However, it should be noted that during its catalytic cycle the transporter undergoes a major conformational change and that it might well be that some inhibitors exert their effect by slow off kinetics rather than by strong binding to the apo state. In this case, LE values derived from IC₅₀ values for transport inhibition should be taken cautiously.

As already outlined, lipophilicity has been shown in numerous studies to be a general predictor for high P-gp inhibitory potency. This most probably is due to the proposed access path of the compounds, which seems to be directly from the membrane bilayer. On the other hand, high lipophilicity is very often associated with poor oral drug-like properties. This led to the assumption that clogP values between 2 and 3 are considered optimal in an oral drug program and prompted Leeson et al., to introduce the concept of lipophilic efficiency.23

**Lipophilic Efficiency (LipE).** LipE is a parameter that combines both potency and lipophilicity and is defined as a measure of how efficiently a ligand exploits its lipophilicity to bind to a given target. Briefly, in a lead optimization series, there is a greater likelihood of achieving good in vivo performance when potency can be increased without increasing logP or logD values. To explore this concept also for P-gp inhibitors, we calculated LipE values for the whole set of...
benzophenones as well as for the compounds used for the LE study (Table 2). The clogP values vary from 2.66 to 15.09, leading to a lipophilic efficiency range between −8.79 and +3.08. This is somewhat surprising, as it has been reported that a lipophilic efficiency greater than 5 combined with clogP values between 2 and 3 is considered optimal for a promising drug candidate.23,24 None of the clinically tested P-gp inhibitors fulfils these requirements. Only the 4-hydroxy-4-phenylpiperidine analogous propafenone GPV062 as well as the dimer 23 exhibit values slightly higher than 3. All other compounds show values lower than 3 (Figure 5). It is tempting to speculate whether this is due to the unique entrance pathway directly from the membrane bilayer, which requires a different logP profile than for compounds which access their binding site directly from the extracellular or intracellular aqueous compartment.

To study in more detail whether the unique access path of P-gp inhibitors directly from the membrane bilayer is linked to this unexpectedly low LipE values, we studied the distribution of LipE profiles for a set of targets showing different access pathways of their ligands: P-glycoprotein (via the membrane bilayer), the serotonin transporter SERT (from the extracellular environment), and the hERG potassium channel (from the cytoplasm) (Figure 6). LipE values of inhibitors of SERT (extracted from the ChemBL database),40 hERG blockers,41 and propafenone-type inhibitors of P-gp (in-house data) were calculated as described in the Materials and Methods section. The LipE distribution profile of SERT inhibitors extracted from the ChemBL database identified about 13% of the compounds that cross the LipE threshold of 5 (Figure 7). These compounds cover a wide range of IC50 (0.01 nM to 10 mM) and clogP (−3.42 to 4.66) (SM Figure 1). Moreover, 15 SERT inhibitors have been identified with clogP ∼2.5, LipE > 5, and IC50 < 10 nM, none of them was listed as a marketed drug. In the case of hERG, only 2.5% of the compounds cross the LipE threshold of 5 that showed a potency distribution from 5 nM to 18 μM and clogP values between −0.77 and 2.21 (SM Figure 1). Only two compounds, almokalant and dofetilide, complied with the desired profile (clogP ∼2.5, LipE > 5, potency values <10 nM). Dofetilide is a registered class III antiarrhythmic agent, while almokalant is in phase II clinical investigations.42,43

LipE profiles of P-gp inhibitors could not identify any compound that reaches the standard threshold value of 5. Most of the ligands fall in the LipE range of 1−2 (39%) or 2−3 (28%), with wide a range in distribution of their clogP (0.40 to 6.02) as well as IC50 (5.60 nM to 1.20 mM) values (SM Figure 1). Thus, the LipE threshold for ligands of P-gp needs to be reconsidered. Nevertheless, from the benzophenone data set presented here, compounds 16, 19, 20, and 23 might be the

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**Table 2. Pharmacological Activities, Ligand Efficiency (LE), and Lipophilic Efficiency (LipE) Profiles of Selected Propafenones and P-gp Inhibitors Which Entered in Clinical Studies**

| compd       | pIC50 | HA | LE  | clogP | LipE      |
|-------------|-------|----|-----|-------|-----------|
| Verapamil   | 6.24  | 33 | 0.27| 4.47  | 1.77      |
| Elacridar   | 7.14  | 42 | 0.24| 4.21  | 2.93      |
| Tariquidar  | 7.48  | 48 | 0.22| 5.55  | 1.93      |
| Zosuquidar  | 7.23  | 39 | 0.26| 4.96  | 2.27      |
| ONT093     | 7.50  | 37 | 0.29| 7.30  | 0.19      |
| Valspordar  | 6.30  | 86 | 0.10| 15.09 | −8.79     |
| Cyclosporine A | 6.99 | 85 | 0.12| 14.36 | −7.37     |
| Niguldipine| 6.15  | 45 | 0.20| 7.80  | −1.65     |
| Propafenone | 6.48  | 25 | 0.37| 3.64  | 2.84      |
| GPV576      | 8.25  | 35 | 0.33| 6.02  | 2.23      |
| GPV062      | 7.24  | 34 | 0.30| 4.15  | 3.09      |
| GPV005      | 6.22  | 27 | 0.33| 4.38  | 1.84      |

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Figure 4. Fit quality scores vs heavy atom counts of benzophenones 6−24, compounds which entered clinical studies, and selected propafenones. FQ score around 1 indicate a near optimal ligand binding affinity for a given number of heavy atoms.

Figure 6. LogP vs LipE distribution of P-gp inhibitors 16−23. The dotted line indicates the LipE = 5 threshold.
most promising ones as their LipE values are between 2 and 3, a range where most of the compounds which entered clinical trials are located.

Docking into a Homology Model of P-Glycoprotein.

To get insights into the potential binding mode of propafenone-type benzophenones, we selected compounds 6, 19, and 20 and the dimer 23 for further in silico studies. Compounds 19, 20, and 23 were selected as they are ranked high both in LipE and FQ scores, and 6 was additionally included as it is top ranked with respect to FQ. Interestingly, this selection resembles the key features observed for propafenone analogues: compound 6 shows a 4-tolylpiperazine substituent (analogous to GPVS576), compound 19 is analogous to GPV062 (4-hydroxy-4-phenyl-piperidine), and derivative 20 is the direct propafenone analogue (N-propyl). The docking protocol follows those previously published44 and is provided in detail in the Materials and Methods section.

The analysis of the interaction pattern of selected docking poses indicates that the benzophenone scaffold interacts with F343 and F303 near the entry gate, whereas the lipophilic
substituents in the vicinity of the basic nitrogen atom are surrounded by hydrophobic amino acid residues L724, I720, V981, I840, I836, and I765 located at TM 7, 8, 9, and 12 (Figure 8). This further supports the importance of high lipophilicity and also is in line with previous studies performed by Pajeva and Wiese, who showed that for a series of inhibitors of P-gp hydrophobicity represents a space directed molecular property rather than a simple overall descriptor. The top ranked cluster of poses are in close vicinity of our previously purposed binding positions for benzopyrano[3,4-b][1,4]oxazines, where compounds having 4aS,10bR configuration interact mainly with amino acid residues of TM 4, 5, and 6 near the entry gate, while compounds having 4aR,10bS configuration are positioned deeper inside the binding cavity, being mainly surrounded by hydrophobic amino acid residues of TM 7, 8, 9, and 12. Interestingly, the top scored dimer 23 is positioned in a way to bridge these two positions (Figure 8). Moreover, this pose might also aid in the explanation for the activity differences of homodimer 23 (0.05 μM) and heterodimer 22 (9.48 μM): The additional benzene ring in the best scored pose of homodimer 23 is surrounded by several hydrophobic amino acids (I836, L720, I840, and L724).

A representative docking pose of the 4-hydroxy-4-phenyl-piperidine derivative 19 showed an H-bond interaction between the 4-hydroxy group and A985 (Figure 9A). This further supports our SAR data and strengthens the importance of 4-hydroxy-4-phenyl-piperidine moieties for high inhibitory potency of propafenones and benzophenones. Furthermore, A985 was also identified as interacting with verapamil and the cyclic peptide (AQZ59-SSS) cocrystallized in mouse P-gp. A binding pocket of 4.5 Å around interacting amino acid residues of TM 7, 8, 9, and 12 showed two small hydrophobic cavities...
(encircled in Figure 9B), occupying the hydrophobic substituents at the basic nitrogen atom of the ligands. A closer look of the overlaid poses shows that the benzophenone substituent in dimer 23 fits well in the hydrophobic pockets, which might explain its high FQ score.

Overall, benzophenones shared a similar interaction profile as propafenones. Amino acids S952, F434, F336, L724, and Y307 have been identified as common interacting amino acid residues of all three classes of propafenone type inhibitors of P-gp (SM Figure 3). Selected benzophenone analogues have been previously used as photoaffinity ligands to characterize the drug-binding domain of propafenone-type analogues. In these studies, TM 3, 5, 6, 8, 10, 11, and 12 were identified as potential interacting helices.30-46 This is well in line with our docking studies, which show main interactions with TM 5 and 6 near the entry gate and TM 7, 8, 9, and 12 deeper inside the cavity (SM Figure 4). No significant cluster of poses has been identified on the second wing (2/11 interface), which might be due to the asymmetry in the template used for building the homology model of P-gp, thus narrowing the available space at this side.

CONCLUSIONS

Calculation of ligand efficiency and lipophilic efficiency values for a set of P-gp inhibitors shows that ligands of P-gp exhibit LipE values below the threshold of 5 considered to be optimal for clinical candidates. This might be due to the unique entrance pathway of these classes of compounds, taking a route for clinical candidates. This might be due to the unique

EXPERIMENTAL SECTION

Chemistry. Material and Methods. The data set used consists of a set of previously published benzophenones 9-7 and 12, 19, and 20 as well as a series of newly synthesized analogues. Melting points were determined on Leica Galen III (ser. no. 1413 WT) and are uncorrected. Purity of the compounds was checked by elemental analysis, and all values were within ±0.3%. Elemental analysis was performed at Microanalytical Laboratory of the Institute of Physical Chemistry (Mag. Johannes Theiner), University of Vienna. The equipment used was a 2400 CHN-Elemental Analyzer from Perkin-Elmer. Mass spectra were recorded on a Maldi-TOF, Kratos Instruments, matrix assisted laser-desorption-ionization time-of-flight, reflection mass spectrometer. NMR spectra were recorded on a Bruker spectrospin for 200 MHz 1H NMR and 50 MHz for 13C NMR. CDCl3 and DMSO at room temperature were used as internal standards. Column chromatographic separations were performed by using silica gel 60 (Particle size 40–63 μm, 230–300 mesh) from J. T. Baker or Merck. Thin layer chromatography (TLC) was performed on silica gel 60F254 TLC plates from Merck.

General Procedure for the Preparation of (3-Oxiranylmethoxy-phenyl)-phenyl-methanone C6H5O2 (2b). First, 5 g (25.25 mmol) of 3-hydroxy-benzophenone was dissolved in epichlorohydrine (60 mL), treated with 1.01 g (25.25 mmol) of sodium hydroxide, refluxed for 6 h, and stirred overnight. Then the residue was filtered off and washed with diethyl ether. After removal of the solvents under reduced pressure, the resulting oil was taken up in diethyl ether and washed with water several times. The organic layers were combined, dried over anhydrous sodium sulfate, and evaporated to dryness yielding yellow oil. For further purification a column chromatography (silica gel, ether/petrol ether, 70 + 30) was performed. Subsequent removal of the solvents under reduced pressure gave white opalescent oil, yield 6 g (93.6%).1H NMR (CDCl3) δ 2.71–4.54 (m, 1H, H3), 5.96–6.61 (m, 3H, H2), 3.12–3.34 (m, 1H, CH), 7.55–8.20 (m, 7H, arom H). 13C NMR (CDCl3) δ 136.60 (CH2-O), 130.28 (CH=CH2), 128.79 (Ar-CO), 120.98 (Ph-CO), 119.93 (CH-CH2), 119.00 (C, H3), 110.75 (CH3), 105.78 (CH, C), 101.97 (C, H3), 101.88 (C, H3), 90.49 (C, H3). 3268 | J. Med. Chem. 2012, 55, 3261–3273

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acetate/diethyl ether gave 904 mg (73.8%) white crystals; mp 92 °C. The obtained solid was filtered off and washed with diethyl ether, giving 1.06 g (94.62%) white crystals; mp 122 °C.

First, 700 mg (2.75 mmol) of (4-fluoro-2-hydroxy-3-morpholine-4-yl-propoxy)-phenyl-methanone (2a) was dissolved in 10 mL of piperidine and refluxed for 2.5 h. Then after removal of piperidine under reduced pressure, 336 mg (36%) yellow oil was obtained; mp 64 °C.

H NMR (CDCl3) δ 1.49–1.72 (m, 2H, CH2), 2.58–2.73 (m, 6H, CH2-CH), 4.05–4.08 (m, 2H, O-CH2), 4.20–4.29 (m, 1H, CH, 4.32 (s, 1H, OH), 6.97 (2H, J = 8.55, H-3, H-5), 7.45–7.82 (m, 7H, arom H).

13C NMR (CDCl3) δ 23.65, 25.33 (CH3), 54.78 (N-CH3), 61.21 (CH2-N), 64.89 (CH3), 70.64 (O-CH3), 114.06, 128.15, 129.67 (arom C), 130.38 (Ar-O), 131.89, 132.48 (arom C), 131.83 (Ph-CO), 162.44 (CO).

MS m/e 340.43 (M+, 100%). Anal. Calc. for C19H21NO3: C, 73.75; H, 7.46; N, 3.08. Found: C, 73.51; H, 7.71; N, 4.11.

[2-(2-Hydroxy-3-morpholine-4-yl-propoxy)-phenyl]phenyl-methanone (15). First, 700 mg (2.75 mmol) of (4-oximino-phenyl)-phenyl-methanone (2c) was dissolved in 15 mL of methanol, treated with 496 mg (2.75 mmol) of p-fluorobenzoic acid, and refluxed for 5 h. Then removal of solvent under reduced pressure left a yellow oil which was crystallized from isopropyl alcohol, yielding 1 g (83.6%) white crystals; mp 96–100 °C.

H NMR (CDCl3) δ 2.61–2.69 (m, 4H, N-(CH3)-), 2.80–2.91 (m, 2H, CH2-N), 3.17 (m, 4H, CH2), 3.93 (CH3-), 4.11–4.14 (m, 1H, CH2), 6.97–7.85 (13H, 13H, arom H). 13C NMR (CDCl3) δ 50.24, 53.28, 62.08 (CH3-N-(CH3)), 65.40 (CH3), 70.33 (O-CH3), 114.07, 115.31, 117.81, 117.96, 128.17, 129.71, 130.42 (Ar-Ph), 130.12, 130.52 (Ph, arom C), 138.15 (Ph-CO), 174.77 (Ar-N), 162.58 (Ar-O), 195.50 (CO). MS m/e 435.3 (M+, 100%). Anal. Calc. for C29H26FN2O3: C, 71.87; H, 6.26; N, 6.45. Found: C, 71.61; H, 6.43; N, 6.41.

[2-(2-Hydroxy-3-morpholine-4-yl-propoxy)-phenyl]phenyl-methanone (16). First, 700 mg (2.75 mmol) of (4-oximino-phenyl)-phenyl-methanone (2c) was dissolved in 15 mL of methanol, treated with 240 mg of morpholine, and refluxed for 7 h. Then...
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subsequent removal of the solvent under reduced pressure left 910 mg (96.8%) of yellow oil; mp 135–141 °C. 1H NMR (CDCl3) δ 2.44–2.71 (m, 6H, CH2-N-(CH2)3), 3.73 (t, 4H, O-CH2-CH), 4.06–4.17 (m, 3H, O-CH3), 6.98 (d, 2H, J = 8.82, H-3, H-5), 7.42–7.56 (m, 3H, arom H), 7.72–7.84 (m, 4H, arom H). 13C NMR (CDCl3) δ 53.69, 60.83 ((CH2)-N-(CH2)-), 65.18 (CH), 66.94 ((CH2)-O), 70.28 (O-C), 70.75 (CH3), 82.12 (N-(CH2)3), 112.12 (1H, J = 22.6 Hz, CH), 120.04 (1H, J = 2.05 Hz, CH), 121.09, 132.49 (arom C), 138.13 (Ph-CHO), 145.98 (CO). MS m/e 342.3 (M+, 95%). Anal. Calc. for C36H38N2O6: C, 70.52; H, 7.14; N, 4.51. Found: C, 70.91; H, 7.13; N, 4.37.

[2-(3-Benzoyl-phenoxy)-2-hydroxy-propyl]-piperazine-1-yl)-2-hydroxy-propoxy)-phenyl-methanone (24). First, 356 mg (1.40 mmol) of (2-oxiranylmethoxy-phenyl)-phenyl-methanone (2a) was dissolved in 20–30 mL of methanol and then 50.8 mg (0.59 mmol) of piperazine was added and the reaction mixture was refluxed for 5 h. Then after removal of the solvent by rotary evaporation, a column chromatography was performed (silica gel, CH2Cl2/methanol/concentrated NH3, 120/50). Subsequent evaporation gave yellow oil, which crystallized from isopropyl alcohol to leave 424 mg (51%) of white solid; mp 125–135 °C. 1H NMR (CDCl3) δ 1.98–2.42 (m, 12H, CH2-(CH2)2), 3.10 (s, 3H, OH), 3.70–3.76 (m, 2H, 2CH), 3.94–4.07 (m, 5H, O-CH2-CH3, O-CH2), 6.99–7.83 (m, 18H, arom H). 13C NMR (CDCl3) δ 56.13 ((CH2)-O), 56.06 (CH2-CH3), 70.68 (CH2-CHO), 112.62, 112.10, 128.28, 129.53, 130.09, 132.82 (arom C), 132.81 (Ar-CO), 134.34 (Ph-CHO), 156.55 (Ar-CO), 196.46 (CO). MS m/e 594.7 (M+, 100%). Anal. Calc. for C24H24N2O7C: 70.75; H, 7.14; N, 4.22. Found: C, 70.91; H, 7.02; N, 4.36.

Computational Studies. Ligand Efficiency (LE). Ligand efficiency (LE = ΔG) values of the data were calculated by normalizing binding free energy of a ligand for number of heavy atoms. Free energy calculation was carried out as described by Hopkins et al. (eq 3). According to Hopkins et al., ΔG 0 from percentage inhibition can be substituted for KD (dissociation constant potentia) which was further confirmed by experimental results of Kuntz and co-workers.38

\[
\Delta G = -RT \ln K_D
\]

(3)

Ligand efficiency calculations were done for a temperature of 310 K and given in kcal per heavy atom (eq 4).

\[
LE = -\Delta G/H_A (\text{non-hydrogen atom})
\]

(4)
A size independent fit quality score was obtained as described by Reynolds et al., by fitting the maximum LE over a large range of molecular size. All calculations regarding ligand efficiency were done by using Excel spreadsheet. IC₅₀ values of the propafenone type inhibitors (GPV576, GPV005, GPV062, and propafenone) were determined experimentally by a daunorubicin efflux essay. Inhibition of rhodamine 123 efflux in the transfectant mouse lymphoma line LS178 VMDRI C.06 were used to characterize the MDR-modulating activity values of verapamil, nigrudipine, and cyclosporine A. IC₅₀ values of tarquidar, elacladric, valpado, osuzquid, and ONT-093 were taken from literature (Table 2). IC₅₀ values for most of the compounds in clinical studies were reported by using rhodamine 123 efflux essays. We use these values, as there is a direct correlation between the IC₅₀ values from daunorubicin and rhodamine 123 efflux essays.

Lipophilic Efficiency (LipE). LipE of benzophenones were calculated (eq 5) and compared with the compounds which reached clinical studies (verapamil, tarquidar, valspoda, elacladric, osuzquid, ONT-093, nigrudipine, and cyclosporine A) as well as with selected propafenone analogues.

\[
\text{LipE} = \text{LLE} = \text{pIC}_{50} - \text{clogP}
\]

\text{clogP} values of the data set were computed by using the Bio-Loom software package, and the LipE calculations were performed by using Excel spreadsheet. To compare the standard threshold of LipE along three different entry pathways of ligands into respective binding pockets of P-gp, hERG and SERT, a data set from literature was used. It includes 744 SERT inhibitors extracted from the ChEMBL database, 313 hERG blockers, and 372 inhibitors of P-gp mediated daunorubicin efflux (in-house data). The data sets are available at our homepage (pharminfo.univie.ac.at) and from Chemspider (www.chemspider.com).

Docking. Compounds 6, 19, 20, and 23 were docked in their neutral form into an open state homology model of human P-gp based on the X-ray structure of mouse P-gp (PDB: 3G5U) by using the software package GOLD. To avoid any bias, we considered the whole transmembrane domain region as binding pocket. Then 100 poses per ligand were obtained, and finally ligand protein complexes were minimized by LigX, a minimization tool implemented in MOE, by using the MMFF94 force field.

A complete work flow of poses selection has been provided in Supporting Information (SM Workflow 1). Briefly, agglomerative Hierarchical Cluster analysis of the consensus rmsd matrix based on the common scaffold of the ligands identified two interesting clusters of poses containing all four ligands. However, additional five clusters have been identified containing three out of four ligands. All seven clusters were occupying the center of the binding cavity mainly interacting with amino acid residues of TM 1, 5, 6, 7, 8, 10, and 11 (SM Figure 2A). For a more detailed analysis of the ligand–protein interaction profiles of selected ligands, we used the two clusters containing all four ligands (SM Figure 2B).

To prioritize among the two clusters, a rescoring of all docking poses by using four different scoring functions in MOE (ASE, affinity energy, ABBREVIATIONS USED

P-gp, P-glycoprotein; LipE, lipophilic efficiency; LE, ligand efficiency; MDR, multidrug resistance; ABC, ATP binding cassette; QSAR, quantitative structure–activity relationship

REFERENCES

(1) Giacomini, K. M.; Huang, S. M.; Tweedie, D. J.; Benet, L. Z.; Brouwer, K. L.; Chu, X.; Dahlin, A.; Evers, R.; Fischer, V.; Hillgren, K. M.; Hoffmaster, K. A.; Ishikawa, T.; Keppler, D.; Kim, R. B.; Lee, C. A.; Niemi, M.; Polli, J. W.; Sugiyama, Y.; Swaan, P. W.; Ware, J. A.; Wright, S. H.; Yee, S. W.; Zamek-Gliszczynski, M. J.; Zhang, L. Membrane transporters in drug development. Nature Rev. Drug Discovery 2010, 9, 215–236.

(2) Lee, E. J.; Lean, C. B.; Limenta, L. M. Role of membrane transporters in the safety profile of drugs. Expert Opin. Drug Metab. Toxicol 2009, 5, 1369–1383.
(3) Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. Targeting multidrug resistance in cancer. Nature Rev. Drug Discovery 2006, 5, 219–234.

(4) Couture, I.; Nash, J. A.; Turgeon, J. The ATP-binding cassette transporters and their implication in drug disposition: a special look at the heart. Pharmacol. Rev. 2006, 58, 244–258.

(5) Szakacs, G.; Varadi, A.; Ozvegy-Laczka, C.; Sarkadi, B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Drug Discovery Today 2008, 13, 379–393.

(6) Colabufo, N. A.; Bezardi, F.; Contino, M.; Niso, M.; Perrone, R. ABC pumps and their role in active drug transport. Curr. Top. Med. Chem. 2009, 9, 119–129.

(7) Gottesman, M. M.; Ling, V. The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research. FEBS Lett. 2006, 580, 998–1009.

(8) Glavinas, H.; Krajesi, P.; Ceserepes, J.; Sarkadi, B. The role of ABC transporters in drug resistance, metabolism and toxicity. Curr. Drug Delivery 2004, 1, 27–42.

(9) Juliano, R.; Ling, V.; Graves, J. Drug-resistant mutants of chinese hamster ovary cells possess an altered cell surface carbohydrate component. J. Supramol. Struct. 1976, 4, 521–526.

(10) Ford, R. C.; Kamis, A. B.; Kerr, I. D.; Callaghan, R. The ABC Transporters: Structural Insights into Drug Transport. In Transporters as Drug Carriers; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2010; pp 1–48.

(11) Broccatelli, F.; Carosati, E.; Neri, A.; Frosini, M.; Goracci, L.; Oprea, T. I.; Cruciani, G. A Novel Approach for Predicting P-Glycoprotein (ABCB1) Inhibition Using Molecular Interaction Fields. J. Med. Chem. 2011, 54, 1740–1751.

(12) Kemper, E. M.; van Zandbergen, A. E.; Cleypool, C.; Mos, H. A.; Booger, W.; Beijnen, J. H.; van Tellingen, O. Increased penetration of paclitaxel into the brain by inhibition of P-Glycoprotein. J. Neuro-oncol. 2003, 59, 2849–2855.

(13) Kuhnle, M.; Egger, M.; Muller, C.; Mahringer, A.; Bernhardt, G.; Fricker, G.; Konig, B.; Buschauer, A. Potent and selective inhibitors of breast cancer resistance protein (ABCG2) derived from the P-glycoprotein (ABCB1) modulator tarirapid. J. Med. Chem. 2009, 52, 1190–1197.

(14) Ford, J. M. Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. Eur. J. Cancer 1996, 32A, 991–1001.

(15) Thomas, H.; Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting P-glycoprotein. Cancer Control 2003, 10, 159–165.

(16) Kannan, P.; John, C.; Zoghbi, S. S.; Haldin, C.; Gottesman, M. M.; Innis, R. B.; Hall, M. D. Imaging the function of P-glycoprotein by activated-pharmacophore photoaffinity labeling and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. Mol. Pharmacol. 2002, 61, 637–648.

(17) Pitha, J.; Szabo, L.; Szurai, Z.; Buchowiecki, W.; Kusiak, J. W. Alkyylating prazosin analoge: irreversible label for alpha 1-adrenerceptors. J. Med. Chem. 1989, 32, 96–100.

(18) Chiba, P.; Ecker, G. F. Interaction field based and hologram based QSAR analysis of propafenone-type modulators of multidrug resistance. Mol. Cancer Res. 2005, 3, 431–444.

(19) Ecker, G. F.; Caszas, E.; Kopp, S.; Plagens, B.; Holzer, W.; Ernst, W.; Chiba, P. Identification of ligand-binding regions of P-glycoprotein by activated-pharmacophore photoaffinity labeling and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. Mol. Pharmacol. 2002, 61, 637–648.

(20) Parveen, Z.; Stockner, T.; Bentle, C.; Pferschy, S.; Kraupp, M.; Freischnitt, M.; Ecker, G. F.; Chiba, P.; Molecular Dissection of Dual Pseudosymmetric Solute Translocation Pathways in Human P-Glycoprotein. Mol. Pharmacol. 2011, 79, 443–452.

(21) Pitha, J.; Szabo, L.; Szurai, Z.; Buchowiecki, W.; Kusiak, J. W. Alkyylating prazosin analoge: irreversible label for alpha 1-adrenerceptors. J. Med. Chem. 1989, 32, 96–100.

(22) Chiba, P.; Ecker, G.; Schmid, D.; Drach, J.; Tell, B.; Goldenberg, S.; Gekeler, V. Structural requirements for activity of propafenone-type modulators in P-glycoprotein-mediated multidrug resistance. Mol. Pharmacol. 1996, 49, 1122–1130.

(23) Tmej, C.; Chiba, P.; Huber, M.; Richter, E.; Hitzler, M.; Schaper, K. J.; Ecker, G. A combined Hансch/Free-Wilson approach as predictive tool in QSAR studies on propafenone-type modulators of multidrug resistance. Arch. Pharm. (Weinheim, Ger.) 1998, 331, 233–240.

(24) König, G.; Chiba, P.; Ecker, G. F. Hydrophobic moments as physicochemical descriptors in structure–activity relationship studies of P-glycoprotein inhibitors. Monatsh. Chem. 2008, 139, 401–405.

(25) BioLoom program, trial version, by BioByte Co.

(26) Sakuratani, Y.; Kasai, K.; Noguchi, Y.; Yamada, J. Comparison of Predictivities of Log P Calculation Models Based on Experimental Data for 134 Simple Organic Compounds. QSAR Comb. Sci. 2007, 26, 109–116.

(27) Chiba, P.; Hitzler, M.; Richter, E.; Huber, M.; Tmej, C.; Giovagnoni, E.; Ecker, G. Studies on Propafenone-Type Modulators of Multidrug Resistance III: Variations on the Nitrogen. Quant. Struct.-Act. Relat. 1997, 16, 361–366.

(28) Konig, G.; Chiba, P.; Ecker, G. F. Hydrophobic moments as physicochemical descriptors in structure–activity relationship studies of P-glycoprotein inhibitors. Monatsh. Chem. 2008, 139, 401–405.

(29) BioLoom program, trial version, by BioByte Co.

(30) Verdonk, M. L.; Rees, D. C. Group efficiency: a guideline for hits-to-leads chemistry. ChemMedChem 2008, 3, 1179–1180.

(31) ChEMBLdb; https://www.ebi.ac.uk/chembldb/ (Accessed April 2010).

(32) Teratogenicity of the class III antiarrhythmic drug almokalant. Role of hydroxipia and reactive oxygen species. Reprod. Toxicol. 1999, 13, 93–101.
Houltz, B.; Darpo, B.; Swedberg, K.; Blomstrom, P.; Brachmann, J.; Crijns, H. J.; Jensen, S. M.; Svernhaege, E.; Vallin, H.; Edvardsson, N. Effects of the Ikr-blocker almokalant and predictors of conversion of chronic atrial tachyarrhythmias to sinus rhythm. A prospective study. *Cardiovasc. Drugs Ther.* 1999, 13, 329–338.

Jabeen, I.; Wetwitayaklung, P.; Klepsch, F.; Parveen, Z.; Chiba, P.; Ecker, G. F. Probing the stereoselectivity of P-glycoprotein-synthesis, biological activity and ligand docking studies of a set of enantiopure benzopyrano[3,4-b][1,4]oxazines. *Chem. Commun.* (Cambridge, U.K.) 2011, 47, 2586–2588.

Pajeva, I.; Wiese, M. Molecular modeling of phenothiazines and related drugs as multidrug resistance modifiers: a comparative molecular field analysis study. *J. Med. Chem.* 1998, 41, 1815–1826.

Pleban, K.; Kopp, S.; Csaszar, E.; Peer, M.; Hrebicek, T.; Rizzi, A.; Ecker, G. F.; Chiba, P. P-Glycoprotein substrate binding domains are located at the transmembrane domain/transmembrane domain interfaces: a combined photoaffinity labeling-protein homology modeling approach. *Mol. Pharmacol.* 2005, 67, 365–374.

Chiba, P.; Mihailek, I.; Ecker, G. F.; Kopp, S.; Lichtarge, O. Role of transmembrane domain/transmembrane domain interfaces of P-glycoprotein (ABCB1) in solute transport. Convergent information from photoaffinity labeling, site directed mutagenesis and in silico importance prediction. *Curr. Med. Chem.* 2006, 13, 793–805.

Hopkins, A. L.; Groom, C. R.; Alex, A. Ligand efficiency: a useful metric for lead selection. *Drug Discovery Today* 2004, 9, 430–431.

Pleban, K.; Hoffer, C.; Kopp, S.; Peer, M.; Chiba, P.; Ecker, G. F. Intramolecular distribution of hydrophobicity influences pharmacological activity of propafenone-type MDR modulators. *Arch. Pharm.* (Weinheim, Ger.) 2004, 337, 328–334.

Roe, M.; Folkes, A.; Ashworth, P.; Brumwell, J.; Chima, L.; Hunjan, S.; Pretswell, I.; Dangerfield, W.; Ryder, H.; Charlton, P. Reversal of P-glycoprotein mediated multidrug resistance by novel antranilamide derivatives. *Bioorg. Med. Chem. Lett.* 1999, 9, 595–600.

Dodic, N.; Dumaitre, B.; Daugan, A.; Pianetti, P. Synthesis and activity against multidrug resistance in Chinese hamster ovary cells of new acridone-4-carboxamides. *J. Med. Chem.* 1995, 38, 2418–2426.

Bachheimer, C. J.; Miller, D. W. A fluorometric screening assay for drug efflux transporter activity in the blood–brain barrier. *Pharm. Res.* 2005, 22, 113–121.

Wang, J. S.; Zhu, H. J.; Markowitz, J. S.; Donovan, J. L.; DeVane, C. L. Evaluation of antipsychotic drugs as inhibitors of multidrug resistance transporter P-glycoprotein. *Psychopharmacology (Berlin, Ger.)* 2006, 187, 415–423.

Shepard, R. L.; Cao, J.; Starling, J. J.; Dantzig, A. H. Modulation of P-glycoprotein but not MRP1- or BCRP-mediated drug resistance by LY335979. *Int. J. Cancer* 2003, 103, 121–125.

Dantzig, A. H.; Shepard, R. L.; Cao, J.; Lawn, K. L.; Ehhardt, W. J.; Baughman, T. M.; Gumol, T. F.; Starling, J. J. Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. *Cancer Res.* 1996, 56, 4171–4179.

Newman, M. J.; Rodarte, J. C.; Benbatoul, K. D.; Romano, S. J.; Zhang, C.; Krane, S.; Moran, E. J.; Uyeda, R. T.; Dixon, R.; Guns, E. S.; Mayer, L. D. Discovery and characterization of OC144-093, a novel inhibitor of P-glycoprotein-mediated multidrug resistance. *Cancer Res.* 2000, 60, 2964–2972.

Chiba, P.; Holter, W.; Landau, M.; Bechmann, G.; Lorenz, K.; Plagens, B.; Hitzler, M.; Richter, E.; Ecker, G. Substituted 4-aclypyrazoles and 4-aclypyrazolones: synthesis and multidrug resistance-modulating activity. *J. Med. Chem.* 1998, 41, 4001–4011.

Ayesh, S.; Shao, Y. M.; Stein, W. D. Co-operative, competitive and non-competitive interactions between modulators of P-glycoprotein. *Biochim. Biophys. Acta* 1996, 1316, 8–18.

Shao, Y. M.; Ayesh, S.; Stein, W. D. Mutually co-operative interactions between modulators of P-glycoprotein. *Biochim. Biophys. Acta* 1997, 1360, 30–38.