Structure−Activity Relationship Studies on Benzofuran Analogs of Propafenone-Type Modulators of Tumor Cell Multidrug Resistance

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A series of benzofurylethanolamine analogs of propafenone (**1a**) have been prepared and evaluated for multidrug resistance-reversing activity in two in vitro assay systems. As for propafenones, an excellent correlation of biological data with calculated lipophilicity values was found for benzofurans, whereby the latter generally had lower activity/lipophilicity ratios. Almost identical slopes of the regression lines were obtained for both propafenones and benzofurans. Multiple linear regression analysis of the complete data set yielded an equation with excellent predictive power ($r^2_{cross-valid} = 0.968$). Interaction measurements with artificial membranes indicated that the differences in activity between these two series of compounds are not due to differences in the interaction pattern with biological membranes.

Introduction

The development of multiple drug resistance represents an increasing problem in cancer treatment as well as in antimicrobial therapy. Within the past decade several mechanisms of pleiotropic drug resistance of tumor cells have been identified.[1] One type of multidrug resistance (MDR) has been shown to be mediated by an energy dependent, membrane-bound efflux pump termed P-glycoprotein (PGP).[2] PGP represents a member of the ATP-binding casette[3] with low substrate specificity. A broad range of cytostatic drugs such as anthracyclines, epipodophyllotoxins, actinomycin D, vinca alkaloids, colchicines, and taxol are eliminated via PGP-mediated efflux.[4] Recently, this mechanism of drug resistance has attracted additional attention since it was proposed to be involved in multidrug resistance of Gram-negative bacteria[5] as well as in fungi[6−8] and host-mediated resistance of *Mycobacterium tuberculosis* against tuberculostatic drugs.[9] Within the past few years a variety of substances have been shown to inhibit PGP-mediated drug efflux and thereby reestablish sensitivity toward chemotherapeutic agents.[10] These include ion channel blockers such as verapamil,[11] amiodarone,[12] propafenone,[13] and some dihydropyridines,[14] antipsychotic drugs like phenothiazines[15] and thioxanthenes,[16] cyclosporines,[17] and some recently identified compounds like the triazinopiperidine S 9788[18] and the acridone carboxamide GF 120918.[19] Preliminary results obtained in clinical studies clearly demonstrate that modulation of MDR might be a successful approach in hematological malignancies, but serious side effects often preclude optimal dosage of modulators.[20] These side effects are due to the modulator's inherent pharmacological effects including cardiac effects, immunosuppression, and nephrotoxicity. Therefore, specifically designed highly active modulators with limited side effects are urgently required.

Although the proposed mechanism of action of MDR modulators is inhibition of PGP, little is known about structure−activity relationships (SAR) of these compounds.[21] Despite considerable structural heterogeneity, most of the modulators share the properties of high lipophilicity and a basic nitrogen atom. In addition, evidence has been published that amphiphilic compounds may also influence membrane surface density and viscosity.[22] Therefore, it seems likely that both direct protein and indirect lipid interactions influence the activity of PGP inhibitors.[23]

We recently identified a series of analogs of propafenone as effective inhibitors of PGP.[24] Propafenone is in clinical use as an antiarrhythmic agent due to its ability to block cardiac sodium channels. The substance also has weak β-adrenoreceptor-blocking activity.

Rhodamine-123 as well as daunomycin efflux studies on a series of closely related structural homologs of propafenone showed a highly significant correlation between lipophilicity and MDR-reversing ability. Nevertheless, altering the acyl side chain by reducing the carbonyl group or changing the substituent's position from ortho to meta or para led to a decrease in activity, for which lipophilicity was no longer the only determinant.[25]

A series of benzofuran analogs with diminished flexibility were synthesized and tested for their chemosensitizing potency in both rhodamine-123 efflux studies and daunomycin cytotoxicity assays in order to gain further insights into structural features required for good PGP-inhibitory activity of propafenone-type MDR modulators. Additionally, membrane interaction measurements of selected compounds with lipid vesicles were performed using nuclear magnetic resonance (NMR) spectroscopy or differential scanning calorimetry (DSC) in order to determine which regions of the molecules interact with biological membranes.

Chemistry

Propafenone analogs **1a**−**n** were synthesized as previously described.[25] The benzofurans **2a**,**b** as well as

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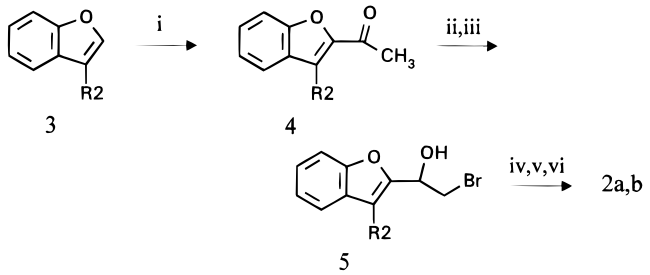
Scheme 1. Synthesis of Benzofurans **2a**,**b**[a]



[a] (i) Ac_2O, BF_3; (ii) $Br_2/AlCl_3$; (iii) $LiAlH_4$; (iv) dihydropyran, *p*-TSA; (v) amine; (vi) etheral HCl.

the enantiomers of **2b** were synthesized as outlined in Scheme 1. Thus, 3-alkylbenzofuran **3** (which can be prepared in three steps from appropriate *o*-hydroxyphenones)[26] was acetylated under Friedel−Crafts conditions to give 2-acetyl-3-alkylbenzofuran **4**, which was brominated with Br_2 and reduced with $NaBH_4$ to yield the bromohydrin **5**. Subsequent protection of the hydroxy group with dihydropyran, reaction with an appropriate amine, and cleavage of the protecting group with hydrochloric acid directly yielded the hydrochlorides of **2a**,[27]**b**[28] in fair overall yields. The enantiomers of **2b** were prepared by using mbe-lactole instead of dihydropyran followed by chromatographic resolution of the diastereoisomers.[29]

The deshydroxy analogs **2d**,**e** were prepared as described recently[30] (Scheme 2). *o*-Hydroxy-3-phenylpropiophenone was alkylated with epichlorohydrin and the epoxide ring opened by reaction with hydrochloric acid to give the chlorohydrin **6**. Oxidation with oxalyl chloride/DMSO and silica gel-mediated cyclization gave the dihydrobenzofuran **7**. Reduction with triethylsilane/$BF_3 \cdot Et_2O$ yielded the 2-(chloroalkyl)benzofuran **8**, which was reacted with *n*-propyl- or isopropylamine to give the desired deshydroxy analogs **2d**,**e**. Synthesis of the arylpiperazine analog **2f** was achieved via acidic dehydration of **7** followed by reaction with (*o*-methoxyphenyl)piperazine. Subsequent reduction of **2f** with $NaBH_4$ yielded **2c**. Alternatively, **2f** can also be prepared by reaction of the corresponding 2-(bromoacetyl)benzofuran (Scheme 1) with (*o*-methoxyphenyl)piperazine. Nevertheless, the synthesis outlined in Scheme 2 generally gave higher overall yields.

MDR-Modulating Activity. 1. Rhodamine-123 Efflux Studies. The rhodamine-123 assay is a well-documented, direct, and reproducible functional assay for measuring PGP dependent efflux.[31] We therefore measured the ability of our compounds to inhibit PGP-mediated rhodamine-123 efflux in the resistant T-lymphoblast cell line CCRF-CEM vcr1000.[32] As shown previously, similar results can be obtained by measuring either rhodamine-123 or daunomycin efflux.[25] The time dependent linear decrease in mean fluorescence of cells was determined in the presence of various concentrations of modifier, and the initial efflux rates were calculated by linear regression analysis. Correction for simple diffusion was achieved by subtracting the efflux rates observed in the parental line. ED_{50} values of all modifiers were obtained from dose−response curves of initial efflux rate vs modifier concentration. Data points of at least two independently performed experiments were fitted according to eq 1, where y is the initial efflux rate determined as a function of modifier concentration c, y_i is the initial efflux rate in the absence of modulator, and ME is the modulator efficacy.

$$y = y_i - \frac{ME \cdot c}{ED_{50} + c} \quad (1)$$

Chemical structures and ED_{50} values of all compounds are given in Table 1.

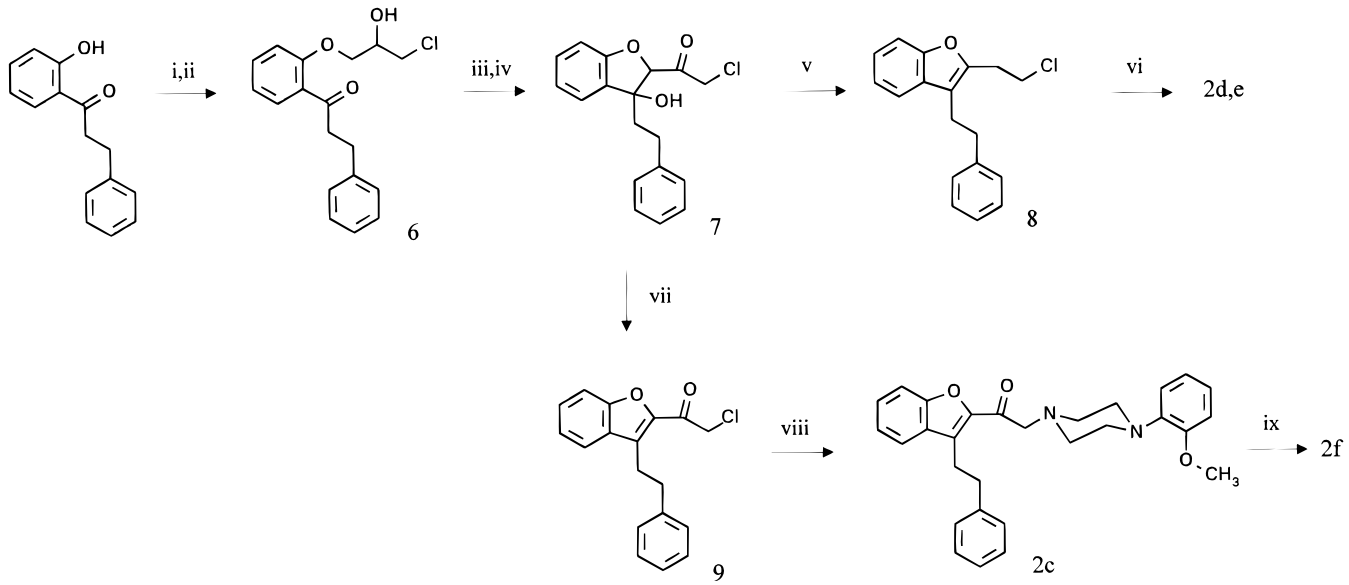
2. Cell Cytotoxicity Assay. Although inhibition of rhodamine-123 efflux is the more direct method for measuring interaction with PGP, MTT-based cytotoxicity assays represent a more general assay for the modulation of MDR, which additionally accounts for intracellular metabolism of modulators. Therefore, we measured the chemosensitizing effect of the propafenone-type modulators with respect to daunomycin using CCRF-CEM vcr1000 cells. Data were processed using the previously established method of combined simultaneous analysis of sigmoidal dose−response curve families, which leads to highly accurate ED_{50} values of modulators[33] (Table 1). Correlation with data obtained in the rhodamine-123 efflux studies gave an r value of 0.946 (Figure 1). Generally, cytotoxicity of all compounds tested did not exceed 20% at the highest modulator concentration used (10 μM).

Membrane Interaction Measurements. 1. Nuclear Magnetic Resonance Experiments. To investigate the influence of membrane lipids on selected modulators, spin−spin relaxation times in the presence of different amounts of lecithin liposomes were measured as described previously.[22] Interaction strength is represented by the slope of the regression line obtained by plotting line broadening vs lecithin concentration (Figure 2) and is listed in Table 2. Within the series of *o*-[(acylaryl)oxy]propanolamines, the strongest interaction was observed with the -O-CH_2- group and somewhat less with protons of the central phenyl ring. In all cases almost no interaction between lecithin and the phenylethyl group was observed. Within the series of benzofuran analogs the deshydroxy derivative **2d** showed strong interaction with lecithin, indicating that the entire (phenylethyl)benzofuryl system is involved. In contrast, the propafenone analogous compound **2b** showed an interaction pattern similar to **1a** with the strongest interaction near the C-OH group.

2. Differential Scanning Calorimetry. Artificial dipalmitoylphosphatidylcholine (DPPC) membranes were used to investigate the influence of selected modulators on membrane lipids. Changes in phase transition properties in the presence of different concentrations of modulators were measured according to Pajeva et al.[22] Generally, the DPPC curve characteristics showed a broadening of the main peak and a shift of T_{max} and T_{trans} to lower temperatures. Peaks were analyzed by a nonlinear least-squares fit using a Gaussian function. The peak width in °C of a molar DPPC to modifier concentration of 1:0.05 was taken as a measure of interaction strength and is presented in Table 3. These values closely correlate with the lipophilicity of the modulators (Figure 3; $r = 0.997$, $n = 6$).

3. Determination of Lipophilicity. The log *P* values were calculated according to the method of Ghose et al.[34] using the software package MOLGEN.[35] As previously shown, these values are in excellent agreement with those obtained experimentally by an HPLC method.[25]

Scheme 2. Synthesis of Benzofurans **2c**−**f**[a]



[a] (i) Epichlorohydrin, NaOH; (ii) concentrated HCl; (iii) $C_2O_2Cl_2$, DMSO; (iv) silica gel; (v) triethylsilane/BF_3; (vi) amine; (vii) H_3PO_4; (viii) amine, MeOH; (ix) $NaBH_4$.

Results and Discussion

As recently demonstrated an excellent correlation between lipophilicity and PGP inhibition was found for a homologous series of propafenone analogs. Using an MDR1-transfected cell line, we showed that inhibition of PGP is indeed the main mechanism of action of propafenone-type MDR modulators. SAR studies indicated that a hydrogen bond acceptor near the central aromatic ring moiety seems to be crucial for maintaining high MDR-modulating activity.[25]

In this paper we extend our studies to a series of analogous, conformationally restricted benzofuran derivatives. Pharmacological activity was determined both in rhodamine-123 efflux studies and in daunomycin cytotoxicity assays. The two assay systems correlate excellently, indicating that the cellular interaction site for benzofurans is indeed PGP. Figure 4 shows the correlation of calculated lipophilicity values and the chemosensitizing activity determined in rhodamine-123 efflux assays. Two regression lines were obtained for propafenones ($\log(1/ED_{50}) = 0.82(\pm0.03)\log P - 3.21(\pm0.11)$; $r = 0.994$, $n = 13$) and benzofurans ($\log(1/ED_{50}) = 0.96(\pm0.10)\log P - 4.94(\pm0.46)$; $r = 0.982$, $n = 5$). Both lines were virtually parallel. Multiple linear regression analysis using an indicator variable for benzofurans ($I_{Bf} = 1$, else $I_{Bf} = 0$) resulted in the following, statistically highly significant, equation:

$$\log(1/ED_{50}) = 0.86(\pm0.03)\log P - 1.16(\pm0.06)I_{Bf} - 3.33(\pm0.13)$$

$$r = 0.990,\ n = 18,\ r^2_{cross-valid} = 0.968$$

Figure 5 shows a plot of predicted vs corresponding observed log potency values ($r = 0.990$). Analogous results were obtained in cytotoxicity assays (propafenones, $r = 0.964$; benzofurans, $r = 0.961$; r (multiple linear regression) $= 0.971$). Generally, incorporation of the ether oxygen of propafenone into a benzofuran moiety results in a remarkable decrease in activity. Since the two regression lines are parallel, this loss of activity is compensated for in part by increasing lipophilicity. **2c** is, for example, equipotent to **1e**.

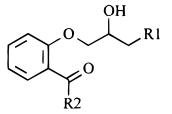
The measured activity of the deshydroxy analogs **2d**,**e** correlated well with the expected values. Thus, the hydroxyl group does not seem to be involved in interaction with PGP. Slight differences in activity of the enantiomers of **2b** in cytotoxicity assays (1.83 ± 0.13 for (*S*)-**2b** and 1.03 ± 0.01 for (*R*)-**2b**) were not found in rhodamine-123 efflux studies. Since incubation times are considerably longer in cytotoxicity assays (72 h) than those of efflux studies (2 min), the slightly lower activity of (*S*)-**2b** in cytotoxicity assays might be due to stereospecific cellular metabolism of the compounds.

Previous experiments showed that propafenones carrying a carbonyl group are more active than the corresponding hydroxy and methoxy derivatives. Therefore, the hydroxyl group of the benzofuran **2c** was converted to a carbonyl group. An increase in the activity/lipophilicity ratio was, however, not observed. Conversely, a decrease in activity in relation to the expected value by a factor of 4 was noted. These data suggest that the distance between the carbonyl group and the basic nitrogen atom might be crucial, which agrees with results previously obtained for propafenone analogs.[25]

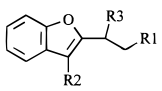
Two desphenyl analogs (**1m** and **2a**) were synthesized and tested in order to evaluate whether the benzyl group influences activity by direct interaction with PGP. The results demonstrate that the phenylethyl group influences activity mainly via its contribution to overall lipophilicity of propafenones and benzofurans and probably not via $\pi-\pi$ interaction of the phenyl ring with aromatic amino acids of PGP.

It has been proposed in the literature, that MDR-modulating activity of amphiphilic drugs depends on their ability to interact with membrane phospholipids.[22] NMR and DSC were employed to characterize the interaction of selected compounds with artificial lipid membranes. NMR−interaction measurements with lecithin liposomes showed that propafenone and benzofuran analogs bearing a hydroxyl group only interact in the vicinity of this substructure, whereas the deshydroxy benzofuran **2d** showed strong interaction over the whole (phenylethyl)benzofuryl region. However, this difference in the interaction pattern of various benzofurans with phospholipid vesicles had no influence on

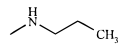
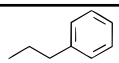
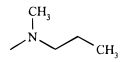
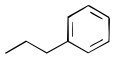
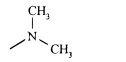
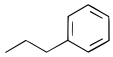
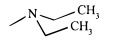
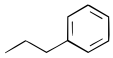
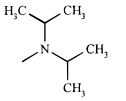
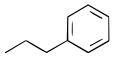
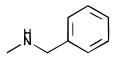
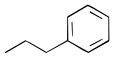
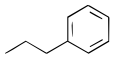
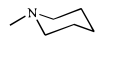
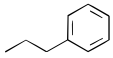
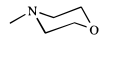
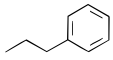
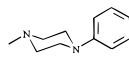
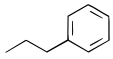
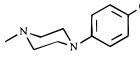
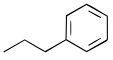
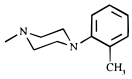
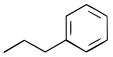
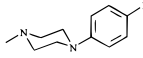
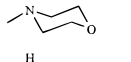
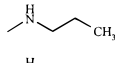
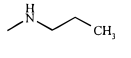
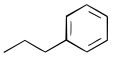
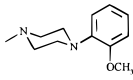
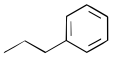
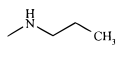
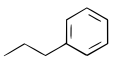
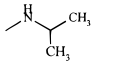
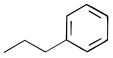
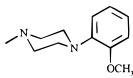
Table 1. Chemical Structure, Calculated Lipophilicity, and Pharmacologic Activity of Compounds **1a**−**n** and **2a**−**f**



General formula for compounds **1a-n**



General formula for compounds **2a-f**

#	R1	R2	R3	calcd. logP	ED_{50} (μmol/l) CCRF-CEM vcr100 Efflux[a]	MTT[b]
1a			-	3.36	3.55	0.29
1b			-	3.77	1.36	0.27
1c			-	2.94	7.12	0.82
1d			-	3.62	1.40	0.39
1e			-	4.25	0.61	0.29
1f			-	4.30	0.41	0.19
1g			-	3.26	2.55	0.52
1h			-	3.67	1.65	0.29
1i			-	2.54	13.56	1.49
1j			-	4.93	0.16	0.15
1k			-	4.65	0.28	0.11
1l			-	5.26	0.06	0.05
1m		$-CH_3$	-	2.67	8.61	0.89
1n			-	0.94	n.a.	n.a.
2a			−OH	3.10	69.60	4.19
2b			−OH	4.07	13.61	1.80
2c			−OH	5.32	0.46	0.21
2d			−H	4.71	2.90	0.87
2e			−H	4.54	5.80	1.10
2f			=O	5.05	4.96	2.02
	Verapamil			5.69	0.54	0.35

[a] Data points of at least two independently performed experiments were used to determine the ED_{50} values. Generally, interexperimental variation was below 10%. [b] Data points were determined in triplicate as described previously;[24] interexperimental variation was below 15%.

their MDR-modulating activity. Thus, the decrease in activity of benzofurans may be related to a decrease in

Figure 1. Correlation of ED_{50} values of modulators obtained in rhodamine-123 efflux assays vs those obtained in cytotoxicity assays (MTT); $r = 0.946$, $n = 19$.

Figure 2. Correlation of line broadening (expressed as peak width in Hz) of the ArO-CH_2- signal in **1h** vs concentration of lecithin liposomes (in mg/mL).

Table 2. Interaction Strength with Lecithin Vesicles (n 1/2), Calculated log P, and MDR-Modulating Activity of Selected Propafenone Analogs

compd	n 1/2[a]	log P	ED_{50}[b]
1a	42.34	3.36	3.55
1d	35.34	3.62	1.40
1e	39.50	3.67	0.61
1h	52.40	4.25	1.65
1i	63.42	2.54	13.56

[a] n 1/2 is represented as the slope of the correlation of peak width of the ArO-CH_2- signal vs concentration of lecithin liposomes (see also Figure 2). [b] ED_{50} values obtained in Rh^{123} efflux assays are presented.

flexibility in comparison to propafenones or to the lack of an appropriate hydrogen-bond acceptor.

Results of DSC−interaction measurements between modulators and phosphatidylcholine vesicles closely correlate with calculated lipophilicity values of both propafenones and benzofurans. This is shown for selected compounds in Figure 3. Data indicate that within the series of compounds tested, calculated lipophilicity of the molecules seems to be a good predictor of partitioning into uncharged membranes. A close correlation between DSC data and biological activity is found within both homologous series. Comparison of the nearly equilipophilic compounds **1j** (propafenone analog) and **2d** (benzofuran derivative) shows that in this series of compounds the DSC method can, how-

Table 3. Interaction Strength with Phosphatidylcholine Vesicles (*b* 1/2), Calculated log *P*, and MDR-Modulating Activity of Selected Compounds

compd	*b* 1/2[a]	log *P*	ED_{50}[b]
1h	1.64	3.67	1.65
1i	1.18	2.54	13.56
1j	2.08	4.93	0.16
1n	0.67	0.94	na
2b	1.83	4.07	13.61
2d	1.95	4.71	2.90

[a] *b* 1/2 is represented as the peak width in °C of the main transition peak at a molar DPPC to modifier concentration of 1:0.05. [b] ED_{50} values obtained in Rh^{123} efflux assays are presented.

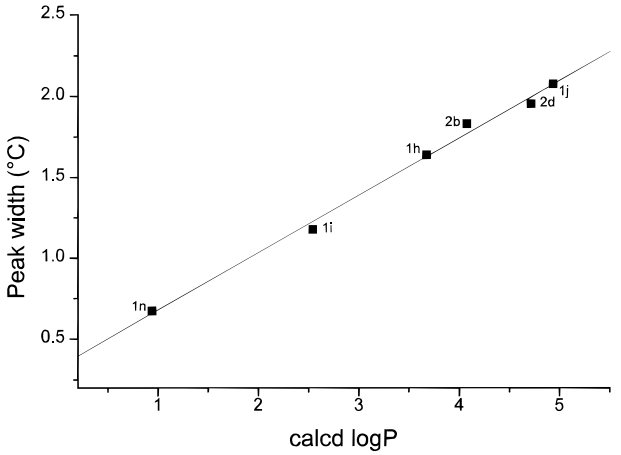


Figure 3. Correlation of interaction strength of selected compounds with phosphatidyl vesicles (expressed as peak width in °C at a molar DPPC to modifier concentration of 1:0.05) vs calculated log *P* values; $r = 0.997$; $n = 6$.

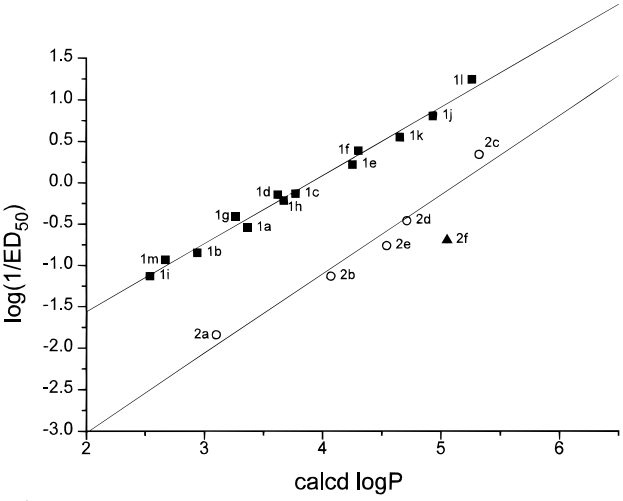


Figure 4. Correlation of MDR-modulating activity (expressed as $\log(1/ED_{50})$ values of modulators determined in Rh^{123} efflux assays) and calculated log *P* values: (■) propafenones **1a**−**n**, (○) benzofurans **2a**−**e**, and (▲) **2f**.

ever, not be used to predict activity for nonhomologous molecules.

Conclusions

A series of benzofurylethanolamine analogs of the class Ic antiarrhythmic agent propafenone was synthesized. These substances were evaluated as modulators of multidrug resistance using both a cytotoxicity assay and rhodamine-123 efflux studies. Lipophilicity of the molecules was calculated and correlated to their chemosensitizing activity. Highly significant correlations were obtained both within the series of propafenone analogs as well as within the series of benzofurans with the regression lines being virtually parallel. Multiple linear regression analysis of combined sets leads to an equation with high predictive power ($r^2_{cross-valid} = 0.968$). Thus, incorporation of the ether oxygen of propafenone into a benzofuran moiety and simultaneous removal of the carbonyl group leads to a remarkable decrease in activity, whereby lipophilicity retains its predictive character. Results obtained for the enantiomers of **2b** and for the deshydroxy analogs **2d**,**e** show that the hydroxyl group does not seem to contribute to PGP interaction. Although it has been previously reported that a carbonyl group near to an aromatic system seems to be important for MDR-modulating activity, oxidation of the hydroxyl group led to a decrease of activity. This suggests that the distance between the carbonyl group and the basic nitrogen seems of utmost importance. Synthesis of desphenyl derivatives of propafenones as well as benzofurans demonstrates that the phenyl ring contributes to pharmacological activity only by influencing lipophilicity. Membrane interaction measurements using NMR spectroscopy show that propafenone analogs as well as benzofurylethanolamines mainly interact within the region of the aryl ether oxygen, whereas the deshydroxy benzofuran derivative **2d** exhibits a strong interaction over the whole (phenylethyl)benzofuryl region. Nevertheless, this difference was not reflected in the MDR-modulating ability of the compounds. DSC measurements showed that the strength of interaction with phosphatidylcholine, the main plasma membrane component, is a good predictor for the lipophilicity of the molecules. Thus, for this set of compounds, calculated lipophilicity of the molecules seems to correspond to their membrane partitioning coefficients. Further membrane interaction studies, employing fluorescein leakage assays and fluorescence spectroscopy, are currently in progress.

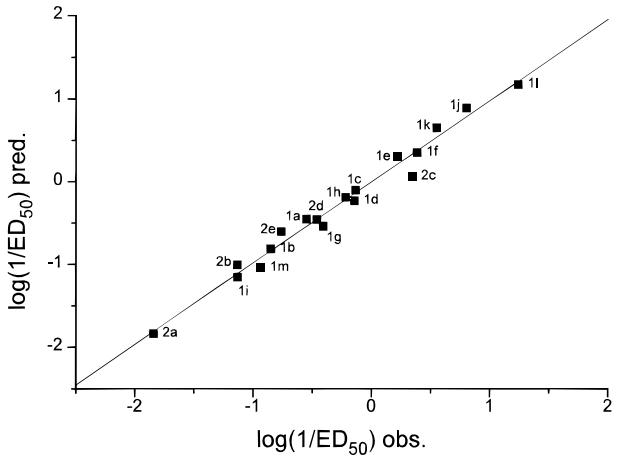


Figure 5. Correlation of predicted vs observed MDR-modulating activity (expressed as $\log(1/ED_{50})$ of modulators in Rh^{123} efflux assays) using the equation: $\log(1/ED_{50}) = 0.86 \log P - 1.16I_{Bf} - 3.33$; $r = 0.990$, $n = 18$.

Experimental Section

Chemistry. Melting points were determined on a Kofler melting point apparatus and are uncorrected. Infrared spectra were recorded as thin films on salt disks on a Perkin Elmer 298 spectrophotometer. Mass spectra were performed on a Shimadzu QP 1000 spectrometer by G. Reznicek (Institut für Pharmakognosie, University of Vienna, Vienna, Austria). GC/

MS spectra were performed by L. Jirovetz (Institut für Pharmazeutische Chemie, University of Vienna, Vienna, Austria) on an HP-5890A GC equipped with an HP-5970 MSD and a 59970 ChemStation data system. NMR spectra were recorded on a Bruker AC 80 spectrometer and a Varian Unity plus 300 system, using tetramethylsilane as internal standard. Microanalyses were done by J. Theiner (Institut für Physikalische Chemie, University of Vienna, Vienna, Austria). Satisfactory C, H, N, and Cl analyses (±0.4%) were obtained for all hydrochlorides.

General Procedure for the Synthesis of the Propafenone Analogs 1f,n. An appropriate epoxide (17.7 mmol)[21] was dissolved in 20−30 mL of amine and refluxed for 6 h. The mixture was evaporated to dryness and the oily residue purified via column chromatography (silica gel, CH_2Cl_2/methanol/concentrated NH_4OH, 200/10/1). Formation of the hydrochlorides was carried out by dissolving the amine in ethyl acetate and adding a 1 M solution of HCl in diethyl ether. The hydrochloride was filtered off and purified via crystallization.

1-[2-[3-(Benzylamino)-2-hydroxypropoxy]phenyl]-3-phenyl-1-propanone (1f): yield 65%; ^{1}H NMR (chloroform-*d*) δ 2.66−3.01 (m, 4H, -CH_2-NH-, -OH), 2.99 (t, 2H, J = 7.8 Hz, -CH_2-Ph), 3.28 (t, 2H, J = 7.8 Hz, -CH_2-CO), 3.67 (d, 1H, J = 13.5 Hz, -N-CH_a-Ph), 3.76 (d, 1H, J = 13.5 Hz, -N-CH_b-Ph), 3.97−4.05 (m, 3H, -O-CH_2-CH(O)-), 6.91 (d, 1H, J = 7.8 Hz, arom H), 6.97 (t, 1H, J = 7.8 Hz, arom H), 7.13−7.31 (m, 10H, arom H), 7.39 (dt, 1H, J = 1.5, 7.8 Hz, arom H), 7.63 (dd, 1H, J = 1.5, 7.8 Hz); ^{13}C NMR (chloroform-*d*) δ 30.12 (PhCH_2-), 44.97 ($COCH_2$-), 51.23, 53.57 (-CH_2-N-CH_2-), 68.03 (CH(OH)), 71.25 (O-CH_2-), 112.97, 125.84, 127.03, 127.99, 128.26, 128.30, 128.34, 130.07, 133.29, 139.67, 141.42, 157.54 (arom C), 201.40 (CO); IR (cm^{-1}) 1670 (CO). Anal. ($C_{25}H_{27}NO_3$) C, H, N.

1f hydrochloride: mp 132−137 °C (ethyl acetate). Anal. ($C_{25}H_{28}NO_3Cl$) C, H, N, Cl.

1-[2-[2-Hydroxy-3-(4-morpholinyl)propoxy]phenyl]-1-propanone (1n): yield 44%; ^{1}H NMR (chloroform-*d*) δ 1.19 (t, 3H, J = 6.7 Hz, -CH_3), 2.33−2.88 (m, 7H, -CH_2-N-$(CH_2)_2$-, -OH), 3.04 (qu, 2H, J = 6.7 Hz, -CH_2CO), 3.76 (t, 4H, J = 4.8 Hz, -CH_2-O-CH_2-), 4.02−4.29 (m, 3H, O-CH_2-CHO)-), 6.93−7.72 (m, 4H, arom H); ^{13}C NMR (chloroform-*d*) δ 8.46 (-CH_3), 36.73 (-CH_2CO), 53.75 (-CH_2-N-), 61.12 (-N-$(CH_2)_2$-), 65.42 (-CH(OH)), 66.89 (-CH_2-O-CH_2-), 71.03 (Ar-O-CH_2-), 112.81, 120.96, 128.67, 130.07, 133.04, 157.45 (arom C), 203.26 (CO); IR (cm^{-1}) 1670 (CO); MS (70 eV) 293 (M$^+$, 1.3), 128 (14), 100 (100). Anal. ($C_{16}H_{23}NO_4$) C, H, N.

1n hydrochloride: mp 155−160 °C (ethyl acetate). Anal ($C_{16}H_{24}NO_4$) C, H, N, Cl.

2-[4-(2-Methoxyphenyl)-1-piperazinyl]-1-[3-(2-phenylethyl)-2-benzofuryl]ethanone (2f). Chloroethanone (**9**) (0.5 g, 1.7 mmol)[26] was dissolved in 10 mL of toluene, and 0.64 g of 1-(2-methoxyphenyl)piperazine (3.4 mmol) was added. The reaction mixture was stirred for 5 h at 60 °C, filtered, and evaporated to dryness. Crystallization with 2-propanol gave 0.53 g (70%) of **2f** as colorless crystals: mp 138−140 °C; ^{1}H NMR (chloroform-*d*) δ 2.82−2.93 (m, 4H, -N-$(CH_2)_2$-), 2.97 (t, 2H, J = 6.5 Hz, Ph-CH_2), 3.16−3.27 (m, 4H, -$(CH_2)_2$-N-), 3.39 (t, 2H, J = 6.5 Hz, -CH_2-Bf), 3.87 (s, 3H, -OCH_3), 3.98 ($COCH_2$-), 6.86−7.56 (m, 13H, arom H); ^{13}C NMR (chloroform-*d*) δ 26.45, 30.90 (-CH_2-CH_2-), 50.51, 53.94 (piperazine CH_2), 55.34 (-OCH_3), 64.49 ($COCH_2$-), 111.13, 112.15, 118.30, 121.01, 121.51, 122.93, 123.31, 126.04, 128.22, 128.32, 128.41, 128.56, 128.93, 141.33, 147.18, 152.28, 153.95 (arom C), 189.20 (CO); IR (cm^{-1}) 1690 (CO); MS (70 eV) 454 (M$^+$, 17), 205 (100), 190 (23), 70 (45). Anal. ($C_{29}H_{30}N_2O_3$) C, H, N.

2f hydrochloride: mp 197−198 °C (ethanol). Anal. ($C_{29}H_{31}N_2O_3Cl$) C, H, N, Cl.

α-[[4-(2-Methoxyphenyl)-1-piperazinyl]methyl]-3-(2-phenylethyl)-2-benzofuranmethanol (2c). Ethanone **2f** (0.5 g, 1.1 mmol) was dissolved in 10 mL of methanol, and 0.05 g of $NaBH_4$ was added. The reaction mixture was diluted with water and extracted twice with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4, and evaporated to dryness. Crystallization from cyclohexane gave 0.46 g (93%) of **2c**: mp 99−100 °C; ^{1}H NMR (chloroform-*d*) δ 2.07 (d, 1H, J = 12 Hz, -OH), 2.54−3.19 (m, 14H, Ph-CH_2-CH_2-, -CH_2-N-, piperazine CH_2), 3.88 (s, 3H, -OCH_3), 4.65 (m, 1H, -CH(O)), 6.85−7.55 (m, 13H, arom H); ^{13}C NMR (chloroform-*d*) δ 26.89, 35.88 (Ph-CH_2-CH_2-), 50.74, 53.03 (piperazine CH_2), 55.35 (-OCH_3), 61.00 (-CH_2-N-), 61.14 (-CH(OH)), 111.17, 111.35, 116.78, 118.22, 119.46, 120.99, 122.28, 123.07, 124.30, 126.19, 128.41, 128.65, 128.79, 141.08, 141.40, 151.53, 152.22, 154.29 (arom C); MS (70 eV) 456 (M$^+$, 0.2), 205 (100), 190 (18), 70 (71). Anal. ($C_{29}H_{32}N_2O_3$) C, H, N.

2c hydrochloride: mp 167−170 °C. Anal. ($C_{29}H_{34}N_2O_3Cl_2$) C, H, N, Cl.

Pharmacology. Cell Lines and Culture Conditions. The CCRF-CEM T-lymphoblast cell line as well as the resistant line were obtained as described previously.[25] 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 the continuous presence of 1000 ng/mL vincristine. The selecting agent was washed out at least 1 week prior to the experiments. PGP expression was shown to be stable for at least 1 month after washout of the selective agent as shown by flow cytometry using the MRK16 antibody (Behring Institut GesmbH, Vienna, Austria) and by cytotoxicity and efflux experiments (data not shown). The cell line used in our studies was selected in the presence of increasing doses of vincristine without prior mutagenization. This cell line has been chosen on the basis of distinct PGP expression and does not show the mutation at codon 185. In addition, no significant contribution of other factors to MDR could be observed (V. Gekeler, unpublished data).

MTT Assay. The assay is dependent on the cellular reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Chemical Co., St. Louis, MO) in mitochondria of viable cells to water insoluble formazan. The assays were performed in 96-well plates essentially as described by Mosmann,[36] with the exception that water insoluble formazan granules were dissolved in 2-propanol containing 0.04 N HCl. Absorbance was read spectrophotometrically using an EL311 Biotek microtiter plate reader (Biotek Instruments Inc., Highland Park, VT). Data points for different modulator concentrations are corrected for modulator toxicity, which generally did not exceed 20% at 10 μM.

Rhodamine Efflux Studies. Rhodamine efflux studies were performed in a modification of published methods.[31] Cells were pelleted, the supernatant was removed by suction, and the cells were resuspended at a density of 1 × 10^6/mL in RPMI1640 medium containing rhodamine-123 (Sigma Chemical Co., St. Louis, MO) at a final concentration of 0.2 μg/mL. Cell suspensions were incubated at 37 °C for 15 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 ranging from 16 nM to 500 μM, depending on solubility and expected potency of the modifier. Eight concentrations (serial dilution 1:2.5) were tested for each modulator. After 30, 60, 90, and 120 s 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); 0 time points were done by immediately pipetting Rh^{123}-preloaded cells into ice cold stop solution. Parental CCRF-CEM cells were used as controls for simple plasma membrane diffusion, whereby initial Rh^{123} 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 1 h on a Becton Dickinson facscalibur flow cytometer (Becton Dickinson, Vienna, Austria). Viable cells were gated on the basis of forward and side scatter; 5000 gated events were accumulated for the determination of mean fluorescence values. Time dependent decrease in mean fluorescence values was linear over time for at least 2 min and is expressed as the percentage of 0 time points, to allow comparison of independent experiments.

Membrane Interaction Measurements. 1. NMR Measurements. Preparation of Liposomes for NMR Experiments. BBPS was used for liposome preparation. Samples

containing 0.01 mg/μL of D_2O were sonicated using a Branson B-12 sonifier (Branson Sonic Power Co., Damburg, CT) three times for 30 s at 40 W. This led to a desired increase in temperature to 35 °C. After centrifugation the liposome preparation was allowed to equilibrate for 24 h at room temperature. This stock solution was diluted for the interaction studies as indicated.

NMR Measurements. Drugs were dissolved in D_2O or phosphate buffer (pH 5.0). The final pH was adjusted to pH 5.0. The drug concentration was 4 mM and stayed constant for the time of the experiment. A chemical shift of the resonance signals and changes in pH were not observed. Liposomes were added in 3−5 μL portions to 500 μL of solution. Acetone served as the standard to control field homogeneity. The experiments were performed with an AM 360L spectrometer (Bruker, Darmstadt, Germany) at a probe temperature of 23 °C. Data acquisition included 32 scans, 32K fid, sweep width at 4098 Hz, and homonuclear presaturation to depress the H_2O signal; peak broadening (change in $1/T_2$) at half-peak height as a function of liposome concentration was determined from the NMR spectra. For calculation of peak half-widths, a curve-fitting program was employed (M. Wiese, Borstel, Germany). This program allows to fit mixed-type Gaussian and Lorentzian curve shapes.

2. DSC. Preparation of Liposomes for DSC Experiments. Liposome suspensions were prepared in phosphate buffer (1/15 M, pH 7.4) using conditions that give multilamellar vesicles.[37] Briefly, DPPC/drug mixtures were prepared by mixing appropriate amounts of the drug dissolved in either methanol or chloroform/methanol (2:1, v/v) and DPPC dissolved in chloroform. All solvents were used in sufficient quantities to ensure complete dissolution. The solvents were evaporated under argon at 30 °C, and the samples were placed in a vacuum desiccator overnight at 4 °C. Phosphate buffer was added to dried samples, and the samples were vortexed for 2 h at 60 °C. In all preparations the incubation temperature was higher than the main phase transition temperature of DPPC (about 42 °C). The vortex intensity used was 1200−1300 min^{-1}. The lipid concentration was 5 mg/mL in all samples, and changes in pH were not observed. The experiments were done at lipid:drug molar ratios from 1:0 (control) to 1:0.1 in most cases.

DSC Measurements. All measurements were performed with a highly sensitive differential scanning microcalorimeter (Setaram, France), equipped with a digital interface and a data acquisition allowing automatic data collection; 200 μL aliquots of the liposome suspension and of the reference solution (phosphate buffer) were added to the relevant pans and heated at a rate of 0.5 °C/min. The sensitivity range setting was 50 μV. Temperature intervals varied from 0 to 70 °C for different phospholipids and phospholipid/drug mixtures. Every experiment was performed in duplicate, and two runs were recorded for selected samples to ensure that the calorimetric response of the system was stable. Peaks were analyzed by a nonlinear least-squares fit using a Gaussian function. The peak width of a molar DPPC to modifier concentration of 1:0.05 was taken as a descriptor of interaction strength.

Calculation of Lipophilicity. The log *P* values were calculated according to the method of Ghose and Crippen[34] using the software package MOLGEN.[35] Molecules were generated using the builder function and energetically minimized using the optimization function. Conformationally independent lipophilicity values were calculated.

Supporting Information Available: NMR spectra of **1a** and **2b** in the absence and presence of lecithin liposomes (1 page). Ordering information is given on any current masthead page.

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