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In silico screening with benzofurane- and benzopyrane-type MDR-modulators

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Abstract

Development of inhibitors of the drug efflux pump P-glycoprotein is a versatile approach to overcome multi drug resistance (MDR) in tumor therapy. In an approach to lower the conformational flexibility of the lead compound propafenone, we synthesized a set of dihydrobenzofuranes and benzopyranones. In the case of the 4 diastereomeric dihydrobenzofuranes, no significant differences in activity regarding the configuration on the side-chains at the dihydrofurane moiety (*cis* or *trans*) was observed. This may be due to the high flexibility of the side-chains, which still allow mutually overlap of pharmacophores. The benzopyranones showed a good correlation between lipophilicity and activity with gnerally lower log potency/log *P* ratios. This decrease may be due to the rigidization of the molecules. In an in silico screening approach, a set of diverse propafenone-type compounds was used to establish a pharmacophore model, which was used to screen the world drug index. Among the hits retrieved there are several compounds, which were previously described as MDR-modulators. This demonstrates the validity of the model.

Keywords: Multidrug resistance; P-glycoprotein inhibitors; Structure-activity relationship; Benzopyranone; In silico screening approach

1. Introduction

Development of multi-drug resistance (MDR) is one of the major reasons for weak responses in tumor therapy and in the treatment of bacterial and fungal infections. One of the mechanisms for the development of multiple drug resistance is the overexpression of plasma membrane associated transport proteins that efflux therapeutically administered xenotoxins and thereby prevent the substances from reaching their intracellular targets [1]. In cancer cells, broad-spectrum resistance to chemotherapeutic agents is mediated by ATP-driven drug transporters such as P-glycoprotein (Pgp) [2]. Pgp is encoded by the *mdr1* gene and transports a broad range of structurally and functionally diverse drugs, such as anthracyclines, vinca alkaloids, epipodophyllotoxins, colchicine and even taxol [3]. Inhibition of Pgp leads to resensitization of multi-drug resistant tumor cells in vitro and was thus considered a promising approach for treatment of multi-drug resistant tumors [4]. Within the past decade, several classes of compounds were identified as inhibitors of Pgp. Among them are, e.g. verapamil, phenothioazines, dihydropyrines, amiodarone, propafenone, steroids, acridonecarboxamides and cyclosporine and the non-immunosuppressive derivative valspodar [5]. Currently, several compounds are in clinical phase III studies [6]. However, most of the compounds tested in a clinical setting gave disappointing results due to severe side effects [7]. These side effects occur because of the inherent pharmacological effects of the modulators, and include cardiac effects, immunosuppression and nephrotoxicity. Additionally, Pgp is important for a proper function of the blood brain barrier and *mdr1* double knock-out mice were shown to accumulate a lot of drugs in the brain, which, under normal conditions, are not able to pass the blood brain barrier [8]. Thus, there is urgent need for specifically designed modulators with higher activity and tissue selectivity.

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Using a series of propafenone analogs (**1**), we identified both predictive physicochemical parameters and pharmacophoric substructures [9]. Despite the advantages of propafenone as lead structure (several pharmacophoric substructures as defined by Klopman [10], easy to synthesize, broad range of structural variability), the high flexibility of the molecules is of major disadvantage when performing 3D-QSAR studies. In this case, rigidization of the molecules is the strategy of choice. Previously performed synthesis of a series of benzofuranes (**2**) showed, that the major structure–activity relationship pattern remains unchanged, although the benzofuranes generally showed lower activity/lipophilicity ratios [11]. Synthesis of the dihydrobenzofuranes (**3**) would lead to compounds with additional two centers of chirality, thus leading to stereoisomeres with remarkable differences in the relative positioning of their pharmacophoric groups (nitrogen and phenyl). Within this paper we describe the synthesis and pharmacological activity of dihydrobenzofurane- and benzopyranone-analogues as well as the use of these compounds as templates for in silico screening of the world drug index (Fig. 1).

2. Chemistry

Dihydrobenzofuranes (**3**) were synthesized as described previously [12] using a modification of the procedure used for synthesis of benzofuranes (**2**). The reaction sequence includes alkylation of 1-(2-hydroxyphenyl)-3-phenyl-1-propanone (**4**) with epichlorohydrine, nucleophilic ring opening by addition of HCl, oxidation of the secondary alcohol with DCC–DMSO to yield **5**, and cyclization with silica gel to yield **6**. Application of the system *tert*-butylamine-borane in the presence of aluminium chloride on the reduction of **6** gave chlorohydrine **7** as main product. A mixture of the four possible racemic diastereomeres of **7a**–**d** was obtained in the reaction, which could be separated by a combination of flash chromatography and medium pressure column chromatography. Reaction of **7** with sodium methoxide gave the distereomeric epoxides **8a**–**d**, which were converted to the desired aminoethanols **3a**–**d** using piperidine in methanol (Scheme 1).

To further investigate the influence of rigidization, also several benzopyranones (**13**) were synthesized. For synthesis of benzopyranones, reduction of chromone-2-carboxylic acid ester (**9**) to the diol **10**, selective

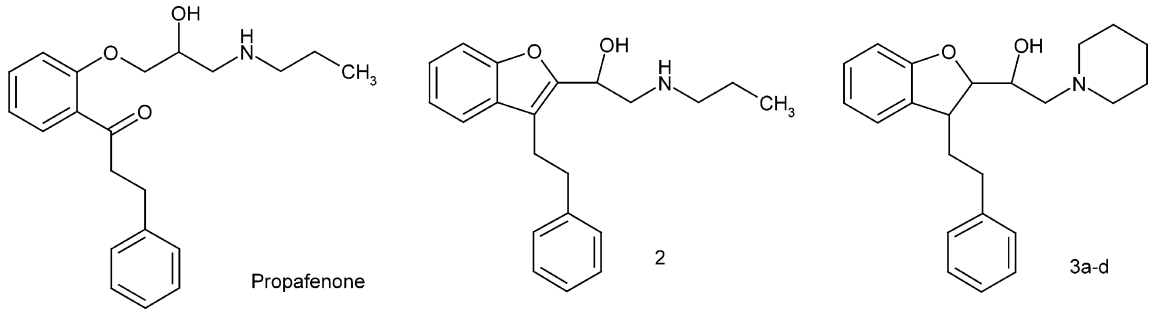
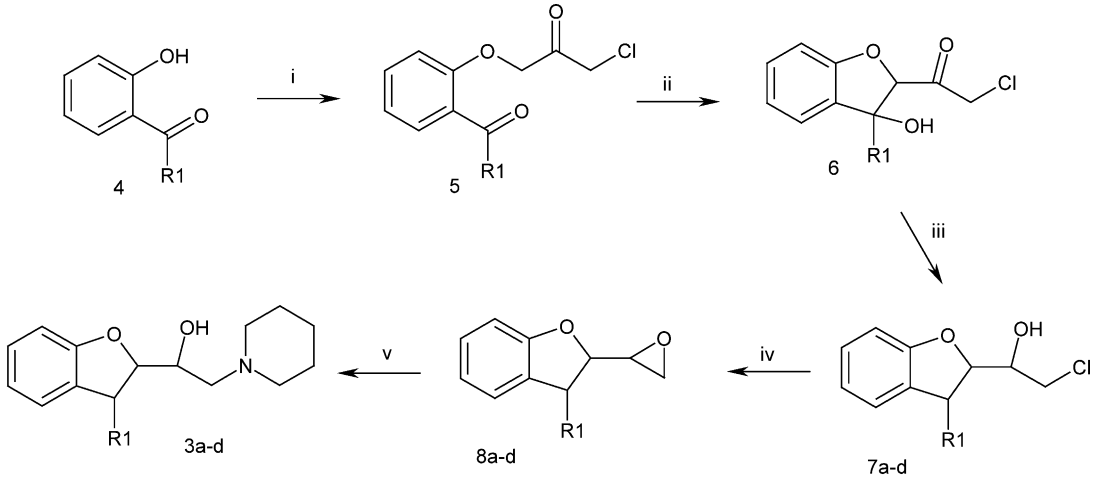
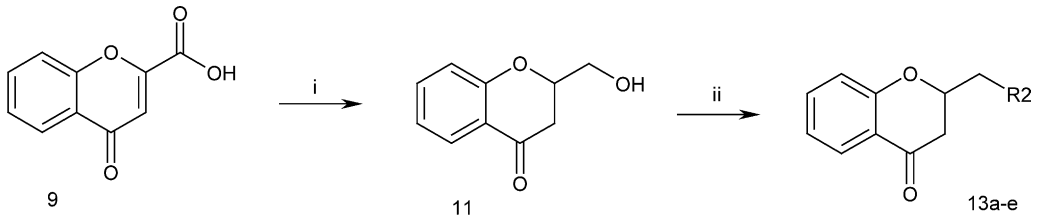


Fig. 1. Chemical structure of propafenones, benzofuranes and dihydrobenzofuranes.



Scheme 1. Synthesis of dihydrobenzofuranes **3a**–**d**; (i): epichlorohydrine, HCl, DCC–DMSO; (ii) silica gel; (iii) *t*-butylamine–borane, $AlCl_3$; (iv) NaOMe; (v) amine.



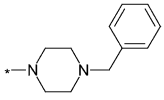
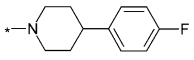
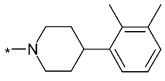
Scheme 2. Synthesis of benzopyranones **13a**–**e**; (i) $SOCl_2$–MeOH, $NaBH_4$; (ii) PCC; (iii) MesCl, amine.

oxidation of the benzylic hydroxy group to **11**, mesylation and reaction with the desired amines gave the target compounds **13a**–**e** (Scheme 2). This procedure for synthesis of the hydroxymethyl-benzopyranone (**11**) gave better yields than the previously published rout via selective reduction of the ester group followed by catalytic hydrogenation of the double bond [13]. Chemical structures, calculated log *P* values and pharmacological activity are shown in Table 1

3. Biological evaluation

Pharmacological activity of the compounds was determined in a zero trans efflux protocol, using daunomycin as the fluorochrome. Briefly, cells were loaded with the fluorescent toxin daunomycin and the time dependent decrease in mean cellular fluorescence was measured in presence of various concentrations of modulator. The first order rate constants ($V_{max.}/K_m$) were calculated by non linear regression analysis and the EC_{50} values of modulators were calculated from dose–response curves of efflux rate ($V_{max.}/K_m$) versus modifier concentration. Thus, the effect of different modulators on the transport rate of Pgp was measured in a direct functional assay.

Table 1
Chemical structure, calculated log *P* values and MDR-modulating activity of compounds **13a**–**e**

nr.	R2	logP	EC_{50} (μM)
13a		0.67	1251.1
13b		1.80	176.3
13c		2.55	8.86
13d		3.05	17.72
13e		3.87	2.06

4. Computational methods

4.1. Calculation of log P values

The log *P* values were calculated using the software package CHEMOFFICE (option: best method). Calculated log *P* values are given in Table 1.

4.2. Pharmacophoric feature modeling

3D structures of the compounds were built interactively using CATALYST version 4.0. The number of conformers generated using the 'best' feature of the program for each substrate was limited within the program to a maximum of 255 with an energy range of 15.00 kcal/mol beyond the calculated potential energy minimum. Ten hypothesis were generated using these conformer structures for the molecules in the training set and the EC_{50} values after selection of the following features: hydrogen bond acceptor (HBA), hydrophobic (H), aromatic hydrophobic (HA), and positive ionizable (PI).

5. Experimental

5.1. Synthesis of compounds

Melting points were determined on a Kofler melting point apparatus and are uncorrected. Infrared spectra were recorded as KBr pellets on a Perkin–Elmer Paragon 1000 spectrophotometer. NMR spectra were recorded on a Bruker Avance 200 and a Varian Unity plus 300 system, using tetramethylsilane as internal standard. Microanalyses were done by J. Theiner (Institute of Physical Chemistry, University of Vienna, Vienna, Austria). The values found for C, H, and N

were ±0.4% of the theoretical ones. Dihydrobenzofuranes **3a**–**d** were prepared as described previously.

*5.1.1. 2,3-Dihydro-2-hydroxymethyl-4H-1-benzopyran-4-ol (**10**)*

To a solution of 4-oxo-4H-1-benzopyran-2-carboxylic acid methyl ester (16.65 mmol) in methanol $NaBH_4$ (3 g) was added. After the reaction was finished (TLC control) water was added and the solution was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated to dryness. Column chromatography (silica gel, MeOH–CH_2Cl_2) gave 2.51 g (84%) **10** as colorless oil, which solidifies slowly. M.p.: 102–104 °C; ^{1}H NMR (MeOD–$CDCl_3$, ppm): δ 1.80 (ddd, 1H, $J = 1.9/6.3/12.8$ Hz, H_a-3), 2.23 (dt, 1H, $J = 10.9/12.8$ Hz, H_b-3), 3.73 (d, 2H, $J = 5.0$ Hz, CH_2-OH), 4.16 (m, 1H, H-2), 4.75–4.95 (m, 3H, CH–OH, –OH), 6.76 (d, 1H, $J = 8.3$ Hz, H-8), 6.88 (t, 1H, $J = 7.5$ Hz, H-6), 7.10 (dd, 1H $J = 7.5/8.3$ Hz, H-7), 7.43 (d, 1H, $J = 7.5$ Hz, H-5); ^{13}C NMR (MeOD–$CDCl_3$, ppm): δ 34.81 (CH_2), 65.58 (CH), 65.67 (CH_2), 76.84 (CH), 117.17 (CH), 121.36 (CH), 127.46 (C), 128.16 (CH), 129.46 (CH), 155.52 (C); MS *m/e* 180 (M^+, 31), 149 (26), 131 (30), 121 (100), 77 (39).

*5.1.2. 2,3-Dihydro-2-hydroxymethyl-4H-1-benzopyran-4-on (**11**)*

To a solution of **10** (10.9 mmol) in CH_2Cl_2 2.35 g pyridiniumchlorochromat was added and the reaction mixture was stirred for 3 h at room temperature. The solvent was evaporated and the resulting oil was purified via column chromatography (silica gel, CH_2Cl_2–MeOH) to give 1.4 g (77%) of **11** as colorless oil. ^{1}H NMR ($CDCl_3$, ppm): δ 2.0–2.2 (br, 1H, OH), 2.63 (dd, 1H, $J = 3.0/16.8$ Hz, H_a-3), 2.95 (dd, 1H, $J = 13.3/16.8$ Hz, H_b-3), 3.84 (dd, 1H, $J = 5.0/11.5$ Hz, CH_a–OH), 4.00 (d, 1H, $J = 11.5$ Hz, CH_b–OH), 4.58 (m, 1H, H-2), 7.00 (d, 1H, $J = 8.0$ Hz, H-8), 7.04 (t, 1H, $J = 7.8$ Hz, H-6), 7.50 (dd, 1H, $J = 7.8/8.0$ Hz, H-7), 7.90 (d, 1H, $J =$ 7.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, ppm) δ 39.01 (CH_2), 64.47 (CH_2), 78.15 (CH), 117.81 (CH), 120.83 (C), 121.62 (CH), 127.01 (CH), 136.13 (CH), 161.07 (C), 191.97 (CO); MS: *m/e* 178 (M^+, 33), 147 (100).

*5.1.3. 2,3-Dihydro-2-methansulfonyloxymethyl-4H-1-benzopyran-4-on (**12**)*

A solution of 0.95 g (5.3 mmol) of **11** in CH_2Cl_2 was cooled in an ice bath and 1 ml triethylamine and 0.45 ml mesylchloride were added. After stirring for 12 h the mixture is evaporated and the resulting oil is purified via column chromatography (silica gel, CH_2Cl_2) to yield 1.23 g (90%) of **12**. ^{1}H NMR ($CDCl_3$, ppm): δ 2.72 (dd, 1H, $J = 3.5/16.8$ Hz, H_a-3), 2.91 (dd, 1H, $J = 12.6/16.8$ Hz, H_b-3), 3.13 (s, 3H, $-CH_3$), 4.47 (dd, 1H, $J = 5.1/11.3$ Hz, H_a-Omes), 4.55 (dd, 1H, $J = 3.5/11.5$ Hz, H_b-Omes), 4.76 (m, 1H, H-2), 7.02 (d, 1H, $J = 8.3$ Hz, H-8), 7.07 (t, 1H, $J = 7.8$ Hz, H-6), 7.52 (dd, 1H, $J = 7.8/8.3$ Hz, H-7), 7.91 (d, 1H, $J = 7.8$ Hz, H-5); ^{13}C NMR ($CDCl_3$, ppm): δ 37.80 (CH_3), 38.77 (CH_2), 69.53 (CH_2), 74.94 (CH), 117.80 (CH), 120.66 (C), 122.07 (CH), 127.04 (CH), 136.34 (CH), 160.44 (C), 190.26 (CO); MS: *m/e* 256 (M^+, 20), 147 (100). *Anal*. ($C_{11}H_{12}SO_5$) C, H, S.

*5.1.4. 2-(4-Morpholinylmethyl)-4H-1-benzopyran-4-on (**13a**)*

To a solution of 0.8 g (3.1 mmol) **12** in acetonitril 3 ml morpholin are added and the reaction mixture is heated to reflux for 3 days. Evaporation of the solvent and purification of the remaining oil via column chromatography (silica gel, petroleum ether–ethylacetate) gave 0.49 g (63%) **13a** as yellowish oil. ^{1}H NMR ($CDCl_3$, ppm): δ 2.35–2.72 (m, 8H, H-3, $-CH_2-N(CH_2)_2$), 3.45–3.58 (m, 4H, CH_2-O-CH_2), 4.40–4.53 (m, 1H, H-2), 6.80 (d, 1H, $J = 8.0$ Hz, H-8), 6.82 (t, 1H, $J = 8.3$ Hz, H-6), 7.28 (dd, 1H, $J = 8.0/8.3$ Hz, H-7), 7.67 (d, 1H, $J = 8.3$ Hz); ^{13}C NMR ($CDCl_3$, ppm): δ 40.86 (CH_2), 53.96 (CH_2), 61.58 (CH_2), 66.42 (CH_2), 75.74 (CH), 117.58 (CH), 120.68 (C), 120.97 (CH), 126.46 (CH), 135.53 (CH), 160.84 (C), 191.68 (CO); MS: *m/e* 247 (M^+, 0.7), 100 (100); *Anal*. ($C_{14}H_{17}NO_3$) C, H, N.

*5.1.5. 2-(1-Piperidinylmethyl)-4H-1-benzopyran-4-on (**13b**)*

To a solution of 0.6 g (2.34 mmol) **12** in acetonitril 3 ml piperidine are added and the reaction mixture is heated under reflux till the reaction is completed (TLC). The solvent is evaporated and the resulting oil is purified via column chromatography (silica gel, CH_2Cl_2–MeOH) to yield 0.22 g (39%) of **13b** as colorless oil. ^{1}H NMR ($CDCl_3$, ppm): δ 1.33–1.64 (m, 6H, $CH_2-CH_2-CH_2$), 2.43–2.54 (m, 4H, $-N(CH_2)_2$), 2.61 (dd, 1H, $J = 5.0/13.3$ Hz, H_a-3), 2.77 (d, 2H, $J = 7.3$ Hz, $-CH_2-N$), 2.80 (dd, 1H, $J = 6.0/13.3$ Hz, H_b-3), 4.55–4.70 (m, 1H, H-2), 6.98 (d, 1H, $J = 7.3$ Hz, H-8), 7.01 (t, 1H, $J = 8.0$ Hz, H-6), 7.46 (dd, 1H, $J = 7.3/8.0$ Hz, H-7), 7.87 (d, 1H, $J = 8.0$ Hz, H-5); ^{13}C NMR ($CDCl_3$, ppm): δ 24.08 (CH_2), 25.94 (CH_2), 41.55 (CH_2), 55.33 (CH_2), 62.45 (CH_2), 76.31 (CH), 117.99 (CH), 121.09 (C), 121.19 (CH), 126.84 (CH), 135.84 (CH), 161.40 (C), 192.50 (CO); MS: *m/e* 245 (M^+, 1.5), 98 (100). *Anal.* ($C_{15}H_{19}NO_2$) C, H, N.

*5.1.6. 2-(4-Benzyl-1-piperazinylmethyl)-4H-1-benzopyran-4-on (**13c**)*

A solution of 0.95 g *N*-benzylpiperazine in 2 ml of acetonitrile is added dropwise to a solution of 0.66 g (2.57 mmol) **12** in acetonitrile. The reaction mixture is heated under reflux for 20 h, evaporated to dryness and the resulting oil is purified via column chromatography (silica gel, CH_2Cl_2–MeOH) to give 0.4 g (46%) **13c** as yellowish oil. ^{1}H NMR ($CDCl_3$, ppm): δ 2.32–2.83 (m,

12 H, H-3, CH_2–N, piperazine CH_2), 3.46 (s, 2H, benzyl CH_2), 4.48–4.64 (m, 1H, H-2), 6.96 (d, 1H, $J = 7.5$ Hz, H-8), 7.00 (t, 1H, $J = 8.1$ Hz, H-6), 7.15–7.47 (m, 6H, phenyl H, H-7), 7.83 (d, 1H, $J = 8.2$ Hz, H-5); ^{13}C NMR ($CDCl_3$, ppm): δ 41.38 (CH_2), 52.95 (CH_2), 53.84 (CH_2), 61.53 (CH_2), 62.96 (CH_2), 76.19 (CH), 117.93 (CH), 121.02 (C), 121.26 (CH), 126.82 (CH), 126.98 (CH), 128.14 (CH), 129.15 (CH), 135.87 (CH), 137.94 (C), 161.25 (C), 192.32 (CO); MS: *m/e* 336 (M^+, 3.9), 189 (100). *Anal*. ($C_{21}H_{24}N_2O_2$) C, H, N.

5.1.7. 2-(4-(4-Fluorphenyl)-1-piperazinylmethyl)-4H-1-benzopyran-4-on (**13d**)

To a solution of 0.52 g (2 mmol) **12** in acetonitrile 0.8 g *N*-(4-fluorphenyl)-piperazin were added and the reaction mixture was heated to reflux for 20 h. The mixture was evaporated to dryness and the resulting oil was purified via column chromatography (silica gel, CH_2Cl_2–MeOH) to yield 0.57 g (84%) **13d** as yellowish oil. ^{1}H NMR ($CDCl_3$, ppm): δ 2.62–2.86 (m, 8H, H-3, –CH_2–N(CH_2)$_2$), 2.98–3.12 (m, 4H, (CH_2)$_2$N–Ph), 4.52–4.67 (m, 1H, H-2), 6.75–6.98 (m, 7H, phenyl H, H-6, H-8),), 7.41 (dd, 1H, $J = 7.5/8.2$ Hz, H-7), 7.83 (d, 1H, $J = 8.2$ Hz, H-5); ^{13}C NMR ($CDCl_3$, ppm): δ 41.22 (CH_2), 49.96 (CH_2), 53.80 (CH_2), 61.34 (CH_2), 76.14 (CH), 115.13 (CH), 115.57 (CH), 117.57 (CH), 117.71 (CH), 117.85 (CH), 120.95 (C), 121.26 (CH), 126.76 (CH), 135.83 (CH), 147.73 (C), 154.60 (C), 159.34 (C), 161.13 (C), 192.09 (CO); MS: *m/e* 340 (M^+, 1.3), 109 (20), 95 (39), 56 (100). *Anal*. ($C_{20}H_{21}FN_2O_2$) C, H, N.

5.1.8. 2-(4-(2,3-Dimethylphenyl)-1-piperazinylmethyl)-4H-1-benzopyran-4-on (**13e**)

To a solution of 0.54 g (2.1 mmol) **12** in 10 ml acetonitrile 0.8 g *N*-(2,3-dimethylphenyl)-piperazin were added. The reaction mixture was heated under reflux for 20 h, evaporated to dryness and the resulting oil was purified via column chromatography (silica gel, CH_2Cl_2–MeOH) to give 0.5 g (68%) **13e** as yellow oil. ^{1}H NMR ($CDCl_3$, ppm): δ 2.23 (s, 3H, –CH_3), 2.26 (s, 3H, –CH_3), 2.67–2.92 (m, 12H, H-3, CH_2–N, piperazin H), 4.58–4.71 (m, 1H, H-2), 6.86–7.11 (m, 5H, phenyl H, H-6, H-8), 7.44 (dd, 1H, $J = 7.8/8.1$ Hz, H-7), 7.89 (d, 1H, $J = 8.1$ Hz, H-5); ^{13}C NMR ($CDCl_3$, ppm): δ 13.73 (CH_3), 20.39 (CH_3), 41.14 (CH_2), 51.83 (CH_2), 54.22 (CH_2), 61.42 (CH_2), 76.05 (CH), 116.33 (CH), 117.74 (CH), 120.86 (C), 121.05 (CH), 124.71 (CH), 125.60 (CH), 126.63 (CH), 130.85 (C), 135.62 (CH), 137.57 (C), 151.21 (C), 161.05 (C), 191.90 (CO); MS: *m/e* 350 (M^+, 7.6), 203 (100). *Anal*. ($C_{22}H_{26}N_2O_2 \cdot 1/3\ H_2O$) C, H, N.

5.2. MDR-modulating activity

The human T-lymphoblast cell line CCRF-CEM and the multidrug resistant lines CCRF VCR1000 and CCRF adr5000 were provided by V. Gekeler (Byk Gulden, Konstanz, Germany). The resistant lines were obtained by stepwise selection in vincristine or daunorubicin containing medium [14]. Cells were kept under standard culture conditions (RPMI1640 medium supplemented with 10% fetal calf serum). The PGP-expressing resistant cell line was cultured in presence of 1000 ng/ml vincristine or 5000 ng/ml daunorubicin. One week prior to the experiments cells were transferred into medium without selective agents or antibiotics.

Daunorubicin efflux studies were performed as described [15]. Briefly, cells were pelleted, the supernatant was removed by aspiration and cells were resuspended at a density of 1×10^6 per ml in PRMI1640 medium containing 3 μmol/l daunomycin. Cell suspensions were incubated at 37 °C for 30 min. After this time a steady state of daunorubicin accumulation was reached. Tubes were chilled on ice and cells were pelleted at $500 \times g$. Cells were washed once in RPMI1640 medium to remove extracellular daunorubicin. Subsequently, cells were resuspended in medium prewarmed to 37 °C, containing either no modulator or chemosensitizer at various concentrations ranging from 3 nM to 500 μM, depending on solubility and expected potency of the modifier. Generally, eight serial dilutions were tested for each modulator. After 1–4 min aliquots of the incubation mixture were drawn and pipetted into four volumes of ice cold stop solution (RPMI1640 medium containing verapamil at a final concentration of 100 μM). Parental CCRF-CEM cells were used to compensate for simple membrane diffusion, which was less than 3% of the efflux rates observed in resistant cells. Samples drawn at the respective time points were kept in an ice water bath and measured within an hour on a Becton Dickinson FACSCalibur (Becton Dickinson, Heidelberg, Germany) flow cytometer as described. Dose–response curves were fitted to the data points using non-linear least squares and EC_{50} values were calculated as described (Chiba et al., 1996). EC_{50} values of individual compounds are given in Table 2 and represent the average of at least triplicate determinations. The coefficient of variation was generally below 20%.

Table 2
Elemental analyses for compounds **12** and **13a**–**e**

	Calcd.				Found			
	C	H	N	S	C	H	N	S
12	51.55	4.72		12.51	51.74	4.80		12.37
13a	68.00	6.93	5.66		67.75	7.18	5.41	
13b	73.44	7.81	5.71		73.23	7.53	5.87	
13c	74.97	7.19	8.33		75.24	7.25	8.24	
13d	70.57	6.22	8.23		70.29	6.13	7.98	
13e	74.33	7.54	7.66		74.13	7.54	7.86	

6. Structure–activity relationship studies

Using a series of propafenone analogs, we identified both predictive physicochemical parameters and pharmacophoric substructures. Due to the vacuum cleaner model proposed by Gottesman, which assumes the interaction site within the membrane, lipophilicity plays a major role in most QSAR models we obtained. Thus, both within series of propafenones and structurally analogous benzofuranes, excellent correlations between lipophilicity of the molecules and MDR-modulating activity were found, whereby benzofuranes generally showed lower activity values than equilipophilic propafenone derivatives. This may be either due to the lack of a carbonyl group (which was in propafenones shown to act as H-bond acceptor) or reflect the influence of conformational rigidization of the molecules. To further clarify this question, we synthesized and tested both a set of dihydrobenzofuranes and a series of benzopyranones, which show a carbonyl group and are conformationally restricted. Synthesis of dihydrobenzofuranes **3a**–**d** gave a set of four diastereomeric racemates. Pharmacological testing of the compounds showed, that the differences in activity between the four diastereoisomeres are quite low. The two *cis*-configurated racemates showed EC_{50} values of 0.65 and 2.10 μM, respectively, whereas the two *trans*-isomers were in between with their activity (1.14 and 1.73 μM). However, conformational studies showed, that the two sidechains are very flexible allowing mutually overlap of the pharmacophoric sub structures (nitrogen atom and phenyl ring) in all configurations.

Comparison of the pharmacological activity of the dihydrobenzofuranes with those of equilipophilic propafenones display a decrease by approximately a factor of 10 for **3a**–**d** (Fig. 2). This decrease is in the same range as those observed for structurally analogous benzofuranes. An almost identical pattern is shown for the benzopyranones. Fig. 2 shows the correlation of calculated lipophilicity values and the chemosensitizing activity for both benzopyranones **13a**–**e** and a series of propafenones. Also within the series of benzopyranones, a good correlation between lipophilicity and MDR-modulating activity exists, whereby the benzopyranones generally show lower activity than equilipophilic propafenone analogs. The decrease of activity is quite in the same range as those for benzofuranes and dihydrobenzofuranes and may thus indeed be due to the rigidization of the molecules.

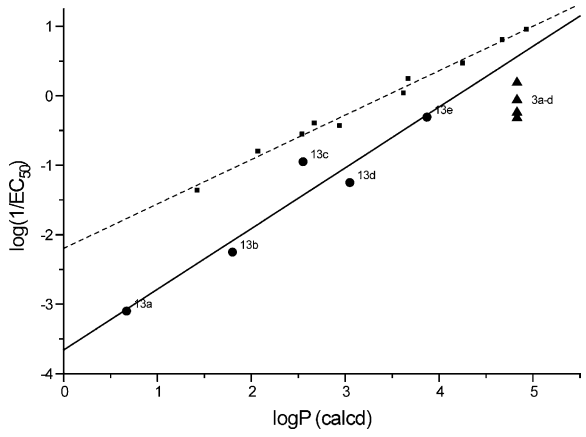


Fig. 2. Correlation of calculated log *P* values and MDR-modulating activity for propafenones (squares, dotted line), benzopyranones **13a**–**e** (circles, solid line) and dihydrobenzofuranes **3a**–**d** (up triangels).

7. In silico screening

Although these results give additional information to the structural requirements necessary for high MDR-modulating activity, a general three-dimensional pharmacophore model for more than one structural class of compounds has not been developed in the past. Data sets of Propafenones and phenothiazines were used for CoMFA and CoMSIA studies [16–18] and very recently two pharmacophore models were published [19,20]. In contrast to other 3D QSAR methods such as CoMFA and CoMSIA, which require accurate alignment of all molecules, models generated by means of pharmacophoric features would allow in silico screening of structurally diverse compound databases.

In a preliminary and ongoing study, we used a training set of structurally diverse propafenone-type modulators (including also conformationally restricted derivatives) to generate a chemical function based pharmacophoric feature model using the software package CATALYST. Validation of the models obtained was performed on basis of our in house library. In silico screening of the Derwent World Drug Index gave 232 hits, which, after refining the model, were reduced to 32. Among them are several compounds, which were shown to act as MDR-modulators (Fig. 3), which supports the validity of the model. Further investigations including pharmacological testing of hitherto not as MDR-modulators described compounds will prove the general applicability of this concept for screening of large virtual compound libraries [21].

Acknowledgements

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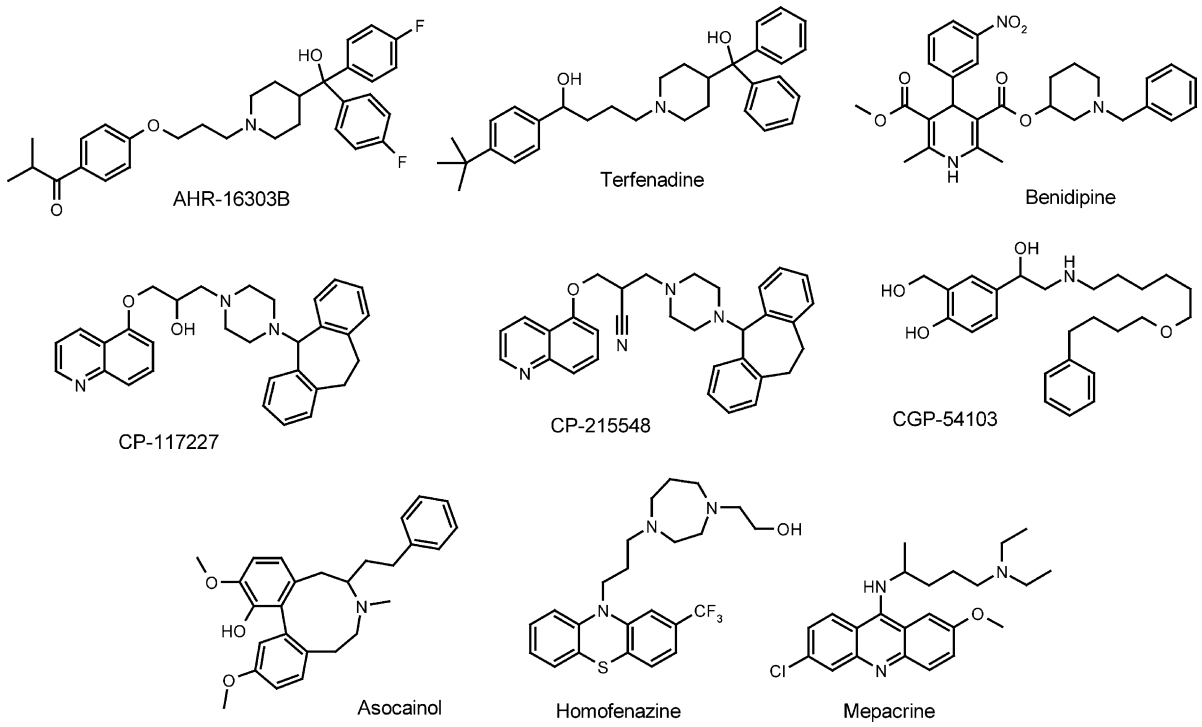

Fig. 3. Hits of the in silico screen of the World Drug Index, which were previously described as MDR-modulators.

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[21] Results of this study including a detailed description of the methods used as well as a more detailed information on our in house library will be published elsewhere.