Studies on Propafenone-type Modulators of Multidrug-Resistance IV[1]: Synthesis and Pharmacological Activity of 5-Hydroxy and 5-Benzyloxy Derivatives

Peter Chiba[a], Barbara Tell[b], Walter Jäger[b], Elisabeth Richter[a], Manuela Hitzler[a] and Gerhard Ecker[b],*

[a] Institute of Medical Chemistry, University of Vienna, Währinger Strasse 10, A-1090 Wien, Austria

[b] Institute of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Wien, Austria

Key Words: *Multidrug resistance; propafenone; P-glycoprotein*

Summary

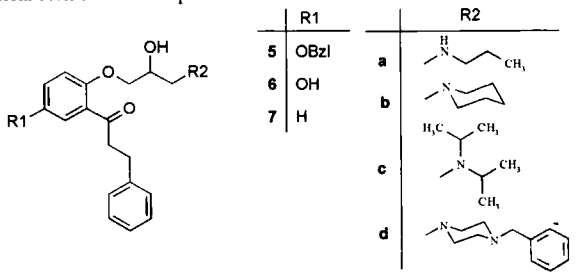
A series of 5-hydroxy and 5-benzyloxy analogs of the antiarrhythmic and multidrug resistance (MDR) modulating drug propafenone was synthesized and the MDR-modulating activity of the compounds was evaluated using a daunomycin efflux assay system. The key step of the synthesis is the selective reduction of the double bond in **1** without cleavage of the benzyl group thus leading to the phenol **3**. Alkylation with epichlorohydrine followed by nucleophilic epoxide ring opening gave the benzylated target compounds **5a–d**. Subsequent cleavage of the benzyl group gave the 5-hydroxy analogs **6a–d**. Structure activity relationship studies showed, that the 5-hydroxy derivates **6a–d** fit the log *P*/log potency correlation line previously established for a series of propafenone analogs. In contrast, all four 5-benzyloxy analogs **5a–d** showed almost identical EC_{50} values, independent of their log *P* value.

Introduction

The multidrug transporter P-glycoprotein (PGP) is a member of the ATP-binding casette[1] and represents an integral membrane protein which in an energy dependent manner effluxes a wide variety of cytotoxic drugs. These include anthracyclines, epipodophyllotoxins, vinca alkaloids, actinomycin D, and taxans[2]. PGP, therefore, mediates the resistance against cytotoxic drugs and antitumour antibiotics. Within the past decade several compounds have been identified as being able to block PGP-mediated efflux of natural product toxins, leading to resensitization of drug resistant tumor cells [3,4]. We recently identified analogs of the class 1c antiarrhythmic agent propafenone (**7a**, Table 1) as highly effective PGP-inhibitors, which reestablish sensitivity of PGP expressing CCRF-CEM vcr1000 tumor cells towards cytostatic drugs [5]. In humans, propafenone is metabolized by the cytochrome P450 system [6]. The main metabolite 5-hydroxypropafenone (**6a**, Table 1) still retains antiarrhythmic properties.[7] In the present study a series of 5-hydroxy analogous propafenone derivatives was synthesized and their chemosensitizing activity was tested in order to verify whether the potential P450 metabolites retain MDR-modulating activity. For means of structure-activity relationship studies the intermediates **5a–d** were also pharmacologically tested. For comparison the propafenone analogs **7a–d**[5] were included in the data set.

Table 1. Chemical structure, lipophilicity, and MDR-modulating activity of compounds **5a–7d**.

General formula for compounds **5a–7d:**



#	calcd. log *P*	EC_{50} (μM)	Analyses
5a	5.00	0.11	C,H,N,Cl
5b	5.28	0.17	C,H,N,Cl[a]
5c	5.86	0.08	C,H,N,Cl
5d	6.04	0.12	C,H,N,Cl
6a	3.00	3.02	Ref [8]
6b	3.29	2.30	C,H,N,Cl
6c	3.87	1.04	C,H,N,Cl
6d	4.04	0.53	C,H,N,Cl[b]
7a	3.39	1.08	Ref [5]
7b	3.67	0.68	Ref [5]
7c	4.25	0.31	Ref [5]
7d	4.43	0.38	C,H,N,Cl

[a] C: calcd 70.64; found 70.05. [b] Cl: calcd 12.54; found 11.87.

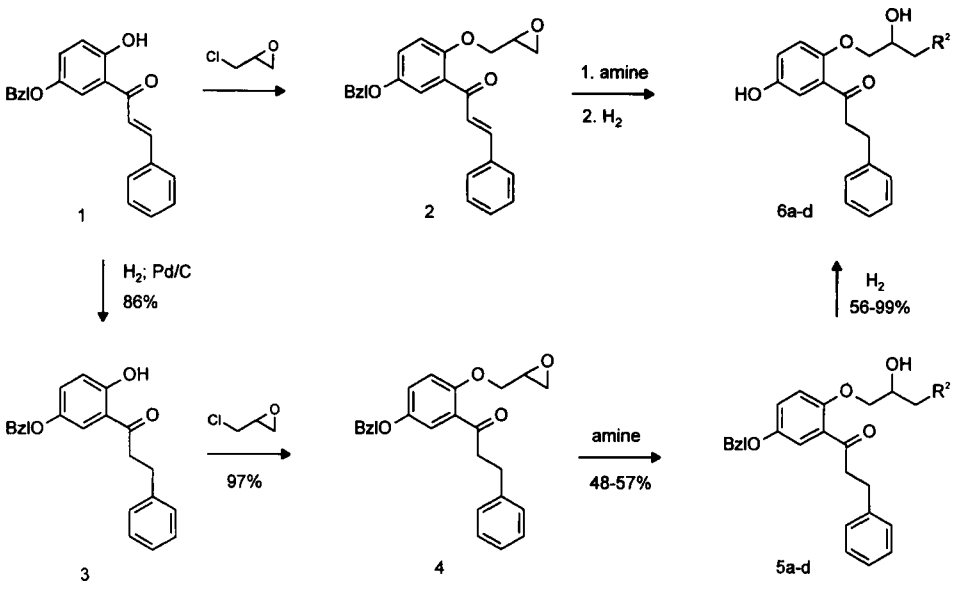
Results and Discussion

Chemistry

The previously described synthesis of 5-hydroxypropafenone (**6a**) proceeds via alkylation of intermediate **1** with epichlorohydrine to give the epoxide **2**. Reaction with *n*-propylamine and catalytic hydrogenation on Pd/C leads to **6a** [8]. The yield for these two reaction steps did not exceed 20%. This might be due to Michael addition on the double bond or retro aldol reaction.

[1] For part III see: P. Chiba, M. Hitzler, E. Richter, M. Huber, C. Tmej, E. Giovagnoni, G. Ecker, *Quant. Struct. Act. Relat.* **1997**, in press.

  0365-6233/97/1111/0343 $17.50 +.50/0



Scheme 1. Synthesis of compounds **5a–d** and **6a–d**.

An alternative route is outlined in a patent on synthesis of 5-hydroxydiprafenone [9]. Thus, intermediate **3** is synthesized via Friedel-Crafts acylation of hydrochinone with dihydrocinnamic acid and selective benzylation of the hydroxy group in position 5.

Our approach is based on the selective reduction of the double bond in **1** without cleavage of the benzylic protecting group, which is absolutely necessary to achieve the desired regioselectivity in the subsequent *O*-alkylation with epichlorohydrine (Scheme 1). Thus, 2,5-dihydroxyacetophenone was selectively benzylated in position 5 according to Pohl et al.[10] and reacted with benzaldehyde to yield the hydroxychalcone derivative **1**. Using catalytic hydrogenation with a maximum of 0.3% catalyst (5% Pd on charcoal) and careful monitoring of the H_2-consumption resulted in formation of **3** in 86% yield. Arylether formation with epichlorohydrine and subsequent nucleophilic epoxide opening with various amines gave the 5-benzyloxypropafenones **5a–d** (Table 1) with excellent overall yields (48–57%). Catalytic hydrogenation on Pd/C led to the desired 5-hydroxypropafenones **6a–d** (Table 1). Compounds **7a–c** were synthesized as described previously [5], and **7d** was synthesized in an analogous manner.

MDR Modulating Activity

Daunomycin efflux is a direct and accurate functional method to measure inhibition of PGP-mediated membrane transport [11]. The resistant human T-lymphoblast cell line CEM vcr1000[12] was used in our studies. The time dependent decrease in mean cellular fluorescence was determined in the presence of various concentrations of modifier and the initial efflux rates were calculated by regression analysis. Correction for simple diffusion was achieved by subtracting the efflux rates observed in the parental line. EC_{50} values of modifiers were calculated from dose response curves of initial efflux rates vs. modifier concentration. Values are given in Table 1.

Structure-Activity Relationship Studies

When comparing molecules with identical nitrogen substituents, potencies of the 5-hydroxy derivatives **6a–d** were generally three fold lower than that of the corresponding propafenone analogs **7a–d**. 5-Benzyloxy derivatives **5a–d** showed remarkably higher activity, with all four compounds exhibiting nearly identical EC_{50} values.

We recently demonstrated an excellent correlation between calculated log *P* values of the compounds and PGP inhibitory activity for a series of propafenone type modulators of multidrug resistance.[11] We, therefore, calculated log *P* values of the compounds using the software packages Molgen[13] and Sybyl®[14], which proved to yield best results in previous studies [15]. Both methods gave highly intercorrelated log *P* values ($r = 0.98$).

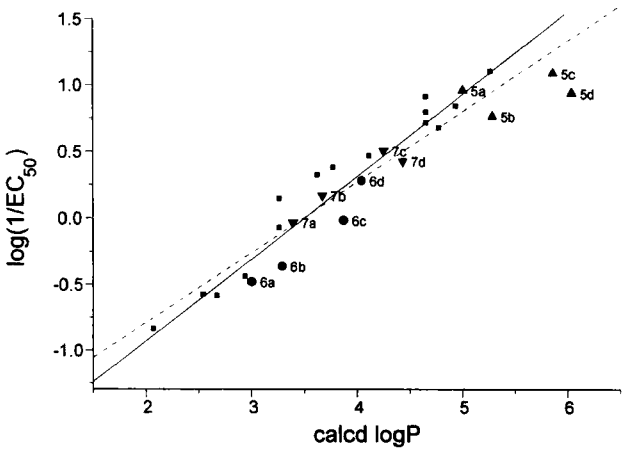


Figure 1: Correlation of calculated log *P* values of compounds **5a–7d** with MDR-modulating activity (expressed as log ($1/EC_{50}$) values); (■) propafenone derivatives[16]; (●) 5-hydroxypropafenone derivatives **6a–d**, (▲) 5-benzyloxypropafenone derivatives **5a–d**, (▼) corresponding propafenone analogs **7a–d**; the solid line represents the correlation obtained with Eq. 1, the dashed line those obtained with Eq. 2

Figure 1 shows the correlation of calculated log *P* values (Molgen) and MDR-modulating activity of the compounds. The 5-hydroxy analogs **6a–d** fit the log *P*/log potency regression line obtained in previous studies [16], whereas 5-benzyloxy analogs **5a–d** with calculated log *P* values exceeding 5.5 seem to exhibit decreased lipophilicity/activity ratios. Including **6a–d** in the data set, we obtained the folowing equation (Figure 1, solid line):

$\log (1/EC_{50}) = 0.62\ (\pm 0.04) \log P - 2.17\ (\pm 0.14)$;
$r = 0.96$, $sd = 0.15$, $n = 23$; $r^2_{cv} = 0.92$

However, recalculation including also benzyloxy derivatives **5a–d** gave an equation with an only slightly decreased predictive power (r^2_{cv}), whereby **5d** showed an activity which was outside the range of two standard deviations of the regression line (Figure 1, dashed line):

$\log (1/EC_{50}) = 0.53\ (\pm 0.04) \log P - 1.85\ (\pm 0.15)$;
$r = 0.95$, $sd = 0.19$, $n = 27$; $r^2_{cv} = 0.87$;

Kubinyi's bilinear model of the dependence of pharmacological activity on lipophilicity[17] predicts the existence of a lipophilicity optimum for the pharmacological activity of compounds. According to this model we suggest that **5d** already passed this lipophilicity optimum for propafenone-type MDR-modulators. Nevertheless, this needs further support by synthesis and evaluation of compounds with log *P* values between 6.5 and 8.0.

Conclusion

A series of 5-hydroxy and 5-benzyloxy analogous propafenone derivatives was synthesized and tested for their MDR-modulating activity. Although the 5-hydroxy derivatives **6a–d** generally showed lower activity than the parent compounds **7a–d**, they excellently fit the lipophilicity/log potency correlation for propafenones. This indicates, that, in analogy to the antiarrhythmic activity, hydroxylation in position 5 of the central aromatic ring, which is the major metabolic route for propafenones, does not remarkably influence PGP-inhibitory activity.

Acknowledgment

The present work was generously supported by the Jubilaeumsfond of the Austrian National Bank (grant #5983).

Materials and Methods

Chemistry

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 AC 80 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). Satisfactory C, H, N, and Cl analyses (± 0.4%) were obtained for all hydrochlorides.

1-(5-Benzyloxy-2-hydroxyphenyl)-3-phenyl-1-propenone (1)[8]

To a solution of 16.1 g (66.5 mmol) 5-benzyloxy-2-hydroxy-acetophenone in 180 ml ethanol 16.09 g (151.8 mmol) benzaldehyde and 32.3 g sodium hydroxide solution (50% in water) was added. After stirring for 1 h 92 ml of 5N HCl was added. The orange precipitate was filtered off and recrystallized from ethanol to give 18.0 g (82%) of **1** as orange needles; mp 103–104 °C. ^{1}H NMR ($CDCl_3$): δ = 5.06 (s, 2H, CH_2), 6.95 (d, 1H, *J* = 9 Hz, aromatic 3-H), 7.19 (dd, 1H, *J* = 2.7/9 Hz, aromatic 4-H), 7.34–7.63 (m, 12H, aromatic H), 7.87 (d, 1H, *J* = 15.6 Hz, =CH-), 12.37 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ = 71.22 (CH_2), 114.61, 119.23, 119.59, 120.02, 124.76, 127.50, 128.11, 128.61, 128.64, 128.96, 130.89, 134.48, 136.79, 145.45, 150.73, 158.00 (aromatic C, CH=CH), 193.23 (CO); IR (KBr): ν = 1642 cm^{-1} (CO);

1-(5-Benzyloxy-2-hydroxyphenyl)-3-phenyl-1-propanone (3)[9]

A suspension of 0.03 g Pd on charcoal (5%) in 20 ml of ethyl acetate was presaturated with H_2. A solution of 12.0 g (36.4 mmol) **1** in 240 ml ethyl acetate was added and hydrogenated. After consumption of 815 ml H_2 the catalyst was filtered off and the resulting solution was evaporated to dryness. Crystallization from ethanol gave 10.28 g (85.2%) **3** as yellow crystalls; mp 77–78 °C. ^{1}H NMR ($CDCl_3$): δ = 3.03 (dd, 2H, *J* = 3.3/7.2 Hz, CH_2-Ph), 3.24 (dd, 2H, *J* = 3.3/7.2 Hz, CO-CH_2), 4.99 (s, 2H, CH_2-O), 6.92 (d, 1H, *J* = 9 Hz, aromatic 3-H), 7.15 (dd, 1H, *J* = 3/9 Hz, aromatic 4-H), 7.22–7.41 (m, 11H, aromatic H), 11.90 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ = 29.94 (CH_2-Ph), 40.09 (CO-CH_2), 71.07 (CH_2), 114.22, 118.73, 119.33, 125.07, 126.30, 127.47, 128.10, 128.36, 128.57, 128.62, 136.63, 140.64, 150.73, 156.97 (aromatic C), 204.01 (CO); IR (KBr) cm^{-1} 1657 (CO);

1-(5-Benzyloxy-2-oxiranylmethyloxyphenyl)-3-phenyl-1-propanone (4)[9]

To a solution of 9.00 g (27.1 mmol) **3** in 40 ml epichlorohydrine 1.30 g NaOH was added and the resulting suspension was refluxed for 1.5 h. The reaction mixture was diluted with water and extracted twice with diethyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to yield 10.2 g (97%) **4** as yellowish oil, which was put into the next reaction step without further purification. ^{1}H NMR ($CDCl_3$): δ = 2.68 (dd, 1H, *J* = 2.4/4.5 Hz, epoxide CH_a), 2.85 (t, 1H, *J* = 4.5 Hz, epoxide CH_b), 3.04 (t, 2H, *J* = 7.5 Hz, CH_2-Ph), 3.26–3.39 (m, 3H, $COCH_2$, epoxide CH), 3.96 (dd, 1H, *J* = 6/10.8 Hz, O-CH_a), 4.26 (dd, 1H, *J* = 3/10.8 Hz, O-CH_b), 5.03 (s, 2H, O-CH_2-Ph), 6.89 (d, 1H, *J* = 9 Hz, aromatic 3-H), 7.05 (dd, 1H, *J* = 3/9 Hz, aromatic 4-H), 7.16–7.43 (m, 11H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 30.22 (CH_2-Ph), 44.47, 45.12 (CO-CH_2-, epoxide CH_2), 49.86 (epoxide CH), 70.17, 70.52 (2 × CH_2-O), 114.52, 115.17, 120.50, 125.82, 127.45, 127.95, 128.29, 128.32, 128.50, 129.09, 136.66, 141.43, 151.74, 153.01 (aromatic C), 201.03 (CO); IR (KBr): ν = 1672 cm^{-1} (CO);

General procedure for preparation of amines 5a–c.

A solution of 5.0 mmol **4** in 15 ml of the corresponding amine was heated to reflux till the reaction was completed (TLC control). The reaction mixture was evaporated to dryness and the resulting oil purified via column chromatography (silica gel; CH_2Cl_2/methanol/NH_3conc. = 200/10/1) or crystallization.

General procedure for preparation of the hydrochlorides of 5a–d,6a–d and 7d.

The amine was dissolved in ethyl acetate and a 1 M solution of HCl in diethyl ether was added. The white precipitate was filtered off and purified via crystallization.

1-(5-Benzyloxy-2-(2-hydroxy-3-propylamino-propyloxy)phenyl)-3-phenyl-1-propanone (5a)

Yield: 71.0%; ^{1}H NMR ($CDCl_3$): δ = 0.91 (t, 3H, *J* = 7.5 Hz, CH_3), 1.50 (sext, 2H, *J* = 7.5 Hz, CH_2), 2.49–2.82 (m, 4H, CH_2-N-CH_2), 3.01 (t, 2H, *J* = 7.5 Hz, CH_2-Ph), 3.25–3.40 (m, 4H, CO-CH_2, OH, NH), 3.92–4.07 (m, 3H, O-CH_2-CH(O)), 5.00 (s, 2H, O-CH_2-Ph), 6.87–7.42 (m, 13H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 11.56 (CH_3), 22.74 (CH_2), 30.15 (CH_2-Ph), 45.01 (CO-CH_2), 51.39, 51.75 (CH_2-N-CH_2), 67.54 (CH), 70.56, 71.96 (O-CH_2,

O-CH_2-Ph), 114.60, 115.42, 120.54, 125.88, 127.45, 127.95, 128.29, 128.33, 128.47, 128.51, 136.65, 141.40, 152.13, 152.70 (aromatic C), 200.93 (CO);

***5a**-hydrochloride*: yield: 80.9%; mp 122–124 °C (ethyl acetate); Anal. ($C_{28}H_{33}NO_4$·HCl): C, H, N, Cl

*1-(5-Benzyloxy-2-(2-hydroxy-3-(1-piperidinyl)propyloxy)phenyl)-3-phenyl-1-propanone (**5b**)*

Yield: 71.7%; ^{1}H NMR ($CDCl_3$): δ = 1.35–1.65 (m, 6H, CH_2-CH_2-CH_2), 2.08–2.58 (m, 6H, CH_2-N-$(CH_2)_2$), 3.02 (t, 2H, *J* = 7.5 Hz, CH_2-Ph), 3.37 (t, 2H, *J* = 7.5 Hz, CO-CH_2), 3.50–3.90 (br., 1H, OH), 3.92–4.12 (m, 3H, O-CH_2-CH(O)-), 5.03 (s, 2H, O-CH_2-Ph), 6.90 (d, 1H, *J* = 9 Hz, aromatic 3-H), 7.07 (dd, 1H, *J* = 3/9 Hz, aromatic 4-H), 7.13–7.43 (m, 11H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 24.10, 26.03 (CH_2-CH_2-CH_2), 30.25 (CH_2-Ph), 45.60 (CO-CH_2), 54.48, 61.39 (CH_2-N-$(CH_2)_2$), 65.06 (CH), 70.57, 71.58 (2 × O-CH_2), 114.19, 115.22, 120.86, 125.81, 127.49, 127.94, 128.31, 128.36, 128.51, 136.77, 141.59, 152.49, 152.71 (aromatic C), 200.72 (CO); IR (KBr): ν = 1657 cm^{-1} (CO);

***5b**-hydrochloride:* yield: 89.2%; mp 169–171 °C (ethyl acetate); Anal. ($C_{30}H_{35}NO_4$·HCl): H, N, Cl; C: calcd 70.64, found 70.05.

*1-(5-Benzyloxy-2-(3-diisopropylamino-2-hydroxy-propoxy)phenyl)-3-phenyl-1-propanone (**5c**)*

Yield: 64.2%; ^{1}H NMR ($CDCl_3$): δ = 0.96 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 1.02 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 2.40 (dd, 1H, *J* = 9.9/13.2 Hz, CH_a-N), 2.65 (dd, 1H, *J* = 4.2/13.2 Hz, CH_b-N), 2.95–3.06 (m, 4H, CH_2-Ph, N-$(CH)_2$), 3.39 (t, 2H, *J* = 7.5 Hz, CO-CH_2), 3.82–4.07 (m, 4H, O-CH_2-CH(O), OH), 5.03 (s, 2H, O-CH_2-Ph), 6.93 (d, 1H, *J* = 9.0 Hz, aromatic 3-H), 7.07 (dd, 1H, *J* = 3.0/9.0 Hz, aromatic 4-H), 7.12–7.43 (m, 11H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 19.42 (CH_3), 22.26 (CH_3), 30.26 (-CH_2-Ph), 45.25, 47.34 (CO-CH_2, CH_2-N), 48.18 (N-$(CH)_2$), 65.32 (CH), 70.59, 72.22 (2 O-CH_2), 114.21, 115.17, 120.78, 125.79, 127.51, 127.95, 128.28, 128.35, 128.53, 128.66, 136.80, 141.54, 152.51, 152.71 (aromatic C), 201.09 (CO); IR (KBr): ν = 1662 cm^{-1} (CO);

***5c**-hydrochloride:* yield: 95.5%; mp 123–125 °C (ethyl acetate); Anal. ($C_{31}H_{39}NO_4$·HCl): C, H, N, Cl.

*1-(2-(3-(4-Benzyl-1-piperazinyl)-2-hydroxy-propoxy)-5-benzyloxy-phenyl)-3-phenyl-1-propanone (**5d**)*

To a solution of 1.5 g (3.9 mmol) **4** in 20 ml methanol 0.68 g (3.9 mmol) of *N*-benzylpiperazine was added and the reaction mixture was heated to reflux till the reaction was completed (TLC control). The solvent was evaporated to dryness and the resulting oil was purified via crystallization to give 1.67 g (76.6%) **5d** as colourless crystalls; mp 114–118 °C; ^{1}H NMR ($CDCl_3$): δ = 2.18–2.68 (m, 10H, CH_2-N, piperazine CH_2), 3.01 (t, 2H, *J* = 7.8 Hz, CH_2-Ph), 3.35 (t, 2H, *J* = 7.8 Hz, CO-CH_2), 3.51 (s, 2H, N-CH_2-Ph), 3.30–3.80 (br., 1H, OH), 3.95–4.08 (m, 3H, O-CH_2-CH(O)-), 5.03 (s, 2H, -O-CH_2-Ph), 6.89 (d, 1H, *J* = 9.0 Hz, aromatic 3-H), 7.06 (dd, 1H, *J* = 3.0/9.0 Hz, aromatic H), 7.11–7.43 (m, 16H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 30.26 (CH_2-Ph), 45.57 (CO-CH_2), 53.05 (piperazine CH_2), 60.59, 62.90 (CH_2-N), 65.26 (CH), 70.60, 71.52 (O-CH_2), 114.34, 115.28, 120.83, 125.84, 127.05, 127.49, 127.96, 128.19, 128.34, 128.53, 129.09, 136.77, 138.03, 141.58, 152.43, 152.78 (aromatic C), 200.09 (CO); IR (KBr): 1667 cm^{-1} (CO);

***5d**-hydrochloride*: yield: 90.2%; mp 168–170 °C (ethyl acetate); Anal. ($C_{36}H_{40}N_2O_4$·2HCl·H_2O): C, H, N, Cl.

*General procedure for preparation of amines **6a–d***

A suspension of 0.05 g Pd on charcoal (5%) was presaturated with H_2. A solution of 1.8 mmol **5** in methanol was added and hydrogenated till completion of H_2 consumption. The catalyst was filtered off and the solvent removed under reduced pressure. The resulting oil was purified via crystallization.

*1-(5-Hydroxy-2-(2-hydroxy-3-propylamino-propoxy)phenyl)-3-phenyl-1-propanone; 5-Hydroxy-propafenone (**6a**)*[8]

Yield: 56.3%; ^{1}H NMR ([D_6] DMSO): δ = 0.89 (t, 3H, *J* = 7.5 Hz, CH_3), 1.45 (sext, 2H, *J* = 7.5 Hz, CH_2-), 2.46 (t, 2H, *J* = 7.5 Hz, N-CH_2), 2.61–2.68 (m, 2H, -CH_2-N), 2.95 (t, 2H, *J* = 7.5 Hz, CH_2-Ph), 3.37 (t, 2H, *J* = 7.5 Hz, CO-CH_2), 3.23–3.42 (br., 1H, NH), 3.91–4.05 (m, 3H, O-CH_2-CH(O)), 4.80–5.20 (br., 2H, OH), 6.93–7.32 (m, 8H, aromatic H); ^{13}C NMR ([D_6] DMSO): δ = 11.72 (CH_3), 22.60 (CH_2), 29.73 (CH_2-Ph), 44.54 (CO-CH_2), 51.28, 52.40 (CH_2-N-CH_2), 68.04 (CH), 71.86 (O-CH_2), 114.75, 115.17, 120.20, 125.71, 128.18, 128.24, 128.37, 141.37, 150.78, 150.88 (aromatic C), 200.78 (CO);

***6a**-hydrochloride*: yield: 89.4%; mp 215–217 °C (ethyl acetate)

*1-(5-Hydroxy-2-(2-hydroxy-3-(1-piperidyl)-propoxy)phenyl)-3-phenyl-1-propanone (**6b**)*

Yield: 91.4%; ^{1}H NMR ([D_6] DMSO): δ = 1.37–1.68 (m, 6H, CH_2-CH_2-CH_2-), 2.32–2.59 (m, 6H, CH_2-N-$(CH_2)_2$-), 2.97 (t, 2H, *J* = 7.8 Hz, CH_2-Ph), 3.39 (t, 2H, *J* = 7.8 Hz, CO-CH_2), 3.95–4.12 (m, 3H, O-CH_2-CH(O)), 4.80–5.20 (br., 1H, OH), 6.96–7.08 (m, 3H, aromatic 3-H, 4-H, 6-H), 7.24–7.34 (m, 5H, phenyl-H), 9.34 (s, 1H, OH); ^{13}C NMR ([D_6] DMSO): δ = 22.14, 25.10 (CH_2-CH_2-CH_2), 29.77 (CH_2-Ph), 44.76 (CO-CH_2), 54.39, 61.50 (CH_2-N-$(CH_2)_2$), 65.95 (CH), 72.03 (O-CH_2), 114.75, 115.21, 120.26, 125.70, 128.18, 128.27, 141.38, 150.79, 150.94 (aromatic C), 200.62 (CO); IR (KBr): ν = 1656 cm^{-1} (CO);

***6b**-hydrochloride*: yield: 76.9%; mp 165–170 °C (ethyl acetate); Anal. ($C_{23}H_{29}NO_4$·HCl): C, H, N, Cl.

*1-(2-(3-Diisopropylamino-2-hydroxy-propoxy)-5-hydroxy-phenyl)-3-phenyl-1-propanone (**6c**)*

Yield: 99.3%; ^{1}H NMR ($CDCl_3$): δ = 0.98 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 1.04 (d, 6H, *J* = 6.6 Hz, 2 CH_3), 2.45 (dd, 1H, *J* = 9.6/13.5 Hz, CH_a-N), 2.67 (dd, 1H, *J* = 3.6/13.5 Hz, CH_b-N), 2.97–3.07 (m, 4H, N-$(CH)_2$, CH_2-Ph), 3.35 (t, 2H, *J* = 6.6 Hz, CO-CH_2), 3.89–4.03 (m, 3H, O-CH_2-CH(O)), 5.31 (br., 2H, 2 OH), 6.76 (d, 1H, *J* = 9.0 Hz, aromatic 3-H), 6.92 (dd, 1H, *J* = 3.0/9.0 Hz, aromatic 4-H), 7.12–7.26 (m, 6H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 19.31, 21.99 (CH_3), 30.20 (CH_2-Ph), 45.19, 47.42 (CO-CH_2, CH_2-N), 48.69 (N-CH), 65.42 (CH), 71.90 (O-CH_2), 114.20, 116.54, 120.83, 125.79, 128.17, 128.34, 141.47, 150.16, 151.88 (aromatic C), 201.53 (CO); IR (KBr): ν = 1668 cm^{-1} (CO);

***6c**-hydrochloride*: yield: 64.9%; mp 120–123 °C (ethyl acetate); Anal. ($C_{24}H_{33}NO_4$·HCl·1/2 H_2O): C, H, N, Cl.

*1-(2-(3-(4-Benzyl-1-piperazinyl)-2-hydroxy-propoxy)-5-hydroxy)phenyl)-3-phenyl-1-propanone (**6d**)*

Yield: 64.4%; ^{1}H NMR ([D_6] DMSO): δ = 2.18–2.48 (m, 10H, CH_2-N, piperazine H), 2.94 (t, 2H, *J* = 7.5 Hz, CH_2-Ph), 3.37 (t, 2H, *J* = 7.5 Hz, CO-CH_2), 3.47 (s, 2H, N-CH_2-Ph), 3.94–4.03 (m, 3H, O-CH_2-CH(O)), 4.86 (br., 1H, OH), 6.94–7.05 (m, 3H, aromatic 3-H, 4-H, 6-H), 7.14–7.40 (m, 10H, aromatic H), 9.30 (br., 1H, OH); ^{13}C NMR ([D_6] DMSO): δ = 29.74 (CH_2-Ph), 44.70 (CO-CH_2), 52.63, 53.36 (piperazine C), 61.08, 62.07 (CH_2-N), 66.38 (CH), 72.12 (O-CH_2), 114.80, 115.18, 120.23, 125.66, 126.81, 128.08, 128.22, 128.32, 128.76, 138.23, 141.35, 150.88 (aromatic C), 200.03 (CO); IR (KBr): ν = 1655 cm^{-1} (CO);

***6d**-hydrochloride*: yield: 88.5%; mp 132–134 °C (ethyl acetate); Anal. ($C_{29}H_{34}N_2O_4$·2HCl·H_2O): C, H, N; Cl: calcd. 12.54, found 11.87.

*1-(2-(3-(4-Benzyl-1-piperazinyl)-2-hydroxy-propoxy)phenyl)-3-phenyl-1-propanone (**7d**)*

For details see **5d**; instead of **4** 1-(2-(2,3-epoxypropoxy)phenyl)-3-phenyl-1-propanone[5] was used; yield: 65.7%; ^{1}H NMR ($CDCl_3$): δ = 2.22–2.61 (m, 10H, piperazine H, -CH_2-N-), 3.02 (t, 2H, *J* = 7.5 Hz, CH_2-Ph), 3.34 (t, 2H, *J* = 7.5 Hz, CO-CH_2), 3.51 (s, 2H, N-CH_2-Ph), 3.58 (s, 1H, -OH), 3.98–4.06 (m, 3H, O-CH_2-CH(O)), 6.94 (d, 1H, *J* = 8.0 Hz, aromatic 3-H), 7.00 (t, 1H, *J* = 8.0 Hz, aromatic 4-H), 7.12–7.36 (m, 10H, phenyl H), 7.43 (dt, 1H, *J* = 2.0/8.0 Hz, aromatic 5-H), 7.70 (dd, 1H, *J* = 2.0/8.0 Hz, aromatic 6-H); ^{13}C NMR ($CDCl_3$): δ = 30.28 (CH_2-Ph), 45.60 (CO-CH_2), 53.07 (piperazine C), 60.63, 62.93 (CH_2-N), 65.22 (CH), 70.86 (O-CH_2), 112.64, 120.99, 125.86,

127.07, 128.21, 128.29, 128.36, 129.11, 130.42, 133.43, 138.07, 141.63, 157.80 (aromatic C), 201.26 (CO); IR (KBr): $\nu = 1670\ cm^{-1}$ (CO);

7d-hydrochloride: yield: 82.4%; mp 159–162 °C (i-PrOH); Anal. ($C_{29}H_{34}N_2O_3 \cdot 2HCl \cdot 1/2\ H_2O$): C, H, N, Cl

MDR-modulating activity

Cell Lines and Culture Conditions

The CCRF-CEM T lymphoblast cell line, as well as the resistant line were obtained as described previously.[12] 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 1000ng/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 one month after washout of the selective agent as shown by flow cytometry using the MRK16 antibody (Behring Institut GesmbH, Vienna, Austria), 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 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).

Efflux Assay

Daunomycin efflux studies were performed as described previously.[11] 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 daunomycin (Sigma Chem. Comp., St. Louis, MO) at a final concentration of 3.0 μM. Cell suspensions were incubated at 37 °C for 30 min. Tubes were chilled on ice and pelleted at 500 ± g in an Eppendorf 5403 centrifuge (Eppendorf, Germany). Supernatants were removed and the cell pellet was resuspended in medium which was prewarmed to 37 °C and contained either no modulator or chemosensitizer at various concentrations depending on solubility and expected potency of the modifier. Eight concentrations (serial dilution 1:2.5) were tested for each modulator. After 1, 2, 3, and 4 min aliquots of the incubation mixture were transferred to tubes containing an equal volume of ice cold stop solution (RPMI1640 medium containing verapamil at a final concentration of 10 μg/ml). Zero time points were done by immediately pipetting daunomycin preloaded cells into ice cold stop solution. Parental CCRF-CEM cells were used as controls for simple plasma membrane diffusion, whereby initial daunomycin fluorescence levels were adjusted to be equal to initial levels observed in resistant cells. Samples drawn at the respective time points were kept in an ice water bath and measured within one hour on a Becton Dickinson Facscalibur flow cytometer (Becton Dickinson, Vienna, Austria). Viable cells were gated on basis of forward and side scatter. 5000 gated events were accumulated for the determination of mean fluorescence values.

The time dependent decrease in mean fluorescence of cells was determined in presence of various concentrations of modifier and the first order rate constants were calculated by fitting an exponential curve to the data points. Correction for simple diffusion was achieved by subtracting the efflux rates observed in the parental, non PGP-expressing line. EC_{50} values of all modifiers were obtained from dose response curve plots of efflux rate vs. modifier concentration. Data points of at least 3 independently performed experiments were fitted according to equation (1), where y is the rate of efflux determined as a function of modifier concentration c, y_i is the efflux rate absence of modulator and ME is the modulator efficacy.

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

Generally, interexperimental variation was below 20%.

References

[1] C.F. Higgins, I.D. Hiles, G.P.C. Salmonel, D.R. Gill, J.A. Downie, I.J. Evans, I.B. Holland, L. Gray, S.D. Buckel, A.W. Bell, M.A. Hermodson, *Nature* **1986**, *323*, 448–450.

[2] J.A. Kellen in *Reversal of multidrug resistance in cancer* (Ed.: J.A. Kellen), CRC Press, Boca Raton, FL, **1994**; pp 1–19.

[3] J.M. Ford, W.N. Hait, *Pharmacol. Rev.* **1990**, *42*, 155–199.

[4] G. Ecker, P. Chiba, *Exp. Op. Ther. Agents* **1997**, *7*, 589–599.

[5] P. Chiba, S. Burghofer, E. Richter, B. Tell, A. Moser, G. Ecker, *J. Med. Chem.* **1995**, *38*, 2789–2793.

[6] H.K. Kroemer, G. Mikus, T. Kronbach, U.A. Meyer, M. Eichelbaum, *Clin. Pharmacol. Ther.* **1987**, *46*, 377–394.

[7] G. v.Philipsborn, J. Gries, H.P. Hofmann, H. Kreiskott, R. Kretzschmar, *Arzneim. Forsch.* **1984**, *34*, 1489–1497.

[8] H. G. Hege, H. Lietz, J. Weymann, *Arzneim. Forsch.* **1984**, *34*, 843–849.

[9] G. Petrik, K. Schubert (Helopharm W. Petrik GmbH & Co. KG). EP 283540. **1988** [*Chem. Abstr.* **1989**, *110*, P75054].

[10] L.R. Pohl, R. Haddock, W.A. Garland, W.F. Trager, *J. Med. Chem.* **1975**, *18*, 513–519.

[11] P. Chiba, G. Ecker, D. Schmid, J. Drach, B. Tell, V. Gekeler, *Mol. Pharmacol.* **1996**, *49*, 1122–1130.

[12] V. Gekeler, G. Frese, A. Noller, R. Handgretinger, A. Wilisch, H. Schmidt, C. Muller, R. Dopfer, T. Klingebiel, H. Diddens, H. Probst, D. Niethammer, *Br. J. Cancer* **1992**, *66*, 507–517.

[13] P. Baricic, M. Mackov, Distributed by Milan Hudecek, P. Horova 18, 841 07 Bratislava, Slowakia.

[14] Tripos GmbH, Munich, Germany 1996

[15] S. Prets, A. Jungreithmair, P. Chiba, G. Ecker, *Sci. Pharm.* **1996**, *64*, 627–636.

[16] G. Ecker, P. Chiba, M. Hitzler, D. Schmid, K. Visser, H.-P. Cordes, J. Csöllei, J.K. Seydel, K.-J. Schaper, *J. Med. Chem.* **1996**, *39*, 4767–4774.

[17] H. Kubinyi, *J. Med. Chem.* **1977**, *20*, 625–629.

Received: June 27, 1997 [FP232]