Synthesis and in Vitro Multidrug Resistance Modulating Activity of a Series of Dihydrobenzopyrans and Tetrahydroquinolines†

Romana Hiessböck,‡ Christian Wolf,‡ Elisabeth Richter,§ Manuela Hitzler,§ Peter Chiba,§ Martin Kratzel,*,‡ and Gerhard Ecker*,‡

Institute of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Wien, Austria, and Institute of Medical Chemistry, University of Vienna, Währingerstrasse 10, A-1090 Wien, Austria

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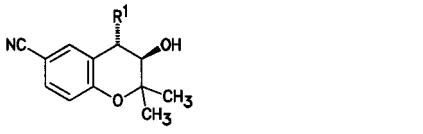
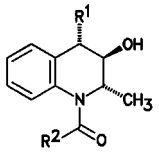
A series of dihydrobenzopyrans and tetrahydroquinolines was synthesized and pharmacologically tested for their ability to inhibit P-glycoprotein mediated daunomycin efflux in multidrug resistant CCRF-CEM vcr1000 cells. Several compounds exhibit activities in the range of the reference compounds verapamil and propafenone. Preliminary structure−activity relationship studies propose the importance of high molar refractivity values of the compounds and the presence of an additional basic nitrogen atom.

Introduction

Resistance of tumor cells to a wide variety of cytotoxic agents represents a major problem in cancer and antimicrobial therapy. The phenomenon that malignant cells acquire cross resistance to a panel of drugs when exposed to a single drug has been termed multidrug resistance (MDR).[1] Multidrug resistant tumors are found to be cross-resistant to a broad, but well-defined spectrum of structurally and functionally unrelated cytotoxic drugs, such as anthracyclines, epipodophyllotoxines, vinca alkaloids, colchicin, and taxanes.[2] In most cases, the cross resistance profile has been shown to be accompanied by a decrease in drug accumulation of the resistant cells, which is due to active efflux of these drugs by the multidrug transporter P-glycoprotein (PGP). PGP and related proteins function as ATP-driven efflux pumps for the aforementioned group of drugs, leading to suboptimal intracellular concentrations of toxins.[3] Inhibitors of PGP thus lead to resensitization of multidrug resistant tumor cells. Within the past decade, several classes of drugs, such as ion channel blockers, calmodulin antagonists, cyclosporine analogues, and even detergents, were identified as potent modulators of MDR, mostly acting via inhibition of PGP.[4] Interestingly, only few systematic structure−activity relationship (SAR) studies on the field of MDR modulators have been published yet, and the number of quantitative structure−activity relationship (QSAR) studies is surprisingly low.[5] Following our ongoing QSAR studies on MDR modulators we have focused our interest on dihydrobenzopyrans (**1**) and tetrahydroquinolines (**2**, **3**). These compounds show several structural features broadly accepted as beneficial for high activity, such as one or more aromatic rings, an aliphatic nitrogen atom, and several hydrogen bond acceptors.[6] Additionally, although an aryloxypropanolamine or arylaminopropanolamine substructure, respectively, is present in the molecules, they possess only low conformational flexibility, which would favor those compounds for three-dimensional (3D) QSAR studies such as CoMFA (comparative molecular field analysis). This paper intends to investigate the ability of compounds of type **1**, **2**, and **3** to inhibit daunomycin efflux from PGP-expressing CCRF-CEM vcr1000 cells and to get first hints on structure−activity relationships within these classes of compounds.



1



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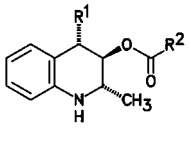

3

Only relative stereochemistry shown throughout

Chemistry

Synthesis of dihydrobenzopyrans **1** was achieved as outlined in Scheme 1 (see Table 1 for definition of substituent group). According to the synthesis of the potassium channel activator cromakalim, the epoxide **6** was prepared via alkylation of 4-hydroxybenzonitril (**4**) with 2-methyl-3-butin-2-ol and trifluoroacetic anhydride/$CuCl_2$, cyclization under heating (→ **5**), and epoxidation with *m*-chloroperbenzoic acid.[7] Subsequent nucleophilic epoxide ring opening with various amines gave the target compounds **1a**−**d** (for preparation of **1c**, see also ref 8). The benzylglycine derivative **1e** was synthesized via benzylation of **1d** using benzyl bromide and diisopropylethylamine. Generally, the corresponding hydrochlorides were prepared and used for pharmacological testing.

† With our best wishes dedicated to Prof. Dr. J. K. Seydel on the occasion of his 70th birthday.

* To whom correspondence should be addressed. Phone: +43-1-31336/8571 (G.E.), -/8577 (M.K.). Fax: +43-1-31336/771. E-mail: ecker@speedy.pch.univie.ac.at (G.E.) or kratzel@speedy.pch.univie.ac.at (M.K.).

‡ Institute of Pharmaceutical Chemistry.

§ Institute of Medical Chemistry.

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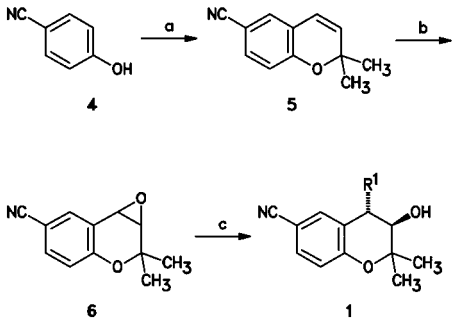
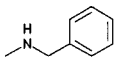
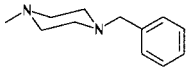
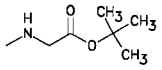
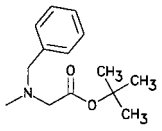
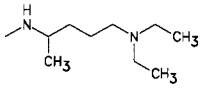
Scheme 1[a]

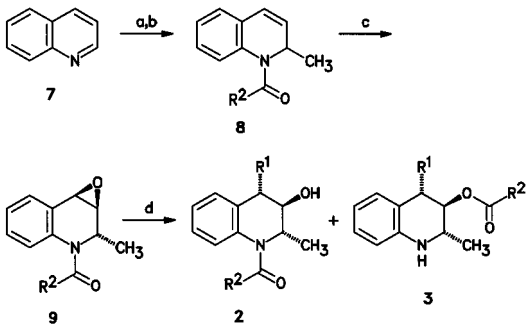


[a] Reagents: (a) 2-methyl-3-butin-2-ol, $(CF_3CO)_2O$, $CuCl_2$; (b) *m*-CPBA, CH_2Cl_2; (c) amine, EtOH.

Table 1

R^1	series		
	**1a**	**2a**	
	**1b**	**2b**	
	**1c**		
	**1d**		
	**1e**		
		**2f**	**3f**
		**2g**	**3g**

Scheme 2[a]



[a] Reagents and conditions: (a) MeLi, THF, 0 °C; (b) R^2COCl, TEA, CH_2Cl_2; (c) *m*-CPBA, CH_2Cl_2; (d) amine, $LiClO_4$, CH_3CN; R^2 = *p*-tolyl.

Compounds **2** (and **3**, respectively), based on a 1,2,3,4-tetrahydroquinoline system, were prepared using epoxide **9** as the key compound as displayed in Scheme 2. This epoxide can readily be prepared starting from quinoline (**7**) after (reductive) 2-alkylation, 1-acylation (→ **8**), and oxidation of the 3,4-double bond by *m*-chloroperbenzoic acid.[9] Contrary to 2-monomethyl-benzopyran congeners,[10] 1,2-dihydroquinolines afford diastereoisomerically pure epoxides **9**.[9] The aminolysis requires catalysis of lithium perchlorate to reach good yields of *vic*-aminols (**2**) which have been just obtained as pure diastereoisomers.[11] Depending on the basicity of the amine used for epoxide cleavage, migration of the acyl group to the hydroxy function can be observed, resulting in the formation of esters **3**.[11]

Table 2. Selected Physicochemical Properties and MDR Modulating Activity of Compounds **1a**−**3g**

no.	log *P*	log k_w	MR	EC_{50} (±sd), μM
1a	3.12	4.35	90.6	59.09 (±6.24)
1b	3.11	5.53	111.7	0.64 (±0.16)
1c	1.24	nd[a]	66.05	1433.21 (±379.23)
1d	1.79	nd	91.67	46.75 (±11.16)
1e	3.76	nd	121.1	2.43 (±0.53)
2a	4.52	5.38	116.8	3.26 (±0.01)
2b	4.51	6.10	138	0.43 (±0.16)
2f	3.34	4.20	104.7	7.98 (±0.52)
2g	4.20	nd	133.9	1.96 (±0.46)
3f	3.92	5.47	105.0	5.64 (±0.75)
3g	4.78	nd	134.2	0.34 (±0.28)

[a] nd: not determined.

Calculation of Physicochemical Properties. Calculation of log *P* values was performed according to the method of Ghose et al.[12] using the fragmentation method based on a topological approach. The software package MOLGEN[13] was used, which has been established to give excellent results when compared to experimentally determined values.[14] The software package is installed on a Intel Pentium 90 MHz personal computer with 64 MB RAM and runs under DOS 6.0. Molecules were generated using the builder function and energetically minimized with the implemented MM2 force field. Conformationally independent log *P* values were calculated. Molar refractivity was calculated in an analogous manner. All parameters calculated are given in Table 2.

Due to the fact that compounds from two different chemical classes were included in the data set, lipophilicity of selected compounds was also determined using an HPLC-based method.[14] Briefly, the log *k′* values were measured on a reversed phase column using mixtures of methanol/buffer, pH 7.4, in different ratios as eluent. A linear relationship between the percentage of organic modifier (methanol) and the log *k′* values allows extrapolation to 0% methanol, which gives the so-called log k_w value. This log k_w value is a measure for lipophilicity of the compounds and is also given in Table 2.

MDR Modulating Activity. The daunomycin efflux assay[15] was used to measure the PGP inhibitory activity of compounds **1a**−**3g**. Thus, PGP expressing CCRF-CEM vcr1000 cells[16] were loaded with daunomycin, and the time-dependent decrease in mean cellular fluorescence was determined in the presence of various concentrations of modifier. The first-order rate constants (V_{max}/K_m) were obtained by nonlinear regression analysis. Correction for simple diffusion was achieved by subtracting the efflux rates observed in parental CCRF-CEM wt cells. EC_{50} values of modulators were calculated from dose−response curves of V_{max}/K_m versus modifier concentration. Values are given in Table 2 and

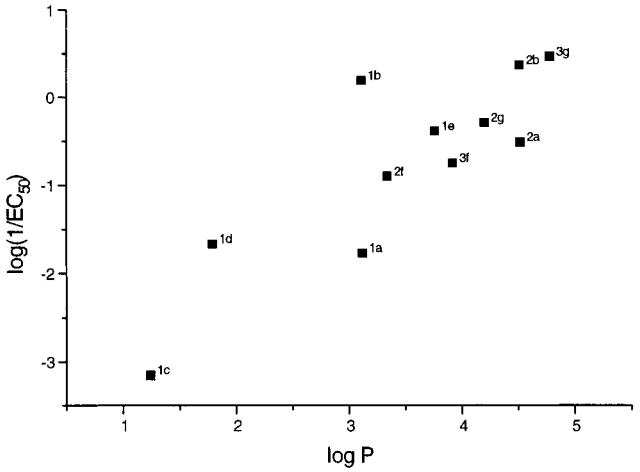


Figure 1. Plot of MDR modulating activity of compounds **1a**−**3g** [expressed as log(1/EC_{50}) values] vs calculated log *P* values.

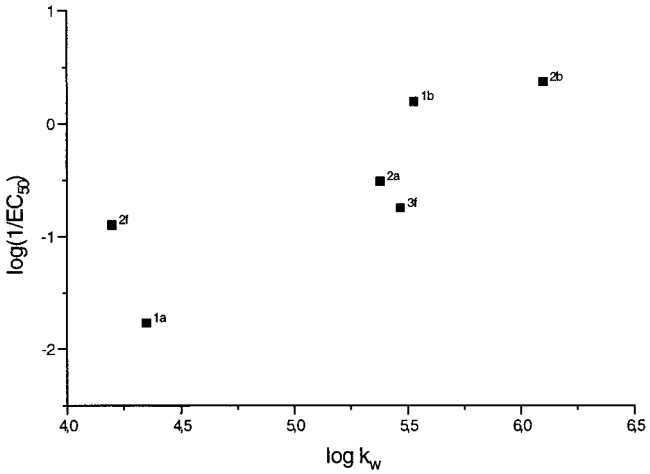


Figure 2. Plot of MDR modulating activity of selected compounds [expressed as log(1/EC_{50}) values] vs log k_w values.

represent the mean (±sd) of at least three independently performed experiments. All compounds tested showed dose−response curves with nearby identical slopes, which indicates similar binding modes.

Results and Discussion

Table 2 shows the MDR modulating activity of compounds **1a**−**3g**. The EC_{50} values for inhibition of PGP mediated efflux of daunomycin range from 0.34 (**3g**) to 1433 μM (**1c**). Highest activity was observed for the *O*-acyl derivative **3g** followed by the benzylpiperazines **2b** and **1b** and the *N*-acyl derivative **2g**. These compounds exhibit activities in the range of propafenone and verapamil.[17] Comparing dihydrobenzopyrans with structurally analogous tetrahydroquinolines, the latter generally show higher activity (**2a** vs **1a** and **2b** vs **1b**). In the series of dihydrobenzopyrans, *N*-benzyl substitution seems to be favorable, which is demonstrated in due course (**1a** vs **1c** and **1e** vs **1d**). Within the series of tetrahydroquinolines, *O*-acyl derivatives are slightly more active than the corresponding *N*-acyl congeners (**3f** vs **2f** and **3g** vs **2g**), although this difference is not statistically significant on the 95% confidence level. Nevertheless, calculation of log *P* values of all compounds showed that these differences in MDR modulating activity are apparently caused by differences in the lipophilicity of the compounds. Figure 1 demonstrates that a trend toward higher activity for compounds with higher lipophilicity is obvious ($n = 11$, $r = 0.85$, $Q^2_{cv} = 0.56$). This behavior was demonstrated for several classes of modulators[18] and is therefore not unique for dihydrobenzopyrans and tetrahydroquinolines. Although this trend is also shown when experimentally determined log k_w values are used (Figure 2; $n = 6$, $r = 83$), this result has to be taken with a grain of salt due to the poor relationship between calculated and experimentally determined lipophilicity values ($n = 6$, $r = 0.66$) and the small set of compounds used.

Recently, we could show that, for a set of 48 compounds structurally related to propafenone, molar refractivity (MR) is superior to lipophilicity as a descriptor for prediction of MDR modulating activity.[19] The plot of log potency vs MR values of compounds **1a**−**3b** shows an excellent correlation (eq 1), and a leave-one-out cross validation demonstrated the high predictivity of the equation obtained (Figure 3). This indicates that polar interactions also contribute to MDR modulating activity of benzopyrans and tetrahydroquinolines.

$$\log(1/EC_{50}) = 0.04(\pm 0.006)MR - 5.86(\pm 0.65) \quad (1)$$

$$n = 11,\ r = 0.94,\ F = 63.79,\ Q^2_{cv} = 0.81$$

Conclusion

A series of dihydrobenzopyrans and tetrahydroquinolines was synthesized and tested in an in vitro assay for their ability to inhibit PGP mediated daunomycin efflux from multidrug resistant CCRF-CEM vcr1000 cells. With the exception of the *N*-methyl derivative **1c**, all compounds displayed moderate to high potency. Preliminary structure−activity relationship studies indicate that high lipophilicity and a second basic nitrogen atom are beneficial for PGP inhibitory activity. The best prediction of MDR modulating activity could be obtained when using molar refractivity as the descriptor. This indicates that polar interactions also take place with PGP. Due to the conformational rigid character of the molecules, these new classes of MDR modulators represent versatile tools for 3D-QSAR studies.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot plate apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity Plus 300 spectrometer. ^{1}H spectra were referenced to tetramethylsilane (δ 0.0); in ^{13}C spectroscopy $CDCl_3$ served as the internal standard (δ 77.0). An asterisk indicates peaks of double intensity. MS spectra were measured on Shimadzu QP 5000, Finnigan 8230, and Finnigan MAT 900S instruments. Flash chromatography was carried out on MERCK silica gel 60, TLC on plastic sheets (MERCK silica gel 60 F_{254}).

General Procedure for the Aminolysis of Dihydrobenzopyran Epoxide 6. Epoxide **6** (2 mmol) was dissolved in 10 mL of ethanol, and the selected amine (2 mmol) was added. The reaction mixture was heated under reflux until the reaction was completed (TLC control) and then evaporated to dryness. Formation of the hydrochlorides was carried out by dissolving the purified amino alcohol in diethyl ether and adding an ethereal solution of HCl (1 M). The hydrochloride was filtered off and purified via crystallization.

(3*RS*,4*SR*)-4-Benzylamino-3-hydroxy-2,2-dimethyl-2*H*-3,4-dihydro-1-benzopyran-6-carbonitril (1a).[20] This com-

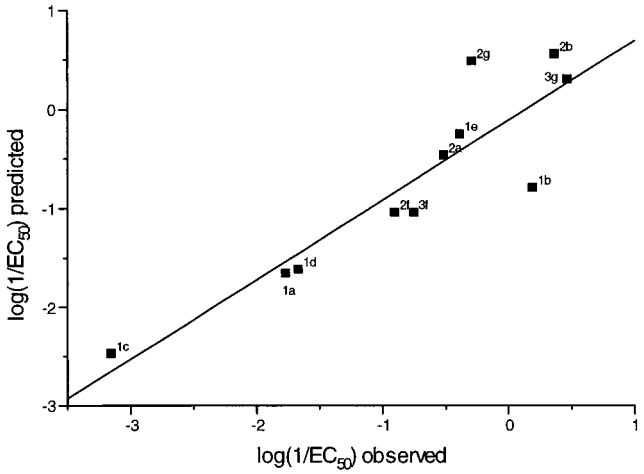


Figure 3. Plot of observed vs predicted MDR modulating activity of compounds **1a**−**3g**. Predicted values were calculated using eq 1.

pound was prepared by reaction with benzylamine (0.22 mL, 2 mmol). Purification by flash chromatography (dichloromethane−methanol−ammonia concentrated; 20:1:0.1) afforded 380 mg (62%) of **1a** as a colorless oil: IR (KBr) 2225 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22, 1.52 (each s, each 3H, 2 × 2-CH_3), 1.85, 2.90 (each br, each 1H, OH, NH), 3.65, 3.80 (each d, each 1H, J = 10.0 Hz, 3-H, 4-H), 3.72, 3.85 (each d, each 1H, J = 13.0 Hz, benzyl-H), 6.86 (d, 1H, J = 8.5 Hz, 8-H), 7.27−7.39 (m, 5H, phenyl-H), 7.44 (dd, 1H, J = 1.5 Hz, J = 8.5 Hz, 7-H), 7.71 (d, 1H, J = 1.5 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 19.4, 27.3 (2 × 2-CH_3), 49.0 (benzyl-C), 56.7 (3-C), 71.6 (4-C), 80.0 (2-C), 104.0 (6-C), 118.8 (8-C), 119.8, 124.7 (4a-C, CN), 127.8, 128.5*, 129.1*, 132.5, 133.0 (arom CH), 140.4 (arom C), 157.9 (8a-C); MS *m*/*z* 236 (28), 91 (100). **1a·HCl:** Yield 85%; mp 239−241 °C (ethyl acetate).

(3*RS*,4*SR*)-4-(4-Benzyl-1-piperazinyl)-3-hydroxy-2,2-dimethyl-2*H*-3,4-dihydro-1-benzopyran-6-carbonitril (1b). This amino alcohol was synthesized by epoxide cleavage with 1-benzylpiperazine (350 mg, 2 mmol). Purification by flash chromatography (dichloromethane−methanol−ammonia concentrated; 20:1:0.1) yielded 160 mg (42%) of **1b** as a yellowish oil: IR (KBr) 2222 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20, 1.50 (each s, each 3H, 2 × 2-CH_3), 1.75 (br, 1H, OH), 2.43−2.55, 2.73−2.97 (each m, each 4H, piperazinyl H), 3.55 (s, 2H, benzyl-H), 3.65, 3.73 (each d, each 1H, J = 10.0 Hz, 3-H, 4-H), 6.82 (d, 1H, J = 8.5 Hz, 8-H), 7.22−7.35 (m, 5H, phenyl-H), 7.41 (dd, 1H, J = 1.8 Hz, J = 8.5 Hz, 7-H), 7.82 (d, 1H, J = 1.8 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 19.0, 27.3 (2 × 2-CH_3), 49.8, 54.5, 63.2, 63.5 (benzyl-C, 3-C, piperazinyl-C), 70.3 (4-C), 79.9 (2-C), 103.8 (6-C), 118.8 (8-C), 120.0, 124.1 (4a-C, CN), 127.5, 128.6*, 129.7*, 132.8, 133.6 (arom CH), 138.1 (arom C), 158.0 (8a-C); MS *m*/*z* 232 (68), 118 (97), 91 (16). **1b·HCl:** Yield 88%, mp 230−231 °C (ethyl acetate).

(3*RS*,4*SR*)-3-Hydroxy-2,2-dimethyl-4-methylamino-2*H*-3,4-dihydro-1-benzopyran-6-carbonitril (1c). The synthesis of this compound is described in ref 8.

(3*RS*,4*SR*)-*N*-(6-Cyano-3-hydroxy-2,2-dimethyl-2*H*-3,4-dihydro-1-benzopyran-4-yl)glycine *tert*-Butyl Ester (1d). This compound was prepared by epoxide cleavage with glycine *tert*-butyl ester (260 mg, 2 mmol) and purified by flash chromatography (dichloromethane−methanol; 70:1) to give 580 mg (87%) of **1d** as colorless crystals, mp 98−103 °C (methanol): IR (KBr) 2227 (CN), 1728 (COOR) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.20, 1.46 (each s, each 3H, 2 × 2-CH_3), 1.50 (s, 9H, C(CH_3)$_3$), 2.75 (br, 1H, NH), 3.32, 3.52 (each d, each 1H, J = 7.3 Hz, N-CH_2-COO), 3.56 (dd, 1H, J = 5.1 Hz, J = 8.9 Hz, 3-H), 3.69 (d, 1H, J = 8.9 Hz, 4-H), 5.54 (d, 1H, J = 5.1 Hz, OH), 6.94 (d, 1H, J = 8.5 Hz, 8-H), 7.63 (dd, 1H, J = 2.1 Hz, J = 8.5 Hz, 7-H), 8.07 (d, 1H, J = 2.1 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 18.9, 27.0 (2 × CH_3), 27.9 (C(CH_3)$_3$), 46.8 (N-CH_2-COO), 57.0 (4-C), 71.4 (3-C), 79.8, 82.4 (2-C, *C*(CH_3)$_3$), 103.6 (6-C), 118.1 (8-C), 119.3 (CN), 124.1 (4a-C), 132.3, 132.5 (5-C, 7-C), 157.5 (8a-C), 174.6 (COO). **1d·HCl:** Yield 76%; mp 170 °C.

(3*RS*,4*SR*)-*N*-Benzyl-*N*-(6-cyano-3-hydroxy-2,2-dimethyl-2*H*-3,4-dihydro-1-benzopyran-4-yl)glycine *tert*-Butyl Ester (1e). To a solution of ester **1d** (500 mg, 1.5 mmol) in dichloromethane small amounts of benzyl bromide and diisopropylethylamine were repeatedly added (total amount of benzyl bromide added: 1.1 mL, total amount of diisopropylamine: 3.5 mL) over a time period of 24 h. Then, the reaction mixture was concentrated and partitioned between water (20 mL) and ethyl acetate (20 mL). After extraction of the aqueous phase with ethyl acetate (2 × 20 mL), the organic layers were combined, dried (Na_2SO_4), and brought to dryness. Flash chromatography (light petroleum−ethyl acetate; 6:1) gave 495 mg (78%) of **1e** as a colorless oil which solidified slowly from light petroleum, mp 131−134 °C: ^1H NMR (DMSO-d_6) δ 1.05, 1.41 (s, 3H, 2 × 2-CH_3), 1.37 (s, 9H, C(CH_3)$_3$), 3.18 (d, 1H, J = 16.8 Hz, N-CH_2-COO), 3.55−3.72 (m, 2H, N-CH_2-COO, benzyl-H), 3.75 (d, 1H, J = 10.0 Hz, 4-H), 3.85 (dd, 1H, J = 5.6 Hz, J = 10.0 Hz, 3-H), 3.98 (br, 1H, benzyl-H), 5.73 (d, 1H, J = 5.6 Hz, OH), 6.85 (d, 1H, J = 8.5 Hz, 8-H), 7.23−7.38 (m, 5H, phenyl-H), 7.55 (dd, 1H, J = 1.7 Hz, J = 8.5 Hz, 7-H), 8.26 (d, 1H, J = 1.7 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 18.9, 27.1 (2 × CH_3), 27.8 (C(CH_3)$_3$), 80.3, 82.3 (2-C, *C*(CH_3)$_3$), 103.4 (6-C), 118.4 (8-C), 119.5 (CN), 123.9 (4a-C), 127.5, 128.6*, 129.0* (arom CH), 132.4, 132.6 (5-C, 7-C), 157.9 (8a-C), 173.9 (1-C). **1e·HCl**: Yield 91%; mp 130−134 °C (diethyl ether).

General Procedure for the Aminolysis of Tetrahydroquinoline Epoxide 9. To a solution of epoxide **9** (1.12 g, 4 mmol) in 10 mL of acetonitrile was added lithiumperchlorate (440 mg, 4 mmol), and then stirred until solution of the salt occurred. After addition of the appropriate amine (4.4 mmol), the mixture was refluxed. Then, the reaction mixture was diluted with water and extracted with dichloromethane (3 × 10 mL), and the combined organic fractions were washed with water, dried (Na_2SO_4), and evaporated in vacuo.

(2*SR*,3*SR*,4*SR*)-3-Hydroxy-2-methyl-4-benzylamino-1-*p*-toluoyl-1,2,3,4-tetrahydroquinoline (2a). This compound was prepared by reaction with benzylamine (0.5 mL, 4.4 mmol), reaction time: 12 h at reflux, 24 h at room temperature. Purification by flash chromatography (diethyl ether) brought back 230 mg (41%) of the starting material and afforded 275 mg (35%) of **2a** as colorless oil, colorless crystals from diethyl ether, mp 138−141 °C: IR (KBr): 1615 (NCO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (d, 3H, J = 6.6 Hz, 2-CH_3), 2.27 (s, 3H, *p*-CH_3), 2.50−3.10 (2H, br, NH, OH), 3.15 (dd, 1H, J = 5.3 Hz, J = 9.5 Hz, 3-H), 3.62 (d, 1H, J = 9.8 Hz, 4-H), 4.03 (2H, AB-system, J = 11.2 Hz, benzyl-H), 4.59 (m, 1H, 2-H), 6.50 (d, 1H, J = 7.5 Hz, arom H), 6.83−7.52 (m, 12H, arom H); ^{13}C NMR ($CDCl_3$) δ 19.3 (2-CH_3), 21.3 (*p*-CH_3), 52.7 (benzyl-C), 56.5 (2-C), 60.4, 79.7 (3-C, 4-C), 123.9, 125.6, 126.6, 126.7, 127.3, 128.1*, 128.5**, 128.8* (arom CH), 132.5, 133.1, 137.7, 139.9, 140.5 (arom C), 169.2 (C═O); MS *m*/*z* 386 (0.4, M^+).

(2*SR*,3*SR*,4*SR*)-3-Hydroxy-2-methyl-4-(4′-benzylpiperazinyl)-1-*p*-toluoyl-1,2,3,4-tetrahydroquinoline (2b). This compound was synthesized with 1-benzylpiperazine (0.6 mL, 3.5 mmol) as amine, reaction time: 48 h. Purification by flash chromatography (ethyl acetate−triethylamine; 9:1) gave 100 mg (18%) of the starting epoxide **9** and 440 mg (48%) of **2b** as spontaneously crystallizing oil. Colorless crystals from diethyl ether, mp 173−175 °C: IR (KBr) 1645 (NCO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H, J = 6.5 Hz, 2-CH_3), 1.76 (br, 1H, OH), 2.27 (s, 3H, *p*-CH_3), 2.44−2.69, 2.80−3.28 (each m, each 4H, piperazinyl-H), 3.58, 3.77 (each s, each 2H, benzyl-H, 3-H, 4-H), 4.55−4.72 (m, 1H, 2-H), 6.50 (d, 1H, J = 7.8 Hz, arom H), 6.78−7.44 (m, 11H, arom H), 7.58 (d, 1H, J = 7.5 Hz, arom H; ^{13}C NMR ($CDCl_3$) δ 18.3 (2-CH_3), 21.2 (*p*-CH_3), 53.8* (piperazinyl-C), 56.4 (2-C), 63.1 (benzyl-C), 65.7, 75.6 (3-C, 4-C), 125.0, 125.8, 126.1, 126.3, 126.9, 128.0*, 128.3*, 128.8*, 129.2* (arom CH), 131.5, 132.5, 137.4, 137.7, 140.2 (arom C), 169.4 (C═O); MS *m*/*z* 455 (2, M^+).

(2*SR*,3*SR*,4*SR*)-3-Hydroxy-2-methyl-4-pyrrolidyl-1-*p*-toluoyl-1,2,3,4-tetrahydroquinoline (2f) and (2*SR*,3*SR*,4*SR*)-

2-Methyl-4-pyrrolidyl-3-*p*-toluoyloxy-1,2,3,4-tetrahydroquinoline (3f). The synthesis of these compounds is described in ref 10.

(2*SR*,3*SR*,4*SR*)-3-Hydroxy-2-methyl-4-(5-diethylamino-2-pentylamino)-1-*p*-toluoyl-1,2,3,4-tetrahydroquinoline (2g) and (2*SR*,3*SR*,4*SR*)-2-Methyl-4-(5-diethylamino-2-pentylamino)-3-*p*-toluoyloxy-1,2,3,4-tetrahydroquinoline (3g). These compounds were obtained using 5-diethylamino-2-pentylamine (700 mg, 4.4 mmol) as reagent, reaction time: 48 h. The addition of amine (400 mg, 2.6 mmol) was repeated after 12 h. Separation by flash chromatography (ethyl acetate−triethylamine; 9:1) yielded 360 mg (41%) of **3g** and 310 mg (35%) of **2g** as yellowish oils. **2g**: IR (KBr/liquid film) 1640 (NCO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89−1.04 (m, 6H, NCH_2CH_3), 1.09/1.19 (d, 3H, J = 6.0 Hz, $NHCH(CH_3)CH_2CH_2$-$CH_2N(C_2H_5)_2$), 1.26 (d, 3H, J = 6.0 Hz, 2-CH_3), 1.40−1.65 (m, 4H, $NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 2.26 (s, 3H, *p*-$CH_3$), 2.40−2.46, 2.46−2.59 (each m, 6H, $NHCH(CH_3)CH_2CH_2CH_2$-$N(CH_2CH_3)_2$), 2.87−3.00, 3.00−3.09 (each m, each 1H, 3-H, $NHCH(CH_3)CH_2CH_2CH_2$-$N(C_2H_5)_2$), 3.60 (t, 1H, J = 10.0 Hz, 4-H), 3.70 (br, 1H, OH), 4.50 (m, 1H, 2-H), 6.46 (d, 1H, J = 7.8 Hz, arom H), 6.88 (t, 1H, J = 7.8 Hz, arom H), 6.92 (d, 2H, J = 7.8 Hz, arom H), 7.03−7.10 (m, 3H, arom H), 7.14−7.26 (m, 3H, arom H);^{13}C NMR ($CDCl_3$) δ 11.3, 11.4 (NCH_2CH_3), 19.3, 20.4, 21.3 (2-CH_3, $CH(CH_3)$, *p*-CH_3), 22.8/23.6, 34.6/36.2 ($NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 46.6, 46.7 ($N(CH_2CH_3)_2$), 52.8/52.9 ($NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 51.6/51.8, 52.2/52.4 (2-C, $CH(CH_3)$), 57.7/58.5 (4-C), 79.5/79.6 (3-C), 112.9/114.0, 117.1/117.2, 123.5/123.8, 125.3/126.2, 128.2*, 128.6* (arom CH), 132.4, 134.1, 137.5, 140.1 (arom C), 168.9 (C═O); MS m/z 437 (2, M^+). **3g**: IR (KBr/liquid film) 1725 (COO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80−0.98 (m, 7.5H, NCH_2CH_3, $NHCH$-$(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 1.11 (d, 1.5H, J = 6.3 Hz, $NHCH$-$(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 1.26 (d, 3H, J = 6.3 Hz, 2-CH_3), 1.22−1.38, 1.40−1.55 (each m, 4H, $NHCH(CH_3)CH_2CH_2CH_2N$-$(C_2H_5)_2$), 2.00−2.06, 2.30−2.56 (each m, 6H, $NHCH(CH_3)CH_2$-$CH_2CH_2N(CH_2CH_3)_2$), 2.46 (s, 3H, *p*-CH_3), 2.85−3.02 (m, 1H, $NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 3.57 (m, 1H, 2-H), 3.95 (d, 1H, J = 8.1 Hz, 4-H), 5.14 (dt, 1H, J = 8.0 Hz, J = 2.3 Hz, 3-H), 6.45 (d, 1H, J = 8.1 Hz, arom H), 6.66 (t, 1H, J = 8.2 Hz, arom H), 6.97 (t, 1H, J = 7.5 Hz, arom H), 7.15−7.18 (m, 2H, arom H), 7.34 (d, 1H, J = 7.5 Hz, arom H), 7.88 (d, 2H, J = 8.1 Hz, arom H); ^{13}C NMR ($CDCl_3$) δ 19.0, 19.2 (NCH_2CH_3), 20.8, 21.1, 21.4 (2-CH_3, $CH(CH_3)$, *p*-CH_3), 23.1/23.2, 35.2/36.0 ($NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 46.4, 46.6 ($N(CH_2CH_3)_2$), 52.7/52.9 ($NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$), 51.1/51.3, 52.2/52.5 (2-C, $CH(CH_3)$), 56.9/57.0 (4-C), 76.9/77.1 (3-C), 113.5, 117.0, 127.6, 128.1/128.2, 128.9*, 129.5* (arom CH), 123.9, 124.2, 127.2 (arom C), 143.4/143.5 (8a-H), 166.0 (C═O); MS m/z 280 (19, M^+ − side chain).

Measurement of Lipophilicity. The chromatographic procedure was performed with a Kontron HPLC system consisting of a Kontron HPLC pump 420, a Kontron HPLC pump 422, a Kontron high-pressure mixing chamber, a 20 μL Rheodyne injection valve, and a Kontron HPLC detector 432 (Kontron Austria, Vienna, Austria). Temperature control was achieved with a peltier column thermostat (ICT Wien, Vienna, Austria). The resulting chromatogramms were processed using a Kontron Datasystem 450 Multitasking v. 2.0 (Kontron Austria, Vienna, Austria). The solvents used were HPLC-grade water and p.a. grade methanol (E. Merck, Vienna, Austria). The solutes were generally monitored at 254 nm. A LiChroCART 75-4 (LiChrospher 100 RP-8 (5 μm); E. Merck, Vienna, Austria) column was used. The pH of the aqueous phase was adjusted to 7.4 using a 1/60 M Soerensen buffer. Control of pH was achieved using a ORION 250A pH meter equipped with a pH-TRIODE (Bartelt, Graz, Austria). Log k_w values were estimated by extrapolation of the log k' values for 70%, 60%, and 50% of methanol to 0% methanol via linear regression analysis.

Pharmacology. 1. Cell Lines. The CCRF-CEM T lymphoblast cell line as well as the resistant lines were obtained as described previously.[15,16] 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. Due to a duplication time of 24 h, 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.[15] This cell line has been chosen on grounds of distinct PGP expression and does not show the mutation at codon 185.[21] As shown previously, EC_{50} values for inhibition of PGP obtained for daunomycin efflux in CCCRF-CEM vcr1000 cells are highly correlated to those obtained for inhibition of rhodamine-123 efflux in *mdr1* transfectant L5178YVMDR1 C.06 cells. This demonstrates that resistance in the vincristin selected cell line is due to PGP expression.

2. Daunomycin Efflux Studies. Daunomycin efflux studies were performed using published methods.[15] 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 Chemical Co., St. Louis, MO) at a final concentration of 3.2 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 ranging from 3 nM to 500 μM, depending on solubility and the expected potency of the modifier. Eight concentrations (serial dilution 1:2.5) were tested for each modulator. After 60, 120, 180, and 240 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). 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 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. The excitation wavelength was 488 nm, and the emission was measured in the FL3 channel (650−780 nm). Five thousand 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. A dose-response curve was obtained when plotting V_{max}/K_m versus modulator concentration, and the respective EC_{50} value was calculated. Means given in Table 2 are means ± standard deviation of EC_{50} values obtained in at least three independently performed experiments.

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