Synthesis, Pharmacologic Activity, and Structure–Activity Relationships of a Series of Propafenone-Related Modulators of Multidrug Resistance†

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A series of [(*o*-acylaryl)oxy]propanolamines have been prepared and evaluated for multidrug resistance-reverting activity in a human tumor cell model. Structure–activity relationship studies indicate that the phenylpropiophenone moiety as well as the substitution pattern at the nitrogen atom is crucial for activity of the compounds. Incorporation of the ether oxygen into a benzofuran substructure, which renders the compound an arylethanolamine, decreased biologic activity. Highest activity could be observed with the arylpiparazines **4f–h**, which not only completely restored daunomycin sensitivity but also showed moderate activity in restoring etoposide toxicity.

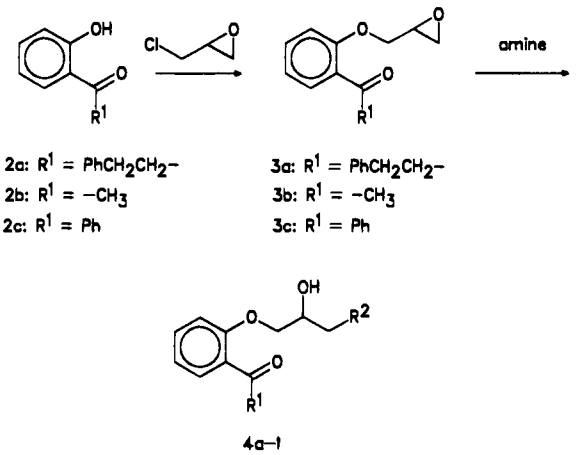
Introduction

Resistance to cytostatic drugs represents a major problem in the treatment of cancer. One cause of the lack of sensitivity of tumor cells to cytostatic drugs is the overexpression of a membrane glycoprotein termed P-glycoprotein (PGP).[1–3] This transmembrane protein functions as an ATP-dependent efflux pump[4] for a large variety of structurally and functionally diverse drugs, such as anthracyclines, epipodophyllotoxines, actinomycin D, vinca alkaloids, colchicine, and taxol. Presently available data suggest that PGP expression contributes to clinical resistance in human tumors.[5–8] Substances capable of reestablishing toxicity of chemotherapeutic agents via inhibition of this efflux pump have been reviewed recently.[9,10] These substances include ion channel blockers, steroids, cyclosporins, antipsychotic drugs like phenothiazines and thioxanthenes, and the antimalaria agent quinine. Selected compounds have been included in experimental chemotherapy regimens and are presently evaluated clinically[11–13] (for a recent review, cf. ref 14).

On basis of previously defined structural requirements,[15–17] we identified the class Ic antiarrhythmic agent propafenone (**1**) to be a highly effective agent in both restoring daunomycin sensitivity and enhancing accumulation of [^{3}H]daunomycin in the human P-glycoprotein expressing T-lymphoblastoid cell line CCRF-CEM vcr 100.[18,19]

The present study was undertaken to further improve the chemosensitizing potency of propafenone derivatives, and a substrate modeling concept was employed to gain further insight in the interaction of these substances with P-glycoprotein. It has recently been published for the group of thioxanthenes that incorporation of the nitrogen atom in a ring structure, such as piperidine or piperazine, enhances activity.[20] In addition, a computer-based analysis of substructures of various response modifiers suggested secondary amino groups, ethers, carbonyl groups, and benzyl groups as possible pharmacophoric substructures.[18] We, therefore, synthesized compounds which are structurally modified at the nitrogen atom and in the phenone moiety of propafenone and tested their chemosensitizing potency in an in vitro model system.

Scheme 1. Synthesis of Compounds **4a–l**



Chemistry

The preparation of the tertiary amines **4a–l** (Table 1) was carried out in analogy to the synthesis of propafenone (Scheme 1).[21] Thus, an appropriate *o*-hydroxyphenone (**2**) was reacted with epichlorohydrine to give the epoxides **3a–c**. Subsequent treatment with an amine yielded the corresponding propanolamines **4a–l**, which were converted to the hydrochlorides using standard procedures. The enantiomers of propafenone were prepared according to ref 22. The structurally constrained benzofurane **5** was prepared as previously described.[23]

MDR-Modulating Activity

Effect of Modulators on the Cytotoxicity of Daunomycin and Etoposide. The chemosensitizing effect of the compounds with respect to the natural product cytotoxic drugs daunomycin and etoposide was evaluated in P-glycoprotein expressing CCRF-CEM vcr 100 cells (Figures 1 and 2). The effect of toxins on

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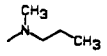
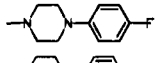
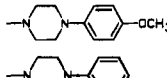
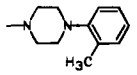
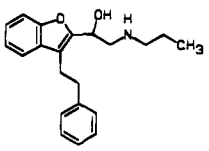
Table 1. Chemical Structure and Physical Data of Compounds 1, 4a–l, and 5

no	R^1	R^2	formula[a]	mp, °C	recryst. solvent
1	$PhCH_2CH_2$-	NC_3H_7	$C_{21}H_{27}NO_3 \cdot HCl$	175-177	MeOH
4a	$PhCH_2CH_2$-	$N(C_2H_5)_2$	$C_{22}H_{29}NO_3 \cdot HCl$	145-147	MeOH
4b	$PhCH_2CH_2$-		$C_{22}H_{29}NO_3 \cdot HCl$	106-108	EtAc
4c	$PhCH_2CH_2$-	$N(i\text{-}C_3H_7)_2$	$C_{24}H_{33}NO_3 \cdot HCl$	126-131[b]	MeOH
4d	$PhCH_2CH_2$-		$C_{22}H_{27}NO_3 \cdot HCl$	162-164	EtAc
4e	$PhCH_2CH_2$-		$C_{23}H_{29}NO_3 \cdot HCl$	153-155[b]	MeOH
4f	$PhCH_2CH_2$-		$C_{28}H_{31}FN_2O_3 \cdot 2HCl$	181-184	MeOH
4g	$PhCH_2CH_2$-		$C_{29}H_{34}N_2O_4 \cdot HCl$[c]	174-176	MeOH
4h	$PhCH_2CH_2$-		$C_{29}H_{34}N_2O_3 \cdot HCl$	162-165	i-PrOH
4i	$PhCH_2CH_2$-		$C_{22}H_{27}NO_4 \cdot HCl$[d]	144-147[b]	EtAc
4k	CH_3-		$C_{16}H_{23}NO_3 \cdot HCl$	131-134[e]	MeOH
4l	Ph-	$N(C_2H_5)_2$	$C_{20}H_{25}NO_3 \cdot HCl$	110-112	MeOH
5			$C_{21}H_{25}NO_2 \cdot HCl$	150-152	EtAc

[a] Satisfactory C, H, N, and Cl elemental analyses (±0.4%) were obtained. [b] See also ref 27. [c] H: calcd, 6.90; found, 6.36. [d] C: calcd, 65.10; found, 64.63. [e] See also ref 28.

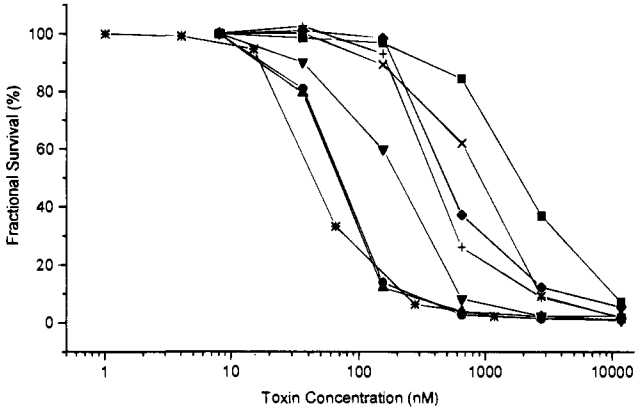


Figure 1. Toxicity of daunomycin to CCRF-CEM vcr 100 cells in the presence of various concentrations of **4f**: (*) wild type CCRF-CEM control, (×) 0.31 μM, (◆) 0.63 μM, (+) 1.25 μM, (▼) 2.5 μM, (●) 5 μM, (▲) 10 μM, and (■) CCRF-CEM vcr 100 control.

parental CCRF-CEM cells was not altered by the addition of propafenone modulators. The reversion potency (RP) expressed as the ratio of the ED_{50} for the toxin in the absence and presence of modifier is shown in Table 2. Since differences in activity of the compounds with respect to daunomycin as toxin are highest at 5 μM, the RP at this concentration of modifier is given. With the exception of **4k**, all substances showed remarkable potency in restoring toxicity of daunomycin in resistant CCRF-CEM vcr 100 cells with the arylpiperazines **4f–h** being the most potent compounds. The latter also proved to enhance toxicity of the epipodophyllotoxin etoposide, whereas all other compounds—including also verapamil—showed almost no activity as modulators of resistance to etoposide.

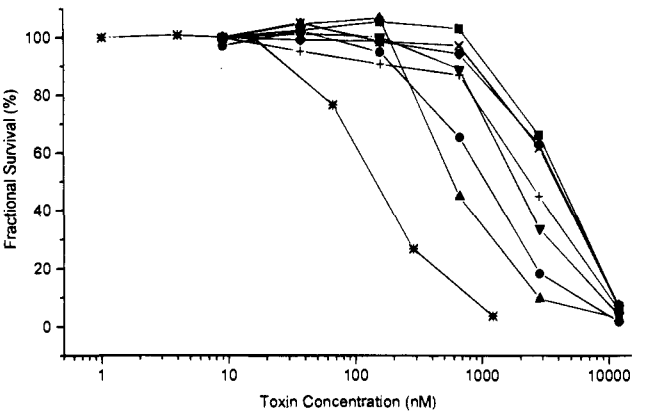


Figure 2. Toxicity of etoposide to CCRF-CEM vcr 100 cells in the presence of various concentrations of **4f**: (*) wild type CCRF-CEM control, (×) 0.31 μM, (◆) 0.63 μM, (+) 1.25 μM, (▼) 2.5 μM, (●) 5 μM, (▲) 10 μM, and (■) CCRF-CEM vcr 100 control.

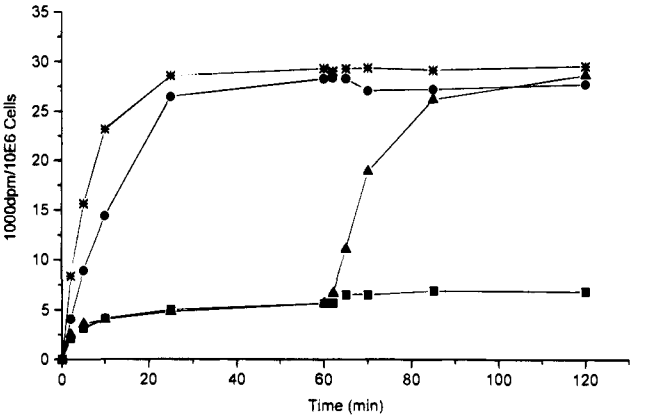


Figure 3. Effect of **4f** (5 μM) on the accumulation of [^{3}H]daunomycin in resistant CCRF-CEM vcr 100 cells: (*) wild type CCRF-CEM control, (●) 5 μM **4f** *30 min prior* to the addition of [^{3}H]daunomycin, (▲) 5 μM **4f** *60 min after* addition of [^{3}H]daunomycin, and (■) CCRF-CEM vcr 100 control.

Effect of Propafenone Analogs on the Accumulation of [^{3}H]Daunomycin in Resistant and Sensitive CCRF-CEM Cells. Uptake of radioactively labeled daunomycin in CCRF-CEM cells was evaluated (Figure 3). The percentage of uptake of [^{3}H]daunomycin into resistant CCRF-CEM vcr 100 cells in comparison to sensitive CCRF-CEM cells in the presence of various modifiers (5 μM) is given in Table 2. Accumulation of daunomycin was enhanced by all compounds tested with the arylpiperazines **4f–h** being the most active derivatives. The activity of modulators in toxicity assay and uptake seems to correlate, which is a strong hint that inhibition of P-glycoprotein is indeed the main mechanism of propafenone type modulators of classical MDR.

Structure–Activity Relationships

Verapamil, which was used as a reference substance in both toxicity and uptake assays, showed slightly higher activity than the lead compound propafenone (**1**). Both enantiomers and the racemate of the latter substance were equally potent, thus indicating lack of stereoselectivity for the modulation of drug resistance. Substances with tertiary amino groups (**4b,c**) had greater potency than propafenone itself. Comparison

Table 2. MDR-Modulating Activity of Compounds 1, **4a**–l, and **5**

no.	ED_{50} (nM)[a] daunomycine	RP	ED_{50} (nM)[a] etoposide	RP	uptake (%)[b]
wt control	33	42	220	15	100
vcr 100	1400		3500		0
verapamil	45	31	3400		79
1	88	16	na		65
4a	115	12	3400		68
4b	55	26	na		nt
4c	65	22	nt		82
4d	70	20	na		nt
4e	46	30	3300	1.1	83
4f	41	34	1024	3.4	92
4g	33	42	948	3.7	96
4h	43	33	648	5.4	96
4i	359	4	na		nt
4k	770	2	nt		nt
4l	150	9	na		nt
5	198	7	na		nt

[a] The ED_{50} values for toxins in the presence of 5 μM modifier are given. Individual data points were determined in triplicate, whereby six different toxin concentrations were used to estimate the individual ED_{50} values (see also Figures 1 and 2). Generally, interexperimental variation was below 15%. [b] The percentage of uptake of [^{3}H]daunomycin in resistant CCRF-CEM vcr 100 cells in comparison to sensitive CCRF-CEM cells is given (see also Figure 3); na, no activity; nt, not tested.

of **4a**,**d** shows that incorporation of the nitrogen into cyclic nonaromatic ring structures proved to enhance modulatory potency. Removal of the phenyl ring led to a compound with only one benzene moiety (**4k**) which showed an almost complete lack of activity. Insertion of an oxygen into the cyclic nitrogen-containing substructure of **4e** (**4i**) dramatically decreased reversion potency. Also, removal of the ethylene moiety between the two aromatic rings (**4l**) resulted in a decrease of activity.

Incorporation of the ether oxygen into a benzofurane moiety (**5**), which renders the compound an arylethanolamine, also gave rise to a decrease of RP. Highest activity could be achieved with the arylpiperazines **4f**–**h** containing three aromatic rings and a piperazine moiety. These compounds also showed moderate activity in restoring etoposide toxicity in multidrug resistant CCRF-CEM vcr 100 cells.

Conclusions

A series of [(*o*-acylaryl)oxy]propanolamines related to the class Ic antiarrhythmic agent propafenone was synthesized. These substances were evaluated as modulators of PGP-related multidrug resistance. The substances proved both to restore daunomycin toxicity and to enhance accumulation of radioactively labeled daunomycin in PGP-expressing CCRF-CEM vcr 100 cells. This indicates that the main mechanism of modulation of MDR by the propafenone analogs evaluated is indeed inhibition of P-glycoprotein. Structure–activity studies indicate that within the set of compounds tested the phenylpropiophenone moiety is important for maintaining high activity. Structural modifications leading to a benzophenone derivative as well as incorporation of the carbonyl C atom into a benzofuran moiety gave rise to a decrease of activity. Different substitution at the nitrogen atom indicates that this part of the molecule also plays a major role for activity. Highest activity could be achieved with the arylpiperazines **4f**–**h**, which showed almost complete reversion of resistance at a concentration of 5 μM. In contrast to verapamil, these derivatives also proved to enhance etoposide toxicity in resistant CCRF-CEM vcr 100 cells. Further studies on various substituted arylpiperazines as well as evaluation of cardiac activity of the new lead compounds are currently under progress.

Materials and Methods

Chemistry. Melting points were determined on a Kofler melting point apparatus and are uncorrected. Infrared spectra were recorded as thin films on salt discs on a Perkin Elmer 298 spectrophotometer. Mass spectra were performed on a Varian MAT 111A spectrometer by A. Nikiforov (Institut für Organische Chemie, Universität Wien, Wien, Austria). NMR spectra were recorded on Bruker AC 80, Bruker AM 400 WB (apparatus supplied by Fond zur Förderung der wissenschaftlichen Forschung, Project P6537C), and Varian Unity plus 300 spectrometers, using tetramethylsilane as internal standard. Microanalyses were determined by J. Zak and J. Theiner (Institut für Physikalische Chemie, Universität Wien, Wien, Austria).

General Procedure for the Synthesis of Epoxides 3a–c. *o*-Hydroxyphenone (**2**) (45.78 mmol) was dissolved in 100 mL of epichlorohydrine, and 50 mmol of powdered NaOH was added. The reaction mixture was refluxed for 12 h and evaporated to dryness. The resulting yellow oil was dissolved in diethyl ether and extracted twice with water. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The resulting yellow oil was put into the next reaction step without further purification.

1-[2-[(Oxiranylmethyl)oxy]phenyl]-3-phenyl-1-propanone (3a): yield 90.6%; ^{1}H NMR (chloroform-*d*) δ 2.60–3.00 (m, 2H, epoxide CH_2), 2.95–3.45 (m, 4H, $-CH_2CH_2-$), 3.20–3.60 (m, 1H, $-CH-$), 3.90–4.45 (m, 2H, $ArOCH_2-$), 6.85–7.80 (m, 9H, arom H).

1-[2-[(Oxiranylmethyl)oxy]phenyl]ethanone (3b): yield 56.2%; ^{1}H NMR (chloroform-*d*) δ 2.68 (s, 3H, $-CH_3$), 2.78 (dd, 1H, J = 2.7, 5.4 Hz, $-CH(O)CH_a-$), 2.95 (t, 1H, J = 5.4 Hz, $-CH(O)CH_b-$), 3.28–3.53 (m, 1H, $-CH(O)-$), 4.02 (dd, 1H, J = 5.4, 10.7 Hz, $ArOCH_a-$), 4.38 (dd, 1H, J = 2.7, 10.7 Hz, $ArOCH_b-$), 6.88–7.18 (m, 2H, H-3, H-5), 7.48 (dt, 1H, J = 2, 8 Hz, H-4), 7.75 (dd, 1H, J = 2, 8 Hz).

1-[2-[(Oxiranylmethyl)oxy]phenyl]-1-phenylmethanone (3c): yield 74.7%; ^{1}H NMR (chloroform-*d*) δ 2.40 (dd, 1H, J = 2.5, 5.0 Hz, $-CH(O)CH_a$), 2.63 (dd, 1H, J = 4.2, 5.0 Hz, $-CH(O)CH_b$), 2.98 (m, 1H, $-CH(O)-$), 3.95 (dd, 1H, J = 7.0, 10.0 Hz, $ArOCH_a-$), 4.15 (dd, 1H, J = 3.8, 10.0 Hz, $ArOCH_b-$), 6.90–7.90 (m, 9H, arom H).

General Procedure for the Synthesis of the Tertiary Amines 4a,c–e,i–l. Epoxide 3 (17.7 mmol) 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 crystallization or 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 dry diethyl ether and adding a 1 M solution of HCl in diethyl ether. The hydrochloride was filtered off and purified via crystallization.

1-[2-[3-(Diethylamino)-2-hydroxypropoxy]phenyl]-3-phenyl-1-propanone (4a): yield 55.6%; mp 63–64 °C (diethyl ether/methanol); ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 6H, 2 $-CH_3$), 2.53 (m, 6H, $-CH_2N(CH_2)_2-$), 2.40–3.20 (br, 1H, $-OH$), 2.87–3.50 (m, 4H, $PhCH_2CH_2-$), 3.83–4.20 (m, 3H, $-OCH_2CH(O)-$), 6.80–7.80 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.66 (2 $-CH_3$), 30.24 ($PhCH_2-$), 45.33 ($COCH_2$), 47.18 ($-CH_2N-$), 56.38 ($-N(CH_2)_2-$), 65.51 ($-CH(OH)$), 70.97 (OCH_2-), 112.58, 120.91, 125.82, 128.31, 128.34, 130.28, 133.36, 141.54, 157.66 (arom C), 201.40 (CO); IR (cm^{-1}) 1665 (CO); MS (70 eV) 355 (M^+, 0.6), 87 (100).

4a hydrochloride: mp 145–147 °C (methanol). Anal. ($C_{22}H_{30}NO_3Cl$) C, H, N, Cl.

1-[2-[3-(Diisopropylamino)-2-hydroxypropoxy]phenyl]-3-phenyl-1-propanone (4c): yield 73.1% (colorless oil); ^{1}H NMR ($CDCl_3$) δ 0.97 (d, 6H, 2 $-CH_3$), 1.15 (d, 6H, 2 $-CH_3$), 2.45 (m, 1H, $-NCH-$), 2.70 (m, 1H, $-NCH-$), 3.20–3.37 (m, 6H, $PhCH_2CH_2-$, $-CH_2N-$), 3.85–4.15 (m, 4H, $-OCH_2CH(OH)-$), 6.95–7.70 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 19.40

(4 $-CH_3$), 22.07 (2 $-CH-$), 30.29 ($PhCH_2-$), 45.20 ($COCH_2-$), 45.72 ($-CH_2N$), 65.38 ($-CH(OH)$), 71.49 (OCH_2-), 112.57, 120.88, 125.81, 128.30, 128.34, 128.57, 130.26, 133.29, 141.55, 157.75 (arom C), 201.62 (CO).

4c hydrochloride: mp 126–131 °C (methanol). Anal. ($C_{24}H_{34}NO_3Cl$) C, H, N, Cl.

1-[2-[2-Hydroxy-3-(1-pyrrolidyl)propoxy]phenyl]-3-phenyl-1-propanone (4d): yield 70.0%; mp 65–67 °C (methanol); ^1H NMR ($CDCl_3$) δ 1.65–2.00 (m, 4H, $-CH_2CH_2-$), 2.25–2.90 (m, 6H, $-CH_2N(CH_2)_2-$), 2.90–3.55 (m, 5H, $PhCH_2CH_2-$, $-OH$), 3.95–4.15 (m, 3H, OCH_2CH-), 6.95–7.90 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 23.56 ($-CH_2CH_2-$), 30.25 ($PhCH_2-$), 45.57 ($COCH_2-$), 53.97 ($-CH_2N$), 58.68 ($-N(CH_2)_2-$), 67.03 ($-CH(OH)$), 71.04 (OCH_2-), 112.64, 120.89, 125.79, 128.25, 128.31, 128.36, 130.35, 133.40, 141.61, 157.80 (arom C), 201.29 (CO); IR (cm^{-1}) 1665 (CO).

4d hydrochloride: mp 162–164 °C (ethyl acetate). Anal. ($C_{22}H_{28}NO_3Cl$) C, H, N, Cl.

1-[2-[2-Hydroxy-3-(1-piperidyl)propoxy]phenyl]-3-phenyl-1-propanone (4e): yield 51.8%; mp 82–83 °C (methanol); ^1H NMR ($CDCl_3$) δ 1.20–1.78 (m, 6H, $-CH_2CH_2CH_2-$), 1.95–2.70 (m, 7H, $-CH_2N(CH_2)_2-$, $-OH$), 2.85–3.55 (m, 4H, $PhCH_2CH_2-$), 3.88–4.28 (m, 3H, $-OCH_2CH(O)-$), 6.88–7.85 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 24.00, 25.90 ($-(CH_2)_3-$), 30.26 ($PhCH_2-$), 45.57 ($COCH_2-$), 54.54 ($-CH_2N-$), 61.49 ($-N(CH_2)_2-$), 64.99 ($-CH(OH)-$), 70.91 ($-OCH_2-$), 112.55, 120.91, 125.82, 128.23, 128.32, 128.36, 130.38, 133.42, 141.62, 157.77 (arom C), 201.26 (CO); IR (cm^{-1}) 1665 (CO); MS (70 eV) 367 (M^+, 1.8), 98 (100).

4e hydrochloride: mp 153–155 °C (methanol). Anal. ($C_{23}H_{30}NO_3Cl$) C, H, N, Cl.

1-[2-[2-Hydroxy-3-(4-morpholinyl)propoxy]phenyl]-3-phenyl-1-propanone (4i): yield 54.9%; mp 90–92 °C (methanol); ^1H NMR ($CDCl_3$) δ 2.10–2.90 (m, 7H, $-CH_2N(CH_2)_2-$, $-OH$), 2.90–3.26 (m, 4H, $PhCH_2CH_2-$), 3.66 (t, 4H, J = 4.8 Hz, $-CH_2OCH_2-$), 3.72–4.33 (m, 3H, $PhOCH_2CH-$), 6.90–7.77 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 30.21 ($PhCH_2$), 45.58 ($COCH_2$), 53.47 ($-CH_2N$), 61.15 ($N(CH_2)_2-$), 65.12 ($CH(OH)$), 66.86 ($-CH_2OCH_2-$), 70.70 ($PhOCH_2$), 112.64, 120.98, 125.51, 125.83, 128.14, 128.31, 130.36, 133.42, 141.58, 157.69 (arom C), 201.05 (CO); IR (cm^{-1}) 1670.

4i hydrochloride: mp 144–147 °C (ethyl acetate). Anal. ($C_{22}H_{28}NO_4Cl$) H, N, Cl; C: calcd, 65.10; found, 64.63.

1-[2-[2-Hydroxy-3-(1-piperidyl)propoxy]phenyl]ethanone (4k): yield 72.4%; ^1H NMR (chloroform-*d*) δ 1.25–1.75 (m, 6H, $-CH_2CH_2CH_2-$), 2.15–2.72 (m, 6H, $-CH_2N(CH_2)_2-$), 2.58 (s, 3H, $-CH_3$), 2.8–3.5 (br, 1H, $-OH$), 3.85–4.12 (m, 3H, $-OCH_2CH(O)-$), 6.80–7.05 (m, 2H, H-3, H-5), 7.37 (dt, 1H, J = 2, 8 Hz, H-4), 7.65 (dd, 1H, J = 2, 8 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 24.06, 25.96 ($-CH_2CH_2CH_2-$), 31.84 ($-CH_3$), 54.69 ($-CH_2N-$), 61.28 ($-N(CH_2)_2-$), 65.24 ($-CH(OH)-$), 71.10 (OCH_2-), 112.59, 120.69, 128.28, 130.28, 133.55, 158.06 (arom C), 199.69 (CO); IR (cm^{-1}) 1675 (CO).

4k hydrochloride: mp 131–134 °C (2-propanol). Anal. ($C_{16}H_{24}NO_3Cl$), C, H, N, Cl.

1-[2-[3-(Diethylamino)-2-hydroxypropoxy]phenyl]-1-phenylmethanone (4l): yield 55.9%; mp 81–82 °C (methanol); ^1H NMR ($CDCl_3$) δ 0.90 (t, 6H, J = 6.7 Hz, 2 $-CH_3$), 2.13 (d, 2H, J = 7.0 Hz, $-CH_2N-$), 2.41 (qu, 4H, J = 6.7 Hz, $-N(CH_2)_2-$), 2.8–3.5 (br, 1H, $-OH$), 3.56 (m, 1H, $-CH(OH)$), 3.80 (dd, 1H, J = 4.8, 8.0 Hz, $-OCH_a-$), 4.03 (dd, 1H, J = 5.3, 8.0 Hz, $-OCH_b-$), 6.90–7.90 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.83 (2 $-CH_3$), 47.10 ($-N(CH_2)_2-$), 55.66 ($-CH_2N$), 65.75 ($-CH(OH)$), 70.77 ($-OCH_2-$), 112.45, 121.20, 128.18, 128.88, 129.44, 132.21, 132.68, 138.37, 156.56 (arom C), 196.50 (CO); IR (cm^{-1}) 1670 (CO).

4l hydrochloride: mp 110–112 °C (methanol). Anal. ($C_{20}H_{26}NO_3Cl$), C, H, N, Cl.

General Procedure for Synthesis of Amines 4b,f–h. A solution of 8.0 mmol of epoxide **3a** and 8.1 mmol of amine in 2-propanol was refluxed for 6 h. The solvent was removed under reduced pressure and the crude product purified via crystallization. Formation of hydrochlorides was achieved as described before.

1-[2-[2-Hydroxy-3-(*N*-methyl-*N*-propylamino)propoxy]phenyl]-3-phenyl-1-propanone (*N*-methylpropafenone, 4b): yield 85%; ^1H NMR ($CDCl_3$) δ 0.75–1.1 (t, 3H, $-CH_3$), 1.1–1.7 (m, 2H, $-CH_2CH_3$), 2.0–2.87 (m, 7H, $-CH_2NCH_2-$, NCH_3), 2.87–3.55 (m, 4H, $PhCH_2CH_2CO$), 3.9–4.2 (m, 4H, $OCH_2CH(OH)-$), 6.75–7.75 (m, 9H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.59 ($-CH_3$), 20.34 ($-CH_2CH_3$), 30.24 ($PhCH_2-$), 41.91 (NCH_3), 45.42 ($COCH_2-$), 59.73, 60.37 ($-CH_2NCH_2-$), 65.66 ($-CH(OH)-$), 71.03 (OCH_2-), 112.58, 120.95, 125.81, 128.31, 128.33, 130.31, 133.41, 141.3, 157.74 (arom C), 201.46 (CO); IR (cm^{-1}) 1680 (CO).

***N*-Methylpropafenone hydrochloride (4b·HCl):** mp 106–108 °C. Anal. ($C_{22}H_{30}NO_3Cl$) C, H, N, Cl.

1-[2-[3-[4-(4-Fluorophenyl)-1-piperazinyl]-2-hydroxypropoxy]phenyl]-3-phenyl-1-propanone (4f): yield 69%; mp 101–103 °C (2-propanol); ^1H NMR ($CDCl_3$) δ 2.35–2.75 (m, 6H, $-CH_2N(CH_2)_2-$), 2.95–3.15 (m, 6H, $-(CH_2)_2NPh$, $PhCH_2$), 3.30–3.40 (m, 2H, $COCH_2-$), 3.40–3.60 (br, 1H, $-OH$), 4.00–4.15 (m, 3H, $-OCH_2CH(O)-$), 6.80–7.07 (m, 6H, arom H), 7.13–7.33 (m, 5H, arom H), 7.45 (dt, 1H, J = 2, 8 Hz, H-4), 7.70 (dd, 1H, J = 2, 8 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 29.71 ($PhCH_2-$), 45.01 ($COCH_2-$), 49.65, 52.57 (4C, piperazine C), 60.09 ($-CH_2N-$), 64.85 ($-CH(O)-$), 70.25 ($-OCH_2-$), 112.16 (C-3), 114.85, 115.07, 117.24, 117.32 (fluorophenyl C), 120.50 (C-5), 127.73 (C-1), 125.31, 127.82 (5C, phenyl C), 129.86 (C-6), 132.89 (C-4), 141.09 ($-CH_2C_{phenyl}$), 147.21 ($-NC_{phenyl}$), 155.47 (FC_{phenyl}), 157.85 (C-2), 200.63 (CO); MS (70 eV) *m/z* 462 (M^+, 4), 219 (12), 193 (100), 150 (17), 91 (16), 70 (60).

4f hydrochloride: mp 181–184 °C (methanol). Anal. ($C_{28}H_{33}N_2O_3FCl_2$) C, H, N, Cl.

1-[2-[2-Hydroxy-3-[4-(4-methoxyphenyl)-1-piperazinyl]-propoxy]phenyl]-3-phenyl-1-propanone (4g): yield 86%; mp 103–104 °C (2-propanol); ^1H NMR ($CDCl_3$) δ 2.35–2.80 (m, 6H, $-CH_2N(CH_2)_2-$), 2.95–3.15 (m, 6H, $-(CH_2)_2NAr$, $-CH_2Ph$), 3.25–3.45 (m, 2H, $COCH_2-$), 3.20–3.80 (br, 1H, $-OH$), 3.77 (s, 3H, $-OCH_3$), 4.05–4.15 (m, 3H, $-OCH_2CH(O)-$), 6.80–7.80 (m, 13H, arom H); ^{13}C NMR ($CDCl_3$) δ 30.29 ($PhCH_2-$), 45.59 ($COCH_2-$), 50.68, 53.26 (4C, piperazine C), 53.27 ($-OCH_3$), 60.68 ($-CH_2N-$), 65.38 ($-CH(O)-$), 70.83 ($-OCH_2$), 112.71, 114.45, 118.22, 121.05, 125.89, 128.30, 128.39, 130.43, 133.46, 141.65, 145.52, 153.90, 157.78 (arom C), 201.22 (CO); IR (cm^{-1}) 1680 (CO).

4g hydrochloride: mp 174–177 °C (methanol). Anal. ($C_{29}H_{35}N_2O_4Cl$) C, N, Cl; H: calcd, 6.90; found, 6.36.

1-[2-[2-Hydroxy-3-[4-(2-methylphenyl)-1-piperazinyl]-propoxy]phenyl]-3-phenyl-1-propanone (4h): yield 66%; mp 83–84 °C (2-propanol); ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H, $-CH_3$), 2.35–2.75 (m, 6H, $-CH_2N(CH_2)_2-$), 2.85–2.95 (m, 4H, $-(CH_2)_2NAr$), 3.0–3.1 (m, 2H, $PhCH_2-$), 3.29–3.45 (m, 2H, $COCH_2$), 3.50–3.80 (br, 1H, $-OH$), 4.05–4.15 (m, 3H, $-OCH_2CH(O)-$), 6.95–7.75 (m, 13H, arom H); ^{13}C NMR ($CDCl_3$) δ 17.84 ($-CH_3$), 30.30 ($PhCH_2-$), 45.69 ($COCH_2-$), 51.76 (4C, piperazine C), 60.82 ($-CH_2N-$), 65.23 ($-CH(O)-$), 70.85 ($-OCH_2-$), 112.64, 118.95, 121.03, 123.25, 125.89, 126.57, 128.23, 128.39, 130.47, 131.07, 132.58, 133.49, 141.67, 151.28, 157.81 (arom C), 201.18 (CO); MS (70 eV) *m/z* 458 (M^+, 6), 189 (100), 146 (12), 70 (32); IR (cm^{-1}) 1675 (CO).

4g hydrochloride: mp 162–165 °C (2-propanol). Anal. ($C_{29}H_{35}N_2O_3Cl$) C, H, N, Cl.

Pharmacology. Cell Lines and Culture Conditions. The human T-lymphoblastoid cell line CCRF-CEM was obtained from the American Type Culture Collection, Rockville, MD. The resistant CCRF-CEM vcr 100 cells were generously provided by V. Gekeler, Dept. of Physiology, University of Tübingen, Germany, and were obtained by stepwise selection in vincristine-containing medium without prior mutagenization.[24] Cells were kept in the presence of 100 ng/mL vincristine until 1 week prior to the experiments. Cells were routinely kept in RPMI 1640 medium supplemented with 10% fetal calf serum and penicillin G (100 U/mL)/streptomycin (100 μg/mL) under standard conditions.

MTT Assay. The assay is dependent on the cellular reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Chemical Corp., 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,[25] 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).

Uptake Experiments. Measurement of uptake of [^{3}H]-daunomycin in sensitive and resistant CCRF-CEM cells was performed as described by Kraupp et al.[26] using a rapid centrifugation method. Cells were suspended in RPMI 1640 medium containing 2% fetal calf serum at a density of 5×10^6 cells/mL. An amount of 0.4 μCi of [^{3}H-G]daunomycin (3.6 Ci/mmol, 1 mCi/mL; NEN, Austria) was added to 1 mL of cell suspension (5×10^6 cells), and unlabeled daunomycin was added to give a final concentration of 1 μmol/L. Modifier stock solutions were prepared in pure DMSO at 100× the final concentration and added 10 min prior to the addition of radioactivity. At 0, 2, 5, 10, 25, and 60 min, 1 mL aliquots were transferred into 1.8 mL Eppendorf tubes (Eppendorf, Hamburg, Germany) containing a bottom layer of dibutyl phthalate/dioctyl phthalate, 4 + 1 (w/w; density 1.035 g/mL), and cells separated from the incubation medium by centrifugation. After centrifugation, medium was removed from the tubes by suction and residual radioactivity was washed away with water. After the second washing step, water was removed together with most of the oil. Pellets were solubilized in 0.5 M NaOH/1% (w/v) SDS, and consecutively cell-associated radioactivity was determined in a LSC.

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