A Combined Hansch/Free-Wilson Approach as Predictive Tool in QSAR Studies on Propafenone-Type Modulators of Multidrug Resistance[1]

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Summary

A series of 48 propafenone-type modulators of multidrug resistance was synthesized and their P-glycoprotein inhibitory activity was measured using the daunomycin efflux assay. Both a Free-Wilson and a combined Hansch/Free-Wilson analysis were performed using log *P*, partial log *P* and molar refraction values as Hansch descriptors. The results of the Free-Wilson analysis show that modifications on the central aromatic ring generally influence pharmacological activity, whereby in almost all cases a decrease in MDR-modulating potency is observed (Q^2_{cv} = 0.66). The combined approach results in equations with remarkably higher predictive power (Q^2_{cv} = 0.83), specifically molar refractivity shows high significance in all equations derived. This indicates that polar interactions also contribute to protein binding.

Introduction

Inhibition of the multidrug transporter P-glycoprotein[2] (PGP) represents a promising approach at least for the treatment of multiresistant haematological malignancies [2, 3]. Although numerous compounds which are able to block PGP-mediated toxin transport are described in the literature, only little information is given on quantitative structure-activity relationships within the class of MDR modulators [4]. Pajeva and Wiese performed a detailed study on 17 thioxanthenes and 17 phenothiazines using the Free-Wilson approach [5]. In both cases, the type of the nitrogen substituent, the distance between the aromatic ring system and the nitrogen atom, and the stereochemistry contribute to high MDR-reversal activity. Recently, Klopman et al. published a study using the MULTICASE software package for structure-activity relationship studies on 609 structurally and functionally diverse MDR reversal agents [6]. Several biophores, (CH_2-CH_2-N-CH_2-CH_2, *o*-dimethoxyphenyl, ...) and biophobes (-COOH, aniline, quaternary ammonium, phenol, ...) were identified and 10 out of 14 test set compounds were correctly predicted. This indicates that a QSAR approach mainly based on indicator variables for substructural features might be successful in the field of MDR modulators.

We recently described a series of propafenone analogs as highly active PGP inhibitors [7]. Within series of analogous derivatives an excellent correlation between inhibition of daunomycin efflux from multidrug resistant CEM vcr1000 cells and overall lipophilicity of the compounds was observed [8]. Additionally we could demonstrate that modifications on the phenone moiety influence pharmacological activity independently of hydrophobicity of the molecules[9]. To obtain additional information on structural requirements necessary for high pharmacological activity, we performed both a Free-Wilson and a combined Hansch/Free-Wilson analysis on a set of 48 compounds. The data set includes substances with variations on the nitrogen atom, on position 1 of the acyl side chain, and on the substitution pattern of the central aromatic ring.

Results and Discussion

Chemistry

Compounds were prepared in analogy to previously described procedures (for references see Table 2). Briefly, an appropriate phenol was reacted with epichlorohydrin to give the corresponding aryl ether. Subsequent reaction with an amine yielded the desired target compounds **1–48**. The chemical structure of compounds **1–48** and the descriptor set for the Free-Wilson analysis is given in Table 1 and Figure 1.

MDR-Modulating Activity

The daunomycin efflux assay is a direct and accurate functional method to measure inhibition of PGP-mediated transmembrane transport. The resistant human T-lymphoblast cell line CEM vcr1000[10] was used in our studies. The time dependent decrease in mean cellular fluorescence was determined in the presence of various concentrations of modifier and the first order rate constants (V_{max}/K_m) were calculated by nonlinear regression analysis. A correction for simple diffusion was achieved by subtracting the efflux rates observed in the parental line. EC_{50} values of modifiers were calculated from dose response curves of V_{max}/K_m *vs.* modifier concentration. Values are given in Table 2 and represent the mean of at least three independently performed experiments. Generally, interexperimental variation was below 20%.

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Table 1: Chemical structure and *X*-descriptor characteristics of compounds **1–48**.

Descr.[a]	–	X_1	X_2	X_3	X_4	X_5	X_6	X_7
–	1[b]	2	3	4	5	6	7	8
X_8		9	10	11	12			
X_9		13	14	15	16			17
X_{10}			18				19	
X_{11}		20		21	22		23	24
X_{12}		25			26		27	
X_{13}			28				29	
X_{14}		30	31	32	33			
X_{15}	34	35				36		
X_{16}	37	38		39		40		
X_{17}		41	42		43			
X_{18}		44	45					
X_{19}		46	47		48			

[a] The descriptors X_1 to X_7 in the first row indicate substituents in R1 position of Figure 1, whereas the descriptors X_8 to X_{19} in the first column indicate substituents in R2 position;

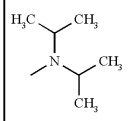
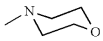
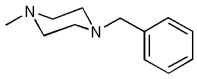
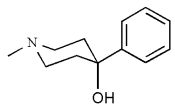
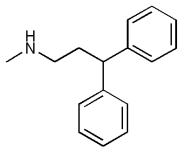
[b] numbers in the table are compound numbers: **1** is the parent molecule propafenone with no structural modification; analog **9** contains the X_1 substituent in R1 position (NH-*n*-Pr replaced by 1-piperidyl) as well as the X_8 substituent in R2 position (*ortho*-COC_2H_4Ph replaced by *ortho*-$CH(OH)C_2H_4Ph$), etc.

Physicochemical Parameters

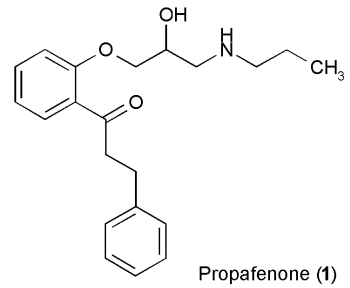
The log P values were calculated according to the method of Ghose and Crippen[11] using the MOLGEN software package [12]. As previously demonstrated on a series of propafenone analogs, the calculated values are in excellent agreement with those obtained experimentally using two different HPLC methods.[13] The molecules were generated using the builder function and were energetically minimized with the optimization tool. Conformationally independent log *P* and MR values were calculated. For the determination of the log *P* contribution of substituents on the nitrogen atom (log P_N) the difference of the corresponding phenylpropiophenone derivative and propafenone was calculated. The log *P* increment of the acyl substituent on the central aromatic moiety (log P_{AC}) was obtained *via* subtraction of the log *P* value of the corresponding phenyloxypropanolamine. All values are given in Table 2. Furthermore, in this table the columns I_m and I_p indicate whether the acyl moiety is shifted from the *ortho*- to the *meta* (I_m) or *para*-position (I_p) to the propoxy group. As can be seen in Table 3, a high level of intercorrelation between MR and the lipophilicity parameters log *P*, log P_N, and log P_{AC} is observed. Additionally, log P_N and log P_{AC} are intercorrelated with log *P*.

Quantitative Structure Activity Relationships

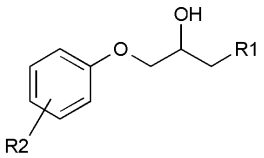
The Fujita-Ban modification of the Free-Wilson method[14] was used in the present study. Multiple linear regression analyses were performed using an in-house software package developed by K.-J. Schaper and M. Wiese. Generally, the 95% confidence intervals are given for each regression coefficient. Activity predictions were obtained by the leave-one-out method.

X_1	
X_2	
X_3	
X_4	
X_5	
X_6	
X_7	

X_8	(ortho) $CH(OH)C_2H_4Ph$
X_9	(ortho) $CH(OCH_3)C_2H_4Ph$
X_{10}	(ortho) $COCH_3$
X_{11}	(ortho) COC_2H_4Naphth
X_{12}	(ortho) COC_2H_5
X_{13}	(ortho) COPh
X_{14}	(ortho → para) COC_2H_4Ph
X_{15}	5-OH
X_{16}	5-OCH_2Ph
X_{17}	(ortho → para) $COCH_3$
X_{18}	(ortho → meta) COC_2H_4Ph
X_{19}	(ortho → meta) $COCH_3$



Propafenone (**1**)



R1: X_1-X_7

R2: X_8-X_{19}

Figure 1: All X-descriptors state the differences to the lead compound propafenone; X_{17}–X_{19} indicate a shift of the acyl group to the *para*- or *meta*-position, whereas X_{15} and X_{16} indicate the presence of OH or OCH_2Ph in *p*-position to the propoxy group (i.e. no change of *ortho*-COC_2H_4Ph).

Table 2: Physicochemical parameters and MDR-modulating activity of compounds **1**–**48**.

No	Anal.[a]	log *P*	log P_{AC}	I_m	I_p	log P_N	MR	EC_{50} (μM)
1	Ref. [23]	3.36	1.57	0	0	0.00	101.20	1.08
2	Ref. [6]	3.67	1.57	0	0	0.31	108.80	0.68
3	Ref. [6]	4.93	1.57	0	0	1.57	147.20	0.14
4	Ref. [6]	4.25	1.57	0	0	0.89	115.90	0.31
5	Ref. [6]	2.54	1.57	0	0	–0.82	115.10	3.75
6	Ref. [14]	4.43	1.57	0	0	1.07	146.00	0.38
7	Ref. [18]	3.98	1.57	0	0	0.62	134.50	0.07
8	Ref. [18]	6.51	1.57	0	0	2.15	150.00	0.72
9	Ref. [8]	3.94	2.48	0	0	0.31	109.40	1.34
10	Ref. [8]	5.20	2.48	0	0	1.57	147.70	0.67
11	C,H,N,Cl	4.52	2.48	0	0	0.89	121.00	1.74
12	C,H,N,Cl	2.81	2.48	0	0	–0.82	115.70	9.54
13	Ref. [8]	4.30	2.84	0	0	0.31	114.10	0.77
14	Ref. [8]	5.56	2.84	0	0	1.57	152.50	0.23
15	C,H,N,Cl	4.88	2.84	0	0	0.89	125.80	0.66
16	C,H,N,Cl	3.17	2.84	0	0	–0.82	120.50	1.80
17	C,H,N,Cl	7.14	2.84	0	0	2.15	155.30	0.75
18	Ref. [18]	2.67	–0.69	0	0	1.57	105.00	3.84
19	Ref. [8]	1.73	–0.69	0	0	0.62	105.50	2.83
20	C,H,N,Cl	4.67	3.21	0	0	0.31	126.00	0.17
21	C,H,N,Cl	5.25	3.21	0	0	0.89	133.00	0.54
22	Ref. [19]	3.54	3.21	0	0	–0.82	123.10	0.68
23	C,H,N,Cl[b]	4.98	3.21	0	0	0.62	151.70	0.07
24	C,H,N,Cl	7.51	3.21	0	0	2.15	167.00	0.72
25	Ref. [18]	2.07	–0.69	0	0	0.31	84.56	6.84
26	Ref. [7]	0.94	0.61	0	0	–0.82	81.68	207.20
27	Ref. [18]	2.38	0.61	0	0	0.62	110.20	0.30
28	Ref. [18]	4.57	1.85	0	0	1.57	125.60	0.42
29	Ref. [18]	3.62	1.85	0	0	0.62	125.10	0.19
30	Ref. [8]	3.67	1.67	0	1	0.31	108.80	1.36
31	Ref. [8]	4.93	1.57	0	1	1.57	147.20	2.53
32	C,H,N,Cl	4.25	1.57	0	1	0.89	115.90	0.92
33	C,H,N,Cl	2.54	1.57	0	1	–0.82	115.10	6.86
34	Ref. [14]	3.00	1.57	0	1	0.00	101.80	3.02
35	Ref. [14]	3.29	1.57	0	1	0.31	110.60	2.30
36	Ref. [14]	4.04	1.57	0	1	1.07	148.60	0.53
37	Ref. [14]	5.00	1.57	0	1	0.00	133.00	0.11
38	Ref. [14]	5.28	1.57	0	1	0.31	140.60	0.17
39	Ref. [14]	5.86	1.57	0	1	0.89	147.60	0.08
40	Ref. [14]	6.04	1.57	0	1	1.07	178.50	0.12
41	C,H,N,Cl	1.42	–0.69	0	1	0.31	79.81	75.90
42	C,H,N,Cl[c]	2.67	–0.69	0	1	1.57	105.00	11.89
43	Ref. [20]	0.28	–0.69	0	1	–0.82	76.94	302.05
44	Ref. [8]	3.67	1.57	1	0	0.31	108.80	0.39
45	Ref. [8]	4.93	1.57	1	0	1.57	147.20	1.12
46	Ref. [20]	1.42	–0.69	1	0	0.31	79.81	9.07
47	C,H,N,Cl[d]	2.67	–0.69	1	0	1.57	105.00	11.36
48	Ref. [20]	0.28	–0.69	1	0	–0.82	76.94	117.69

[a] Satisfactory C, H, N, and Cl elemental analyses (±0.4%) were obtained; [b] Cl: calcd 6.49, found 5.96;
[c] C: calcd 59.05, found 58.38; [d] Cl: calcd 15.92, found 15.35.

Free-Wilson Analysis

As shown in Table 1, 19 indicator variables were used to describe the chemical structure of 48 propafenone analogs. Propafenone (**1**) was used as the reference molecule. All compounds exhibit an aryloxypropanolamine backbone and differ in the substituents on the central aromatic ring system and on the nitrogen atom. Multiple linear regression analyses followed by *X*-descriptor reduction using the t-test resulted in equation (1). The final equation shows that among the R1 substituents morpholine (X_4) leads to a statistically significant decrease of activity, whereas 4-hydroxy-4-phenylpiperidyl (X_6) remarkably enhances the MDR-modulating potency. Among the descriptors describing variations on the central aromatic ring system (X_8–X_{19}), 8 out of 12 proved to significantly influence pharmacological activity. With the exception of a 5-benzyloxy substituent (X_{16}) all modifications gave rise to a decrease of activity. Thus, reduction of the carbonyl group in the propiophenone moiety, exchange by acetyl or propionyl, and a shift of the substituent to *meta* or *para* position of the ether oxygen negatively influence PGP inhibitory activity. Also hydroxylation in position 5 of the central aromatic ring system, which is expected to be the main metabolic pathway of propafenone-type compounds, decreases activity. This is in accordance to results obtained in QSAR studies using Hansch analysis [9, 15].

$$\log(1/EC_{50}) = -0.74(0.28)X_4 + 0.69(0.38)X_6 - 0.45(0.39)X_8 - 0.84(0.56)X_{10} - 1.21(0.45)X_{12} - 0.51(0.39)X_{14} - 0.53(0.44)X_{15} + 0.59(0.39)X_{16} - 1.91(0.44)X_{17} - 1.46(0.44)X_{19} + 0.34(0.17) \quad (1)$$
$$r = 0.94,\ s = 0.35,\ F = 25.87,\ Q^2_{cv} = 0.66,\ n = 48$$

The final Free-Wilson equation obtained describes the pharmacological activity of the compounds in a good way (Figure 2; $r = 0.935$) and exhibits good predictive power. Given the fact that the ratio of cases to adjustable coefficients is 4.4, this is a quite good result. Additionally, no intercorrelation is observed between the variables used (highest *r* value: $X_6/X_{12} = 0.194$). Figure 3 shows the plot of predicted *vs.* observed activity obtained for equation (1) using a leave-one-out procedure. Only the *ortho* acetyl derivatives **18** and **19** and two propionyl analogs (**26** and **27**) are not properly predicted by the model, which might be due to the fact, that both X_{10} and X_{12} are represented by only two compounds.

Hansch Analysis

Previously performed QSAR studies showed, that lipophilicity is a major predictive parameter for MDR-modulating activity of propafenone-type compounds. Recently performed studies demonstrated that this is also the fact for MDR-modulating dihydropyridines, pyrazoles and thienothiazines [16]. Within the series of propafenone analogs there is also evidence, that the substituent on the nitrogen atom influences activity mainly via its contribution to overall lipophilicity of the compounds. Thus, we extended our Free-Wilson analysis using both overall lipophilicity (log *P*) and partial lipophilicity values of the substituents on the nitrogen atom (log P_N) and on the central aromatic ring system (log P_{AC}) as descriptors. In case of log P_{AC} also the indicator variables I_m (substitution *meta* to the ether oxygen) and I_p (substitution *para* to the ether oxygen) were used to describe the substitution pattern on the central aromatic ring. Additionally, the molar refraction (MR) of the compounds, which also takes into account polar interactions, was included in our studies. Using these physicochemical parameters alone or in various combinations, generally lower predictivity was obtained when compared to the Free-Wilson approach (Table 3). The best cross validated Q^2 was obtained with log P_{AC} and MR. However, the contribution of log P_{AC} was not significant on the 95% level. Thus, using MR alone as descriptor, the following equation [eq. (2)] was obtained:

$$\log(1/EC_{50}) = 0.027(0.007)MR - 3.36(0.87);$$
$$\text{scaled: } \log(1/EC_{50}) = 0.75(0.20)MR \quad (2)$$
$$r = 0.75,\ s = 0.59,\ F = 58.60,\ Q^2_{cv} = 0.51,\ n = 48$$

$$\log(1/EC_{50}) = 0.41(0.11)\log P - 1.71(0.47);$$
$$\text{scaled: } \log(1/EC_{50}) = 0.74(0.20)\log P \quad (3)$$
$$r = 0.74,\ s = 0.59,\ F = 55.38,\ Q^2_{cv} = 0.49,\ n = 48$$

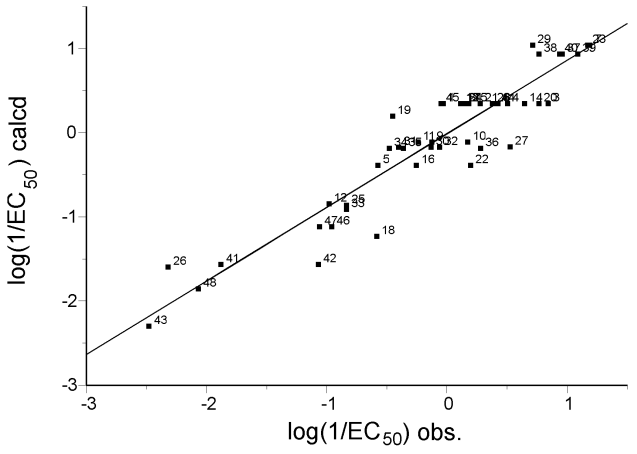


Figure 2: Plot of calculated *vs.* observed MDR-modutating activity (expressed as log ($1/EC_{50}$) values) for compounds **1**–**48** according to equation (1); $r = 0.94$, $s = 0.35$, $n = 48$.

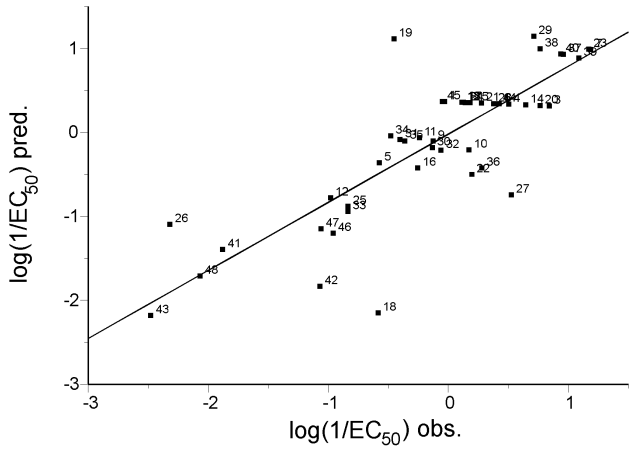


Figure 3: Plot of predicted *vs.* observed MDR-modulating activity (expressed as log ($1/EC_{50}$) values) for compounds **1**–**48** according to equation (1). The predicted values were obtained using a leave one out procedure; $r = 0.94$, $s = 0.35$, $Q^2_{cv} = 0.66$, $n = 48$.

Table 3: Intercorrelation matrix of physicochemical parameters and statistical parameters of Hansch equations.

	log P_N	log P_{AC}	MR	log P	log (1/EC_{50})	s	F	Q^2_{cv}
log P_N	1.000				0.409	0.81	9.23	0.08
log P_{AC}	0.091	1.000			0.618	0.69	28.49	0.33
MR	0.592	0.637	1.000		0.748	0.59	58.60	0.51
log P	0.628	0.717	0.870	1.000	0.739	0.59	55.38	0.49

Generally, the r values are given. Column log (1/EC_{50}) shows the r value of the correlation between the corresponding physicochemical parameter and the MDR-modulating activity (expressed as log (1/EC_{50}); s, F, and Q^2_{cv} are the statistical parameters for these correlations.

Nevertheless, using log P as descriptor, nearly the same predictiveness was obtained [eq. (3)].

The slightly higher significance of MR *vs*. log P might indicate that also polar interactions take place [17]. This is supported by recent results from Konings et al., who showed that the dipole moment seems to be an indicator for substrate and inhibitor properties of compounds interacting with P-glycoprotein [18].

Combined Approach

Combined Free-Wilson/Hansch analysis is a versatile tool in medicinal chemistry using both physicochemical parameters and substructure related indicator variables to describe the biological activity of series of compounds. Thus, we used the Free-Wilson descriptors X_1–X_{19} and combined them with the Hansch descriptors log P, log P_N, log P_{AC} and MR in all possible combinations. When log P_{AC} was used as descriptor, the Free-Wilson descriptors X_{10}–X_{13} were omitted and X_{14} and X_{17}–X_{19} were replaced by the indicator variables I_m (I_m = 1 for X_{18} or X_{19} = 1, else = 0) and I_p (I_p = 1 for X_{14} or X_{17} =1, else = 0). Thus, in this case all acyl substituents on the central aromatic ring are described by their contribution to the lipophilicity of the molecule and their relative position to the ether oxygen. In analogy to the Free-Wilson analysis, several subsequent runs were performed in the multiple linear regression analysis to reduce the number of X descriptors by elimination of non-significant variables.

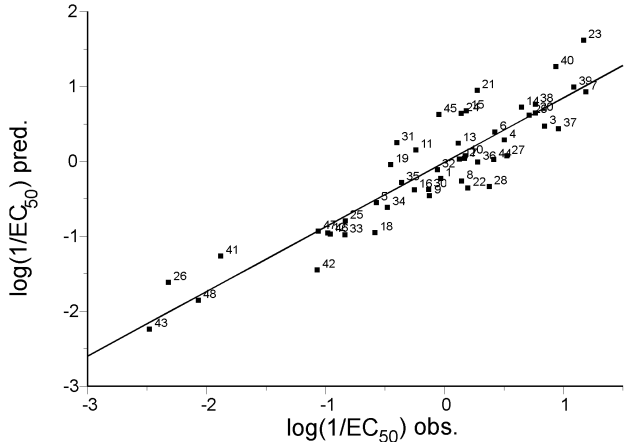


Figure 4: Plot of predicted *vs*. observed MDR-modulating activity (expressed as log (1/EC_{50}) values for compounds **1**–**48** according to equation (4). The predicted values were obtained using a leave-one-out procedure; r = 0.94, s = 0.33, Q^2_{cv} = 0.83, n = 48.

Generally, the results obtained showed higher crossvalidated Q^2 values than those from the Free-Wilson and Hansch analysis alone. When log P_N was used together with X_1–X_7, only X_6 and X_7 showed statistically significant contributions. This indicates that 4-hydroxy-4-phenylpiperidine (X_6) and diphenylpropylamine (X_7) influence MDR-modulating activity independently of their contribution to lipophilicity of the molecules, which is in accordance with previously obtained results [19]. Due to the high intercorrelation of MR and log P and the slightly higher significance of MR in the Hansch analysis [eq. (2)], log P was omitted from the descriptor set. The best equation was obtained when starting either with (X_1–X_9, X_{15}, X_{16}, MR, log P_{AC}, I_m, I_p) or (X_1–X_9, X_{15}, X_{16}, MR, log P_{AC}, I_m, I_p) or with (X_1–X_9, X_{15}, X_{16}, MR, log P_{AC}, I_m, I_p, log P_N). In all three cases, the following equation [eq. (4), Figure 4] was obtained:

$$\log(1/EC_{50}) = 0.035(0.005)MR - 0.40(0.22)I_p - 0.87(0.29)X_2 - 0.82(0.27)X_4 - 0.95(0.47)X_5 - 1.62(0.47)X_7 - 0.43(0.36)X_8 - 3.76(0.64);$$
$$\text{scaled: } \log(1/EC_{50}) = 0.98(0.15)MR - 0.21(0.18)I_p - 0.38(0.12)X_2 - 0.37(0.12)X_4 - 0.27(0.13)X_5 - 0.45(0.13)X_7 - 0.14(0.11)X_8 \quad (4)$$
$$r = 0.94,\ s = 0.33,\ F = 41.98,\ Q^2_{cv} = 0.83,\ n = 48$$

MR clearly shows the highest statistical significance, followed by X_7, X_4, and X_2. Interestingly, I_p (but not I_m) remains in the descriptor set, although log P_{AC} is not present in the final equation. This indicates that the information "*para*-acyl instead of *ortho*-acyl" seems to be important for describing the PGP-inhibitory activity of the molecules. Additionally, reduction of the carbonyl group (i.e. X_8=1) also significantly decreases activity, which is a further hint of the importance of a phenone moiety.

Conclusions

Both Free-Wilson and combined Free-Wilson/Hansch analyses were performed on a set of 48 MDR modulators structurally related to propafenone. Using Free-Wilson analysis alone (19 descriptors), a QSAR equation with moderate, but nevertheless significant predictiveness was obtained. From the descriptors indicating various substructures on the nitrogen atom only those for morpholine and 4-hydroxy-4-phenylpiperidine remained in the final equation as

Table 4: Statistical parameters of combined Free-Wilson/Hansch approaches.

Descriptors	*r*	*s*	*F*	Q^2_{cv}	Descriptors in final equation
FW,log *P*	0.88	0.43	29.56	0.70	log *P*,X_2,X_4,X_6,X_7
FW,log P_{AC},I_m,I_p	0.92	0.41	13.00	0.72	log P_{AC},X_4–X_6,X_8,X_9,X_{15}
FW,log P_N	0.96	0.35	27.22	0.81	log P_N,X_6–X_8,X_{10},X_{12}–X_{17},X_{19}
FW,MR	0.94	0.39	34.63	0.81	MR,X_2,X_4,X_5,X_7,X_8,X_{14},X_{17}
FW,log *P*,log P_{AC},I_m,I_p,	0.90	0.41	28.80	0.74	log P_{AC},*P*,X_4,X_6,X_8,X_{16}
FW,log *P*,log P_N	0.96	0.34	27.40	0.78	log *P*,log P_N,X_6–X_8,X_{10}–X_{17},X_{19}
FW,log P_{AC},log P_N,I_m,I_p	0.92	0.44	29.60	0.78	log P_{AC},log P_N,X_6–X_8,X_{16}
FW,log P_{AC},MR,I_m,I_p	0.94	0.33	41.98	0.83	MR,I_p,X_2,X_4,X_5,X_7,X_8
FW,log P_N,MR	0.96	0.34	26.20	0.80	log P_N,MR,X_6–X_8,X_{10}–X_{17},X_{19}
FW,log *P*,log P_{AC},log P_N,I_m,I_p	0.91	0.45	31.99	0.77	log P_{AC},log P_N,X_6–X_8,X_{16}
FW,log P_{AC},log P_N,MR,I_m,I_p	0.94	0.33	41.98	0.83	MR,I_p,X_2,X_4,X_5,X_7,X_8

statistically significant. With the exception of 5-benzyloxy, variations on the central aromatic ring generally negatively influence MDR-modulating activity, as indicated by the fact that 8 out of 12 descriptors are statistically significant and show negative regression coefficients. This demonstrates that the *o*-alkoxyphenone moiety seems to be crucial for high PGP-inhibitory potency. The combined approach using substructural log *P* values and molar refraction as additional *X* variables, generally resulted in equations with higher predictive power. MR showed high significance in all equations, indicating that polar interactions also contribute favorably to binding to P-glycoprotein.

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Experimental

Materials and Methods

Chemistry

Melting points were determined on a Reichert-Kofler hot-stage microscope and are uncorrected. Elemental analyses were performed by Mikroanalytisches Laboratorium, Institute of Physical Chemistry, University of Vienna. NMR spectra were recorded on a Varian UnityPlus spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C) using $CDCl_3$ solutions at 28 °C. The center of the solvent signal was used as an internal standard which was related to TMS with δ 7.26 ppm (^{1}H) and δ 77.0 ppm (^{13}C). Column chromatographic separations were performed on Merck Kieselgel 60 (70 – 230 mesh). Yields given below are not optimized and refer to analytically pure material.

General Procedure for Preparation of Amines

To a solution of 5.0 mmol of the corresponding epoxide (for **11**, **12**, **15**–**17**, **32**, **33** see ref. [9], for **20**, **21**, **23**, **24** see ref.[20], and for **41**, **42**, **47** see ref.[21]) in 20 mL methanol 5.1 mmol of the desired amine was added. The reaction mixture was heated at 50 °C till the reaction was completed (tlc control). The solvent was evaporated and the resulting oil was purified via column chromatography (silica gel, CH_2Cl_2/methanol/NH_3 conc 200/10/1 – 400/10/1).

General Procedure for Formation of the Hydrochlorides

1.0 mmol of the amine was dissolved in ethyl acetate and 1.2 mL of a 1 M solution of HCl in diethyl ether was added. The resulting precipitate was filtered off and recrystallized.

*3-(N,N-Diisopropylamino)-1-(2-(1-hydroxy-3-phenyl-propyl)phenoxy)-2-propanol (**11**)*

Mixture of diastereoisomers; yield: 60.4%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 1.01 (d, 6H, *J* = 6.4 Hz, 2 CH_3), 1.08 (d, 6H, *J* = 6.4 Hz, 2 CH_3), 2.09–2.30 (m, 2H, ar-CH(O)-C*H*$_2$), 2.46–2.92 (m, 4H, CH_2-ph, CH_2-N), 3.07 (sept, 2H, *J* = 6.4 Hz, N-$(CH)_2$), 3.49–3.58 (m, 1H, OH), 3.86–3.94 (m, 1H, CH(O)), 4.02 (dd, 1H, *J* = 4.7/10.3 Hz, O-CH_a), 4.09 (dd, 1H, *J* = 3.8/10.3 Hz, O-CH_b), 3.85–4.25 (br, 1H, OH), 4.77–4.90 (m, 1H, ar-CH(O)), 6.90 (d, 1H, *J* = 8.1 Hz, aromatic 3-H), 6.95 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.14–7.28 (m, 7H, aromatic H); ^{13}C NMR ($CDCl_3$): δ 19.26 (CH_3), 22.37 (CH_3), 32.43 (CH_2), 38.00, 38.47 (CH_2), 46.68 (CH_2), 48.07 (CH), 65.42 (CH), 70.52, 71.38 (CH), 70.91, 71.21 (CH_2), 112.56, 121.13, 125.55, 127.16, 127.44, 128.26, 128.35, 128.39 (aromatic CH), 132.99 (C), 142.17, 142.24 (C), 156.43, 156.52 (C).

***11**-hydrochloride*: yield: 60.5%; mp 117–119 °C (diethyl ether); Anal. ($C_{24}H_{35}NO_3$•HCl): C, H, N, Cl.

*1-(2-(1-Hydroxy-3-phenyl-propyl)phenoxy)-3-(4-morpholinyl)-2-propanol (**12**)*

Mixture of diastereoisomers; yield: 77.9%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 2.05–2.27 (m, 2H, CH(O)-C*H*$_2$), 2.39–2.89 (m, 8H, CH_2-N-$(CH_2)_2$, CH_2-ph), 3.10–3.40 (br, 1H, OH), 3.40–3.60 (br, 1H, OH), 3.65–3.78 (m, 4H, CH_2-O-CH_2), 3.93–4.12 (m, 3H, O-CH_2-CH(O)), 4.83, 4.86 (2 t, 1H, *J* = 6 Hz, ar-CH(O)), 6.88 (d, 1H, *J* = 8.1 Hz, aromatic 3-H), 6.97 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.14–7.30 (m, 7H, aromatic H); ^{13}C NMR ($CDCl_3$): δ 32.36 (CH_2), 38.20, 38.46 (CH_2), 53.64 (CH_2), 60.84 (CH_2), 65.34 (CH), 66.83 (CH_2), 70.29, 70.61 (CH), 70.47, 70.75 (CH_2), 112.36, 112.41, 121.23, 125.62, 127.22, 127.30, 128.20, 128.23, 128.30, 128.36, 128.37 (aromatic CH), 132.88 (C), 142.11 (C), 156.07, 156.09 (C).

***12**-hydrochloride*: yield: 71.9%; mp 148–158 °C (ethyl acetate); Anal. ($C_{22}H_{29}NO_4$•HCl): C, H, N, Cl.

*3-(N,N-Diisopropylamino)-1-(2-(1-methoxy)-3-phenyl-propyl)phenoxy)-2-propanol (**15**)*

Mixture of diastereoisomers; yield: 65.7%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 1.02 (d, 6H, *J* = 6.4 Hz, 2 CH_3), 1.07 (d, 6H, *J* = 6.4 Hz, 2 CH_3), 1.96–2.07 (m, 2H, CH(O)-C*H*$_2$), 2.43–2.83 (m, 4H, CH_2-N, CH_2-ph), 3.06 (sept, 2H, *J* = 6.4 Hz, N-$(CH)_2$), 3.25, 3.27 (2 s, 3H, OCH_3), 3.81–4.06 (m, 4H, O-CH_2-CH(O), OH), 4.64, 4.67 (2 t, 1H, *J* = 6.2 Hz, ar-CH(O)), 6.88 (d, 1H, *J* = 8.1 Hz, aromatic 3-H), 6.99 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.12–7.27 (m, 6H, aromatic H), 7.37 (d, 1H, *J* = 7.5 Hz, aromatic 6-H); ^{13}C NMR ($CDCl_3$): δ 19.57, 22.12 (CH_3), 31.96, 32.10 (CH_2), 38.09, 38.37 (CH_2), 47.25 (CH_2), 48.20 (CH), 56.77 (CH_3), 65.60 (CH), 70.82, 70.96 (CH_2), 76.77 (CH), 111.36, 111.50, 120.85, 120.89, 125.50, 126.45, 126.59, 128.02, 128.06, 128.10, 128.14, 128.34, 128.41 (aromatic CH), 130.45 (C), 142.24 (C), 156.26, 156.35 (C).

***15**-hydrochloride*: yield: 70.4%; mp 94–96 °C (diethyl ether); Anal. ($C_{25}H_{37}NO_3$•HCl): C, H, N, Cl.

*1-(2-(1-Methoxy-3-phenyl-propyl)phenyloxy)-3-(4-morpholinyl)-2-propanol (**16**)*

Mixture of diastereoisomers; yield: 64.5%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 1.94–2.10 (m, 2H, CH(O)-C*H*$_2$), 2.39–2.84 (m, 8H, CH_2-ph, CH_2-N-$(CH_2)_2$), 3.24, 3.26 (2 s, 3H, -CH_3), 3.15–3.35 (br, 1H, -OH), 3.66–3.79 (m, 4H, CH_2-O-CH_2), 3.91–4.07 (m, 3H, O-CH_2-CH), 4.53, 4.55 (2 t, 1H, *J* = 6.8/7.1 Hz, CH(OCH_3)), 6.86 (d, 1H, *J* = 8.3 Hz, aromatic 3-H), 6.99 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.13–7.38 (m, 7H, aromatic H); ^{13}C NMR ($CDCl_3$): δ 32.03 (CH_2), 37.94, 38.10 (CH_2), 53.81 (CH_2), 56.76 (CH_3), 61.20 (CH_2), 65.64 (CH), 66.87 (CH_2), 70.48, 70.67 (CH_2), 76.99, 77.36 (CH), 111.68, 111.81, 121.09, 125.59, 125.61, 126.77, 126.98, 128.12, 128.42, 128.44, 130.34, 130.43 (aromatic CH), 142.13, 142.19, 156.09, 156.23 (aromatic C).

***16**-hydrochloride*: yield: 91.2%; mp 84–86 °C (ethyl acetate); Anal. ($C_{23}H_{31}NO_4$•HCl): C, H, N, Cl.

*3-(3,3-Diphenylpropylamino)-1-(2-(1-methoxy-3-phenyl-propyl)phenoxy)-2-propanol (**17**)*

Mixture of diastereoisomers; yield: 55.0%; yellow oil; ^{1}H NMR ($CDCl_3$): δ 1.5–2.3 (br, 2H, OH, NH), 1.93–2.09 (m, 2H, CH(O)-C*H*$_2$), 2.25 (qu, 2H, *J* = 7.5 Hz, CH(ph)$_2$-C*H*$_2$), 2.58–2.74 (m, 6H, CH_2-N-CH_2, CH_2-ph), 3.21, 3.22 (2 s, 3H, OCH_3), 3.81–3.92 (m, 3H, O-CH_2-CH(O)), 4.02 (t, 1H, *J* = 7.5 Hz, CH(ph)$_2$), 4.41–4.48 (m, 1H, CH(OCH_3)), 6.82 (d, 1H, *J* = 8.1 Hz, aromatic 3-H), 6.98 (t, 1H, *J* = 7.3 Hz, aromatic 5-H), 7.12–7.34 (m, 17H, aromatic H); ^{13}C NMR ($CDCl_3$): δ 31.99 (CH_2), 35.89 (CH_2), 37.76, 37.97 (CH_2), 48.24 (CH_2), 48.95 (CH), 51.62, 51.79 (CH_2), 56.71 (CH_3), 68.30 (CH), 70.62, 70.96 (CH_2), 77.14, 77.72 (CH), 111.86, 112.00, 121.05, 125.59, 125.62, 126.18, 126.97, 127.30, 127.69, 128.20, 128.29, 128.44, 128.51 (aromatic CH), 130.12, 130.30 (C), 142.10 (C), 144.60 (C), 156.11, 156.35 (C).

***17**-hydrochloride*: yield: 64.1%; mp 120–126 °C (ethyl acetate); Anal. ($C_{34}H_{39}NO_3$•HCl): C, H, N, Cl.

*1-(2-(2-Hydroxy-3-(1-piperidyl)-propoxy)phenyl)-3-(1-naphthyl)-1-propanone (**20**)*

Yield: 72.5%; yellow oil; ^{1}H NMR ($CDCl_3$): δ 1.30–1.55 (m, 6H, CH_2-CH_2-CH_2), 1.82–1.98 (m, 2H, CH_2-N), 2.15–2.37 (m, 4H, N-$(CH_2)_2$), 3.43–3.55 (m, 4H, CO-CH_2-CH_2), 3.20–3.80 (br, 1H, OH), 3.86–3.98 (m, 2H, O-CH_2), 4.00–4.11 (m, 1H, CH(O)), 6.94 (d, 1H, *J* = 8.4 Hz, aromatic 3-H), 7.05 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.35–7.57 (m, 5H, aromatic H), 7.71 (dd, 1H, *J* = 4.5/4.8 Hz, aromatic H), 7.78–7.86 (m, 2H, aromatic H), 8.06 (d, 1H, *J* = 8.1 Hz, aromatic 6-H); ^{13}C NMR ($CDCl_3$): δ 23.85 (CH_2), 25.76 (CH_2), 27.34 (CH_2), 45.08 (CH_2), 54.17 (CH_2), 61.53 (CH_2), 64.73 (CH), 70.78 (CH_2), 112.45, 120.87, 123.62, 125.41, 125.52, 125.83, 125.87, 126.64, 128.71, 130.49, 133.58 (aromatic CH), 127.84 (C), 131.77 (C), 133.80 (C), 137.71 (C), 157.89 (C), 200.10 (CO).

***20**-hydrochloride*: yield: 75.6%; mp 171–174 °C (ethyl acetate); Anal. ($C_{27}H_{31}NO_3$•HCl): C, H, N, Cl.

*1-(2-(3-(N,N-Diisopropylamino)-2-hydroxy-propoxy)phenyl)-3-(1-naphthyl)-1-propanone (**21**)*

Yield: 63.7%; yellow needles, mp 74–75 °C (diethyl ether/petroleum ether); ^{1}H NMR ($CDCl_3$): δ 0.85 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 0.92 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 2.33 (dd, 1H, *J* = 7.5/9.9 Hz, CH_a-N), 2.53 (dd, 1H, *J* = 3.9/9.9 Hz, CH_b-N), 2.90 (sept, 2H, *J* = 6.6 Hz, N$(CH)_2$), 3.51 (s, 4H, CH_2-CH_2), 3.80 (m, 1H, CH(O)), 3.93 (dd, 1H, *J* = 5.1/9.3 Hz, O-CH_a), 4.10 (dd, 1H, *J* = 5.4/9.3 Hz, O-CH_b), 3.85–4.25 (br, 1H, -OH), 6.98 (d, 1H, *J* = 8.7 Hz, aromatic H-3), 7.02 (t, 1H, *J* = 7.2 Hz, aromatic H-5), 7.36–7.52 (m, 5H, aromatic H), 7.68–7.85 (m, 3H, aromatic H), 8.89 (d, 1H, *J* = 7.8 Hz, aromatic H-6); ^{13}C NMR ($CDCl_3$): δ 19.31 (CH_3), 22.08 (CH_3), 27.35 (CH_2), 44.57 (CH_2), 47.41 (CH_2), 48.22 (CH), 65.21 (CH), 71.23 (CH_2), 112.56, 120.85, 123.65, 125.39, 125.51, 125.77, 125.79, 126.65, 128.70, 130.36, 133.39 (aromatic CH), 128.39 (C), 131.83 (C), 133.87 (C), 137.58 (C), 157.89 (C), 201.56 (CO).

***21**-hydrochloride*: yield: 72.5%; mp 148–150 °C (ethyl acetate); Anal. ($C_{28}H_{35}NO_3$•HCl): C, H, N, Cl.

*1-(2-(2-Hydroxy-3-(4-hydroxy-4-phenyl-1-piperidyl)propoxy)phenyl)-3-(1-naphthyl)-1-propanone (**23**)*

Yield: 91.5%; yellow oil, which solidifies slowly; ^{1}H NMR ($CDCl_3$): δ 1.57–1.72 (m, 2H, -OH), 1.92–2.15 (m, 4H, piperidine 3-H, 5-H), 2.25–2.58 (m, 6H, CH_2-N-$(CH_2)_2$), 3.42–3.58 (m, 4H, CO-CH_2-CH_2), 3.88–4.13 (m, 3H, O-CH_2-CH(O)), 6.95 (d, 1H, *J* = 8.4 Hz, aromatic 3-H), 7.03 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.14–7.54 (m, 10H, aromatic H), 7.70 (dd, 1H, *J* = 3.3/6.0 Hz, aromatic H), 7.82 (m, 2H, aromatic H), 8.08 (d, 1H, *J* = 7.5 Hz, aromatic H-6); ^{13}C NMR ($CDCl_3$): δ 27.79 (CH_2), 38.90 (CH_2), 45.37 (CH_2), 48.23 (CH_2), 51.27 (CH_2), 61.20 (C), 65.58 (CH_2), 71.23 (CH_2), 113.02, 121.40, 124.13, 124.90, 125.91, 126.03, 126.32, 127.10, 127.47, 128.76, 129.15, 130.96, 134.05 (aromatic CH), 128.38 (C), 132.23 (C), 134.22 (C), 138.17 (C), 148.50 (C), 158.35 (C), 201.56 (CO).

***23**-hydrochloride*: yield: 78.6%; mp 147–149 °C (diethyl ether/acetone); Anal. ($C_{33}H_{35}NO_4$•HCl): C, H, N; Cl: calcd 6.49, found 5.96.

*1-(2-(3-(3,3-Diphenylpropylamino)-2-hydroxy-propoxy)phenyl)-3-(1-naphthyl)-1-propanone (**24**)*

Yield: 26.6%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 2.21 (qu, 2H, *J* = 7.5 Hz, CH(ph)$_2$-C*H*$_2$), 2.37–2.66 (m, 4H, CH_2-N-CH_2), 2.80–3.20 (br, 2H, OH, NH), 3.38–3.51 (m, 4H, CO-CH_2-CH_2), 3.81–3.98 (m, 4H, O-CH_2-CH(O), CH(ph)$_2$), 6.91 (d, 1H, *J* = 8.4 Hz, aromatic 3-H), 7.00 (t, 1H, *J* = 7.5 Hz, aromatic 5-H), 7.12–7.52 (m, 15 H, aromatic H), 7.66–7.72 (m, 2H, aromatic H), 7.81 (d, 1H, *J* = 6.9 Hz, aromatic H), 8.02 (d, 1H, *J* = 7.8 Hz, aromatic 6-H); ^{13}C NMR ($CDCl_3$): δ 27.23 (CH_2), 35.14 (CH_2), 44.25 (CH_2), 47.93 (CH_2), 48.80 (CH), 51.62 (CH_2), 67.20 (CH), 71.07 (CH_2), 112.98, 120.96, 123.57, 125.48, 125.60, 125.90, 126.23, 126.73, 127.65, 127.88, 128.46, 128.76, 130.31, 133.60 (aromatic CH), 127.67 (C), 131.65 (C), 133.77 (C), 137.49 (C), 144.36 (C), 157.61 (C), 201.40 (CO).

***24**-hydrochloride*: yield: 65.6%; mp 76-80 °C (ethyl acetate); Anal. ($C_{37}H_{37}NO_3$•HCl•H_2O): C, H, N, Cl.

*1-(4-(3-(N,N-Diisopropylamino)-2-hydroxy-propoxy)phenyl)-3-phenyl-1-propanone (**32**)*

Yield: 26.8%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 1.02 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 1.06 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 1.20–1.40 (br, 1H, OH), 2.48 (dd, 1H, *J* = 10.2/13.5 Hz, CH_a-N), 2.71 (dd, 1H, *J* = 4.2/13.5 Hz, CH_b-N), 3.03–3.15 (m, 4H, CH_2-ph, N-$(CH)_2$), 3.25 (t, 2H, *J* = 7.5 Hz, CO-CH_2), 3.90–4.05 (m, 3H, O-CH_2-CH(O)), 6.96 (d, 2H, *J* = 8.7 Hz, aromatic 3-H, 5-H), 7.20–7.33 (m, 5H, aromatic H), 7.94 (d, 2H, *J* = 8.7 Hz, aromatic 2-H, 6-H); ^{13}C NMR ($CDCl_3$): δ 19.49 (CH_3), 22.12 (CH_3), 30.22 (CH_2), 40.00 (CH_2), 46.80 (CH_2), 48.32 (CH), 65.26 (CH), 70.91 (CH_2), 114.19, 125.96, 128.32, 128.39, 130.16 (aromatic CH), 129.95 (C), 141.36 (C), 162.72 (C), 197.08 (CO).

***32**-hydrochloride*: yield: 87.7%; mp 132–134 °C (diethyl ether); Anal. ($C_{24}H_{33}NO_3$•HCl): C, H, N, Cl.

*1-(4-(2-Hydroxy-3-(4-morpholinyl)-propoxy)phenyl)-3-phenyl-1-propanone (**33**)*

Yield: 51.1%; yellow oil; ^{1}H NMR ($CDCl_3$): δ 2.23–2.75 (m, 6H, CH_2-N-$(CH_2)_2$), 3.05 (t, 2H, *J* = 8.1 Hz, CH_2-ph), 3.25 (t, 2H, *J* = 8.1 Hz, CO-CH_2), 3.35–3.55 (br, 1H, OH), 3.67–3.80 (m, 4H, CH_2-O-CH_2), 4.02–4.18 (m, 3H, O-CH_2-CH(O)), 6.95 (d, 2H, *J* = 8.7 Hz, aromatic 3-H, 5-H), 7.18–7.34 (m, 5H, aromatic H), 7.94 (d, 2H, *J* = 8.7 Hz, aromatic 2-H, 6-H); ^{13}C NMR ($CDCl_3$): δ 30.21 (CH_2), 40.05 (CH_2), 53.66 (CH_2), 60.80 (CH_2), 65.17 (CH), 66.89 (CH_2), 70.24 (CH_2), 114.18, 126.01, 128.33, 128.42, 130.21 (aromatic CH), 130.15 (C), 141.33 (C), 162.44 (C), 197.71 (CO).

***33**-hydrochloride*: yield: 62.8%; mp 94–97 °C (ethyl acetate); Anal. ($C_{22}H_{27}NO_4$•HCl): C, H, N, Cl.

*1-(4-(2-Hydroxy-3-(1-piperidyl)-propoxy)phenyl-1-ethanone (**41**)*

Yield: 72.0%; colorless needles, mp 60–61 °C (dichloromethane/methanol); ^{1}H NMR ($CDCl_3$): δ 1.44–1.65 (m, 6H, CH_2-CH_2-CH_2), 2.38–2.63 (m, 6H, CH_2-N$(CH_2)_2$), 2.54 (s, 3H, CH_3), 3.5–3.9 (br, 1H, -OH), 4.03–4.12 (m, 3H, O-CH_2-CH(O)), 6.96 (d, 2H, *J* = 9.0 Hz, aromatic H-3, H-5), 7.93 (d, 2H, *J* = 9 Hz, aromatic H-2, H-6); ^{13}C NMR ($CDCl_3$): δ 24.05 (CH_2), 25.93

(CH_2), 26.17 (CH_3), 54.60 (CH_2), 60.87 (CH_2), 65.11 (CH), 70.52 (CH_2), 114.11, 130.38 (aromatic CH), 130.33 (C), 162.60 (C), 196.54 (CO).

***41**-hydrochloride*: yield: 65.8%; mp 167–169 °C (ethyl acetate); Anal. $C_{16}H_{23}NO_3$•HCl): C, H, N, Cl.

*1-(4-(3-(4-(4-Fluorphenyl)-1-piperazinyl)-2-hydroxy-propoxy)phenyl)-1-ethanone (**42**)*

Yield: 47.1%; yellowish needles, mp 105–106 °C (methanol); ^{1}H NMR ($CDCl_3$): δ 2.56 (s, 3H, CH_3), 2.58–2.70 (m, 4H, $N(CH_2)_2$), 2.83–2.89 (m, 2H, CH_2-N), 3.08–3.20 (m, 4H, $(CH_2)_2$N), 3.45–3.65 (br, 1H, -OH), 4.07–4.19 (m, 3H, O-CH_2-CH(O)), 6.86–7.00 (m, 6H, aromatic H), 7.94 (d, 2H, *J* = 9.0 Hz, aromatic H-2, H-6); ^{13}C NMR ($CDCl_3$): δ 26.26 (CH_3), 50.17 (CH_2), 53.26 (CH_2), 60.27 (CH_2), 65.45 (CH), 70.33 (CH_2), 114.17, 130.55 (aromatic CH), 115.46 (d, CH, J_{CF} = 22.5 Hz), 117.81 (d, CH, J_{CF} = 7.6 Hz), 147.71 (C), 157.01 (d, C, J_{CF} = 215 Hz), 162.53 (C), 196.03 (CO).

***42**-hydrochloride*: yield: 76.6%; mp 188–189 °C (ethyl acetate); Anal. ($C_{21}H_{25}FN_2O_3$•1.5 HCl): H, N, Cl; C: calcd 59.05, found 58.38.

*1-(3-(3-(4-(4-Fluorphenyl)-1-piperazinyl)-2-hydroxy-propoxy)phenyl)-1-ethanone (**47**)*

Yield: 40.2%; colorless needles, mp 133–135 °C (dichloromethane/methanol); ^{1}H NMR ($CDCl_3$): δ 2.53 (s, 3H, CH_3), 2.49–2.64 (m, 4H, $N(CH_2)_2$), 2.75–2.82 (m, 2H, CH_2-N), 2.98–3.16 (m, 4H, $(CH_2)_2$N), 3.25–3.62 (br, 1H, -OH), 3.98–4.12 (m, 3H, O-CH_2-CH(O)), 6.79–6.93 (m, 4H, pF-phenyl H), 7.09 (dd, 1H, *J* = 2.1/8.1 Hz, aromatic H-4), 7.31 (dd, 1H, *J* = 7.5/8.1 Hz, aromatic H-5), 7.45 (d, 1H, *J* = 2.1 Hz, aromatic H-2), 7.48 (d, 1H, *J* = 7.5 Hz, aromatic H-6); ^{13}C NMR ($CDCl_3$): δ 26.63 (CH_3), 50.18 (CH_2), 53.25 (CH_2), 60.27 (CH_2), 65.53 (CH), 70.39 (CH_2), 113.12, 119.97, 121.36, 129.54 (aromatic CH), 115.45 (d, CH, J_{CF} = 22.2 Hz), 117.81 (d, CH, J_{CF} = 7.7 Hz), 138.42 (C), 147.72 (C), 157.30 (d, C, J_{CF} = 215 Hz), 158.90 (C), 197.05 (CO).

***47**-hydrochloride*: yield: 93.6%; mp 100–102 °C (ethyl acetate); Anal. ($C_{21}H_{25}FN_2O_3$•2HCl): C, H, N; Cl: calcd 15.92, found 15.35.

Cell Lines

The CCRF-CEM T lymphoblast cell line, as well as the resistant line were obtained as described previously[9,10]. Cells were kept in RPMI1640 medium supplemented with 10% fetal calf serum under standard culture conditions. The resistant CCRF vcr1000 cell line was kept in medium containing 1000 ng/mL vincristine. The selecting agent was washed out at least 1 week prior to the experiments. The cell line used in our studies was selected in the presence of increasing doses of vincristine without prior mutagenization[10]. This cell line has been chosen because of distinct PGP-expression and does not show the mutation at codon 185[22]. In addition, no significant contribution of other factors to MDR was observed.

Efflux Assay

Daunomycin efflux studies were performed using modifications of published methods [9]. Cells were pelleted, the supernatant was removed by aspiration and the cells were resuspended at a density of 1×10^6/mL in RPMI1640 medium containing daunomycin (Sigma Chem. Comp., St. Louis, MO) at a final concentration of 3.0 μM. Cell suspensions were incubated at 37 °C for 30 min. Tubes were chilled on ice and pelleted at 500 *g* in an Eppendorf 5403 centrifuge (Eppendorf, Germany). Supernatants were removed and the cell pellet was resuspended in medium which was prewarmed to 37 °C and contained either no modulator or chemosensitizer at various concentrations dependent on solubility and expected potency of the modifier. Eight concentrations (serial dilution 1:2.5) were tested for each modulator. After 1, 2, 3, and 4 min, aliquots of the incubation mixture were transferred to tubes containing an equal volume of ice cold stop solution (RPMI1640 medium containing verapamil at a final concentration of 10 μg/mL). Zero time-points were done by immediately pipetting daunomycin preloaded cells into ice cold stop solution. Non PGP expressing parental CCRF-CEM cells were used as controls for simple plasma membrane diffusion, whereby initial daunomycin fluorescence levels were adjusted to be equal to initial levels observed in resistant cells. Samples drawn at the respective time points were kept in an ice water bath and measured within one hour on a Becton Dickinson FACSCALIBUR flow cytometer (Becton Dickinson, Vienna, Austria). Viable cells were gated on the basis of forward and side scatter. The excitation wavelength was 488 nm and the emission was measured in the FL3 channel (650–780 nm). 5000 gated events were accumulated for the determination of mean fluorescence values. Time points were fitted by an exponential curve and the first order rate constant (V_{max}/K_m) was determined as the slope of the curve at the zero time point.

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