Substituted 4-Acylpyrazoles and 4-Acylpyrazolones: Synthesis and Multidrug Resistance-Modulating Activity†

Peter Chiba,§ Wolfgang Holzer,*,‡ Marion Landau,‡ Gerhard Bechmann,‡ Karin Lorenz,‡ Brigitte Plagens,‡ Manuela Hitzler,§ Elisabeth Richter,§ and Gerhard Ecker*,‡

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

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A series of 4-acyl-3-pyrazolone derivatives with a 3-substituted 2-hydroxy-3-aminopropyl chain attached to pyrazole *N*-1 (**7**−**20**) as well as isomeric 4-acyl-5-(3-substituted 3-amino-2-hydroxypropoxy)pyrazole derivatives (**5**, **6**) were synthesized, and their multidrug resistance (MDR)-modulating activity was measured using the daunomycin efflux assay. Reaction of N^1-substituted 4-acyl-3-pyrazolones (tautomer to 4-acyl-5-hydroxypyrazoles) with excessive epichlorohydrin and successive treatment with an appropriate amine resulted in *N*-alkylation and thus afforded the target pyrazolone derivatives **7**−**20**. In contrast, *O*-alkylation occurred upon reaction with 1 equiv of epichlorohydrin and subsequent treatment with amine leading to the corresponding 4-acyl-5-pyrazolyl ethers **5** and **6**. QSAR studies showed a good correlation of MDR-modulating activity with lipophilicity of the compounds. Inclusion of hydrogen bond acceptor strength and steric parameters as descriptors led to a QSAR equation with remarkably increased predictive power ($r^2_{cv} = 0.92$). Additionally, ortho substitution of the propanolamine side chain and the acyl moiety is favorable. Detailed NMR spectroscopic investigations were carried out with the title compounds.

Introduction

Development of unspecific mechanisms of resistance represents an increasing problem in cancer and antimicrobial therapy. The phenomenon that malignant cells acquire cross-resistance to a broad panel of drugs when exposed to only one of these agents has been termed multidrug resistance (MDR).[1] In most cases, MDR has been shown to be accompanied by a decrease in drug accumulation of resistant cells.[2] It seems to be widely accepted that one of the reasons of this decrease in intracellular drug levels is active efflux caused by P-glycoprotein (PGP), a membrane-bound protein of the ABC-transporter family.[3] PGP functions as an ATP-driven efflux pump, transporting a great variety of structurally and functionally diverse drugs, such as anthracyclines, epipodophyllotoxins, vinca alkaloids, colchicine, actinomycin D, and taxol.[4] Within the past decade several inhibitors of PGP-mediated drug efflux have been identified.[5] These so-called modulators of MDR lead to resensitization of multidrug-resistant tumor cells and include ion channel blockers such as verapamil,[6] dexniguldipine,[7] and amiodarone,[8] steroids,[9] cyclosporines,[10] phenothiazines,[11] and thioxanthenes[12] as well as the triazinoaminopiperidine S 9788[13] and the acridone carboxamide GF 120918.[14] Additionally, a large number of compounds are described in the patent literature.[15] We recently identified the class Ic antiarrhythmic agent propafenone (Figure 1) as being capable to restore drug sensitivity in multidrug-resistant CCRF-CEM vcr100 cells.[16] Structure−activity relationship studies showed that modifications in the phenone moiety such as reduction of the carbonyl group or different substitution patterns on the central aromatic ring decrease modulatory potency.[17] To gain further insights in the structural requirements necessary for interaction with PGP, we synthesized a series of structurally related pyrazole derivatives (**5**−**20**) and tested their ability to inhibit PGP-mediated efflux of daunomycin.

Chemistry and Structural Assignment

The synthesis of the compounds investigated in this study is outlined in Schemes 1 and 2, respectively. Precursors **1**−**4** can exist in several tautomeric forms, among them the OH (form A) and the NH (form B) isomers (Scheme 1).[18−20] The alkylation of such ambident species can lead to different substitution products, whereby the regioselectivity of the reaction strongly depends on the substrate, the alkylating agent, and the reaction conditions. Frequently, mixtures of regioisomeric reaction products are obtained. We found that reaction of **1**−**4** (after transformation into the corresponding sodium salts) with excessive epichlorohydrin and successive treatment of the intermediate epoxides with appropriate amines afforded the *N*-substituted pyrazolones **7**−**20**. In contrast, the use of only 1 equiv of epichlorohydrin in DMF and subsequent treatment of the intermediate epoxides **21** and **22** with amine led to the formation of *O*-alkyl products **5** and **6**. A possible explanation for this reaction behavior might be the fact that under equilibrium conditions (excessive epichloro-

† Dedicated to Prof. Dr. Gottfried Heinisch with best personal wishes on the occasion of his 60th anniversary.

* To whom correspondence should be addressed. Tel: +43-1-31336-8023 (W.H.), -8571 (G.E.). Fax: +43-1-31336-771. E-mail: holzer@merian.pch.univie.ac.at or ecker@speedy.pch.univie.ac.at.

‡ Institute of Pharmaceutical Chemistry.

§ Institute of Medical Chemistry.

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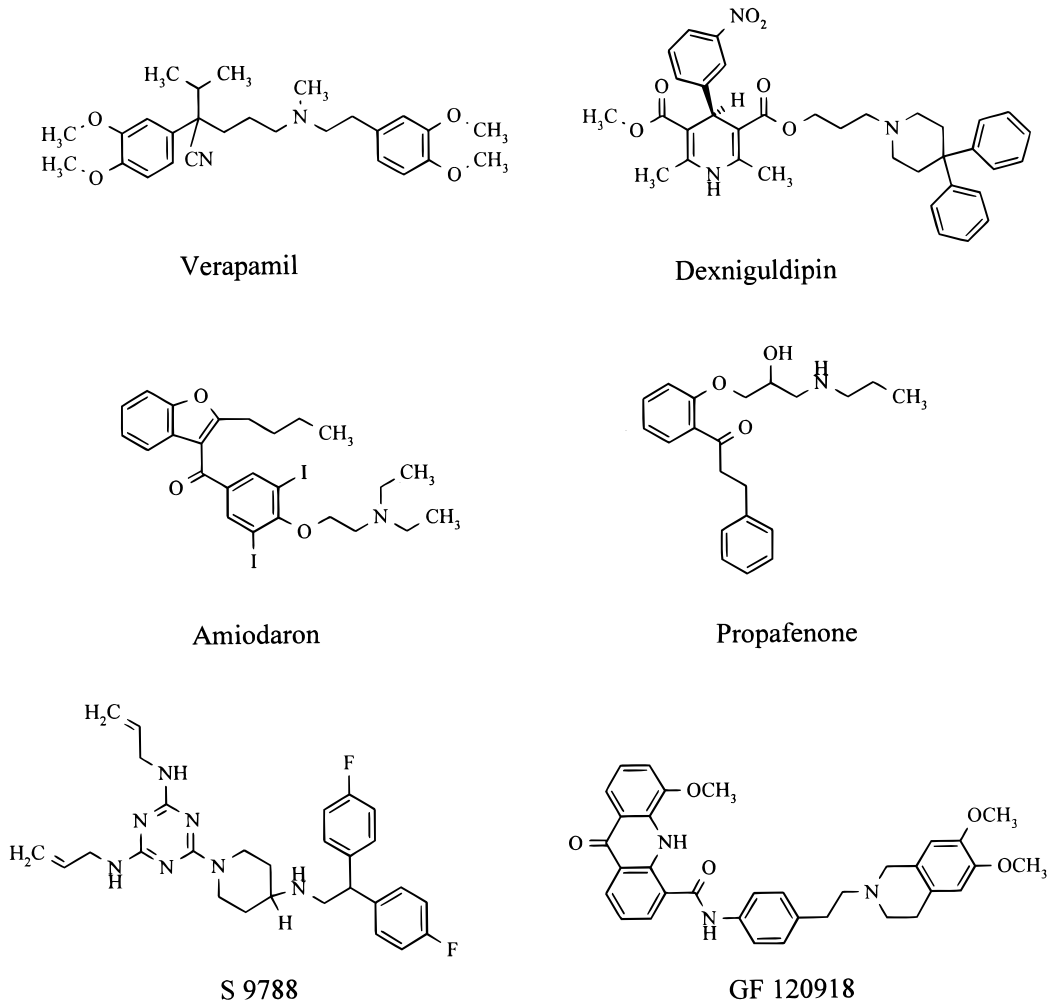
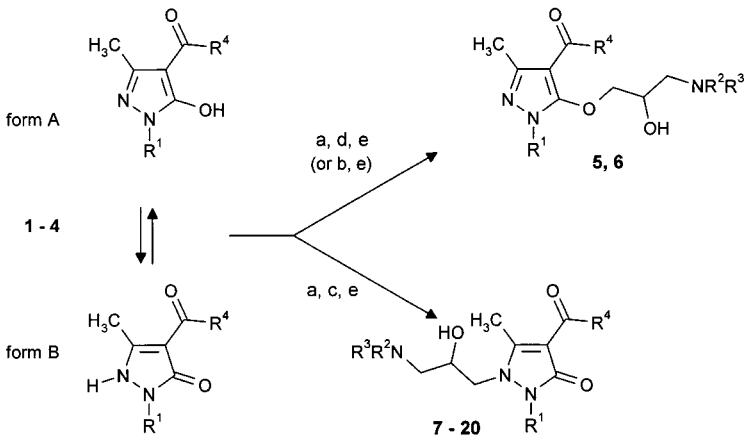


Figure 1. Chemical structures of selected MDR modulators.

Scheme 1[a]

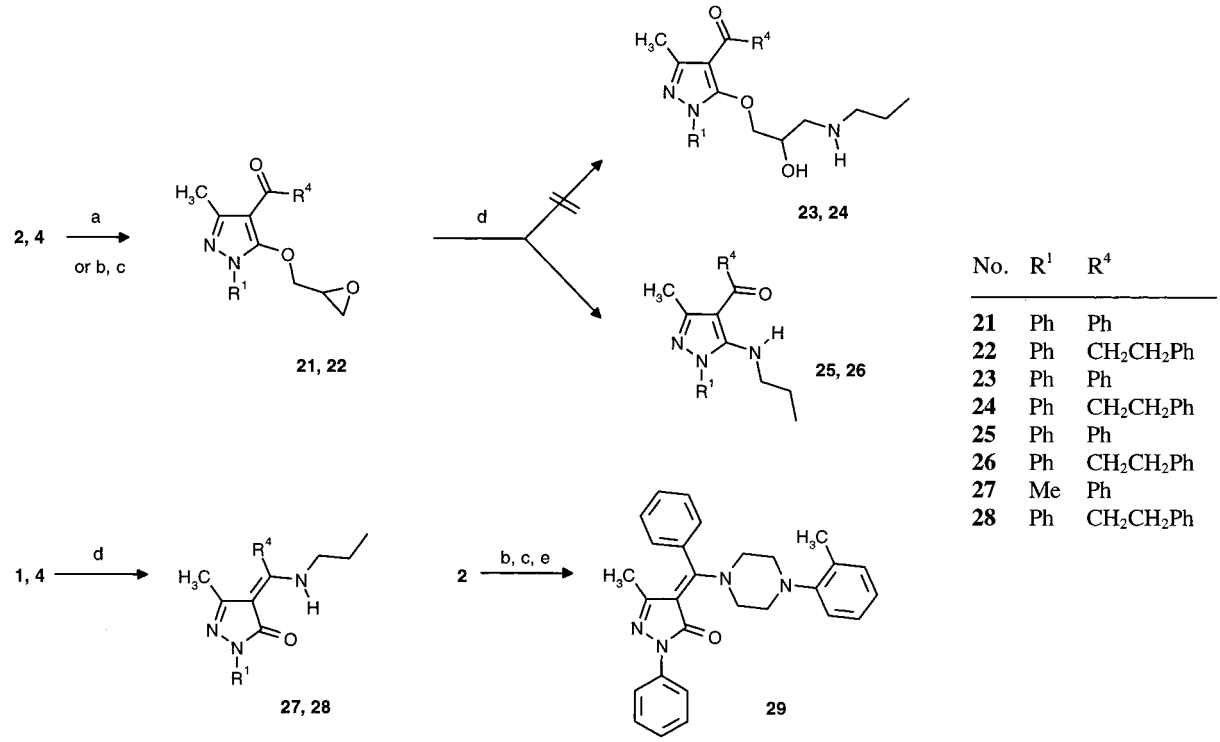


No.	R^1	NR^2R^3	R^4
1	Me	--	Ph
2	Ph	--	Ph
3	Ph	--	2-thienyl
4	Ph	--	CH_2CH_2Ph
5	Me	$N(i\text{-}Pr)_2$	Ph
6	Ph	$N(i\text{-}Pr)_2$	Ph
7	Me	$N(i\text{-}Pr)_2$	Ph
8	Ph	NH(n-Pr)	Ph
9	Ph	NH(i-Pr)	Ph
10	Ph	$N(i\text{-}Pr)_2$	Ph
11	Ph	NH(t-Bu)	Ph
12	Ph	4-(o-tolyl)-piperazin-1-yl	Ph
13	Ph	NH(n-Pr)	2-thienyl
14	Ph	NH(i-Pr)	2-thienyl
15	Ph	NH(t-Bu)	2-thienyl
16	Ph	NH(n-Pr)	CH_2CH_2Ph
17	Ph	NH(i-Pr)	CH_2CH_2Ph
18	Ph	NH(n-Bu)	CH_2CH_2Ph
19	Ph	NH(t-Bu)	CH_2CH_2Ph
20	Ph	4-(o-tolyl)-piperazin-1-yl	CH_2CH_2Ph

[a] (a) NaOMe/MeOH; (b) 2,3-epoxypropanol/diethyl azodicarboxylate/Ph_3P/THF (Mitsunobu conditions); (c) epichlorohydrin (excessive); (d) epichlorohydrin (1 equiv)/DMF; (e) R^2R^3NH.

hydrin) the reaction yields the more stable alkylation product. Semiempirical MO calculations (AM1)[21] revealed the primary *N*-alkylation products to be thermodynamically more stable than their corresponding *O*-isomers of type **21** and **22,** whereas under kinetic control obviously *O*-alkylation takes place.

Scheme 2[a]



No.	R^1	R^4
21	Ph	Ph
22	Ph	CH_2CH_2Ph
23	Ph	Ph
24	Ph	CH_2CH_2Ph
25	Ph	Ph
26	Ph	CH_2CH_2Ph
27	Me	Ph
28	Ph	CH_2CH_2Ph

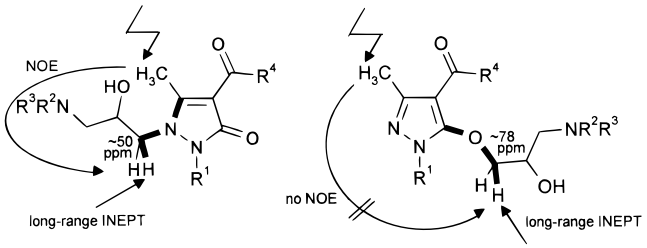
[a] 2,3-Epoxypropanol/diethyl azodicarboxylate/Ph_3P/THF (Mitsunobu conditions); (b) NaOMe/MeOH; (c) epichlorohydrin (1 equiv)/DMF; (d) *n*-propylamine; (e) *N*-(*o*-tolyl)piperazine.

An alternative, selective approach to **21** and **22** was also possible via reaction of **2** and **4**, respectively, with 2,3-epoxypropanol under Mitsunobu conditions according to ref 22 (Scheme 2). Surprisingly, this synthetic route did not permit access to the corresponding *n*-propylamino derivatives **23** and **24** as treatment of **21** and **22** with *n*-propylamine—under formal loss of the 2,3-epoxypropoxy group—led to the 5-(propylamino)-pyrazoles **25** and **26**. The latter compounds were not available from treatment of precursors **1** and **4** with *n*-propylamine, as this reaction afforded 4-[(propylamino)methylene]pyrazoles **27** and **28**, which formally are structural isomers to **25** and **26**. However, besides traces of *N*-substitution product **12**, 4-(aminomethylene)pyrazole **29** was isolated as the main component of the reaction mixture resulting from treatment of the sodium salt of **2** with 1 equiv of epichlorohydrin and successive heating with *o*-tolylpiperazine.

The structure of all novel compounds was confirmed by IR, MS, and mainly NMR spectroscopic methods. Complete and unambiguous assignments for all ^1H and ^{13}C resonances could be achieved on the basis of chemical shift considerations, coupling information (APT[23] and gated decoupled ^{13}C NMR spectra), and NOE difference,[24] COSY-45,[25] HMQC,[26] and 1D-TOCSY[27] spectra as well as on 1D-HETCOR[28] and long-range INEPT experiments[29] with selective DANTE excitation (Chart 1).

Discrimination between *N*-substitution products **7**−**20** and *O*-substitution products **5** and **6** was achieved considering ^{13}C chemical shifts (**7**−**20**, δ N1-**C**H_2- ~ 50 ppm; corresponding δ C5-O**C**H_2- of **5**, **6** ~ 78 ppm), NOE difference experiments (positive NOEs on signals of protons due to the amino alcohol chain upon irradiation of the 5-Me resonance with compounds **7**−**20**, Chart 2),

Chart 1. NOEs and Long-Range ^1H,^{13}C Correlations Used for the Structural Assignment of Compounds **7**−**20**, **5**, and **6**



and selective long-range INEPT experiments (detection of the long-range couplings ^3J(**C**-5,N1-C**H**$_2$) for **7**−**20** and ^3J(**C**-5,5-OC**H**$_2$) for **5** and **6**, Chart 1). *Z*-Configuration (regarding the exocyclic C=C bond) of compounds **27**−**29** follows from NOEs beween 5-Me and Ph H-2″,6″ (**27**−**29**) and CH_2-CH_2Ph (**28**). The large chemical shift (δ > 11 ppm) of the NH proton in compounds **27** and **28** provides a hint that these compounds are stabilized by an intramolecular hydrogen bond between NH and pyrazolone C=O.[18]

Calculation of Physicochemical Parameters

The log *P* values were calculated according to the method of Ghose and Crippen[30] using the software package MOLGEN.[31] Molecules were generated using the builder function and energetically minimized with the optimization function. Conformationally independent lipophilicity values were calculated.

The hydrogen bond acceptor strength of the exocyclic carbonyl oxygen was calculated using the software package HYBOT.[32] The corresponding C_a values were taken as a measure of the hydrogen bond acceptor strength.

Chart 2. Numbering of Nuclei Used for the Assignment of NMR Spectra

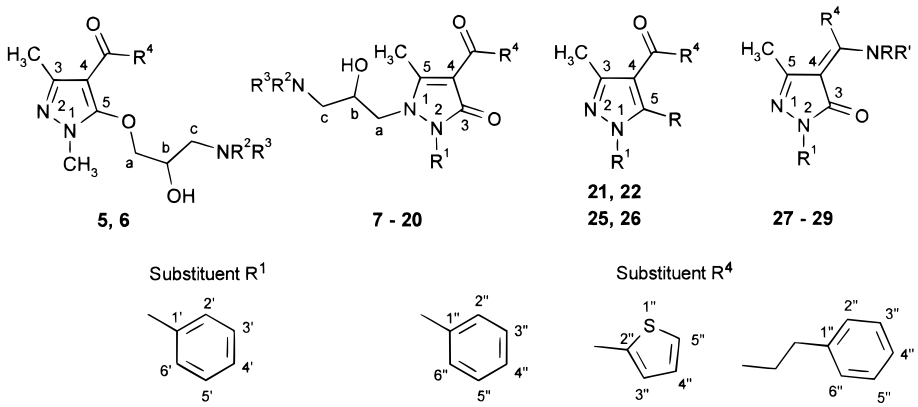


Table 1. Physicochemical Parameters and MDR-Modulating Activity of Compounds **5**−**20**

					EC_{50} (μM; ±SD)	
no.	calcd log *P*	C_a	*Ch*	*L*	daunomycin[a]	rhodamine 123[b]
5	2.62	1.68	−0.402	3.00	1.21 (±0.12)	nd[c]
6	4.28	1.68	−0.402	6.28	1.06 (±0.15)	2.22 (±0.34)
7	1.20	1.68	−0.402	3.00	24.12 (±7.31)	nd
8	1.99	1.68	−0.402	6.28	28.21 (±9.89)	64.55 (±23.25)
9	1.82	1.68	−0.402	6.28	83.18 (±8.11)	225.51 (±45.50)
10	2.86	1.68	−0.402	6.28	12.59 (±0.78)	21.91 (±0.97)
11	2.04	1.68	−0.402	6.28	81.28 (±10.28)	167.52 (± 52.50)
12	3.86	1.68	−0.402	6.28	0.89 (±0.16)	2.43 (±0.06)
13	1.59	1.68	−0.409	6.28	72.44 (±7.31)	173.61 (±112.60)
14	1.01	1.68	−0.409	6.28	123.03 (±7.93)	648.05 (±176.45)
15	1.22	1.68	−0.409	6.28	72.44 (±8.98)	94.74 (±11.43)
16	2.45	1.73	−0.412	6.28	6.31 (±0.54)	15.19 (±0.50)
17	2.28	1.73	−0.412	6.28	11.75 (±1.20)	34.19 (±10.41)
18	2.86	1.73	−0.412	6.28	2.69 (±0.20)	4.02 (±0.70)
19	2.50	1.73	−0.412	6.28	7.76 (±0.61	17.84 (±2.71)
20	4.32	1.73	−0.412	6.28	0.21 (±0.05)	nd
propafenone					0.32 (± 0.04)	nd
verapamil					0.12 (± 0.03)	nd
niguldipine					0.06 (±0.01)	nd

[a] Inhibition of daunomycin efflux from multidrug-resistant CEM vcr1000 human T-lymphoblasts. [b] Inhibition of rhodamine 123 efflux from *mdr1* transfectant L5178Y VMDR1 C.06 mouse lymphoma cells. [c] nd, not determined.,

The partial charge of the exocyclic carbonyl oxygen was calculated using the molecular modeling software package SYBYL.[33] Thus, the compounds were generated and minimized with the Powell algorithm (1000 iterations) using the Tripos force field and Gasteiger−Hückel charges. Charges were calculated using the Gasteiger−Hückel option.

Multiple linear regression analysis was performed using an in-house software package developed by K.-J. Schaper, Borstel, Germany. Values in parentheses brackets generally correspond to the 95% confidence interval.

MDR-Modulating Activity

The efflux assay is a direct and accurate functional method to measure inhibition of PGP-mediated transmembrane transport.[17] Both inhibition of daunomycin efflux in the resistant human T-lymphoblast cell line CEM vcr1000[34] as well as inhibition of rhodamine 123 efflux in the transfectant mouse lymphoma line L5178Y VMDR1 C.06[17] were used to characterize the MDR-modulating activity of our compounds. The time-dependent decrease in mean cellular fluorescence was determined in the presence of various concentrations of modifier, and the first-order rate constants (V_{max}/K_m) were calculated by nonlinear 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 V_{max}/K_m vs modifier concentration. Values are given in Table 1 and represent the mean of at least five independently performed experiments. Generally, interexperimental variation was below 20%.

Results and Discussion

A series of phenylpyrazolone analogous propafenone derivatives were synthesized and tested for their MDR-modulating activity. Table 1 summarizes the EC_{50} values for inhibition of PGP-mediated rhodamine 123 efflux in transfectant L5178YVMDR1 C.06 cells. These values are highly correlated with those obtained for daunomycin efflux in CEM vcr1000 cells, which were selected stepwise in vincristine-containing medium (Figure 2). This demonstrates that the EC_{50} values are a measure of modulator activity and are both toxin- and cell line-independent. In addition, resistance in the vincristine-selected cell line is due to PGP expression. The most active compound tested is the arylpiperazine **12** followed by the diisopropyl derivative **6**. Similar results were found for propafenones with the analogous nitrogen substituents.[16] In general, activities of phenylpyrazolones are lower than those observed for cor-

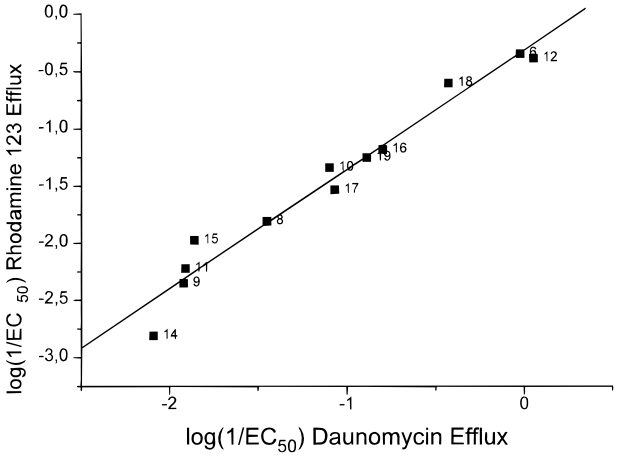


Figure 2. Plot of $\log(1/EC_{50})$ values of compounds **5**−**19** obtained in rhodamine 123 efflux studies from transfectant L5178Y VMDR1 C.06 mouse lymphoma cells vs those obtained in daunomycin efflux studies from CEM vcr1000 human T-lymphoblast cells. An excellent correlation ($\log(1/EC_{50})_{Rh123} = 1.04 \log(1/EC_{50})_{dauno} - 0.31$; $r = 0.98$, $n = 13$) is obtained.

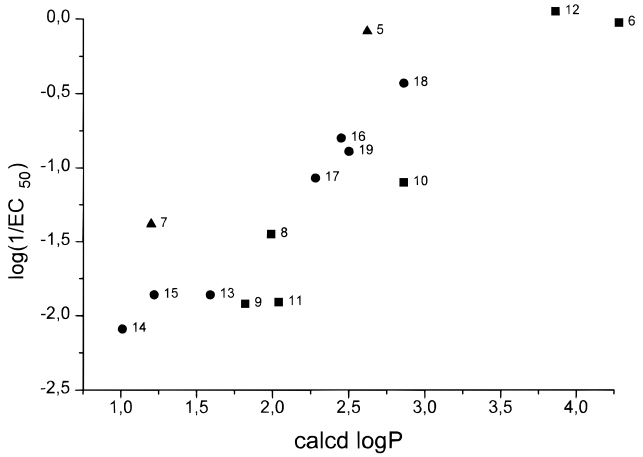


Figure 3. Correlation of MDR-modulating activity of compounds **5**−**19** (expressed as $\log(1/EC_{50})$ values of daunomycin efflux inhibition) vs lipophilicity of the molecules: (■) benzoyl derivatives **6** and **8**−**12**, (●) phenylpropionyl (**16**−**19**) and thienoyl (**13**−**15**) derivatives, (▲) *N*-methyl analogues **5** and **7**.

responding propafenones. Benzophenone derivatives **5**−**12** were obtained by shortening the distance between the two aromatic systems. In comparison to phenylpropionyl derivatives **16**−**19**, this modification results in a decrease of activity by a factor of 5−10 (**8** vs **16**, **9** vs **17**, and **11** vs **19**). Replacement of the benzoyl residue by 2-thienoyl (**15** vs **11**, **14** vs **9**, and **13** vs **8**) leads to a further decrease of potency. Replacement of the *N*-phenyl group by a methyl group does not remarkably influence activity (**5** vs **6** and **7** vs **10**). A pairwise comparison of **10** and **6** on one hand and **7** and **5** on the other hand indicates that the pyrazolyloxypropanolamines **5** and **6** (benzoyl substituent in ortho position to the ether oxygen) are more active by at least an order of magnitude than the isomeric pyrazolones **7** and **10** (benzoyl substituent and propanolamine side chain in meta position). The requirement for both an ether oxygen linker group between the heteroaromatic ring and the propanolamine side chain or the different substitution pattern on the heteroaromatic ring could account for this result. For propafenones the latter has been shown to lead to decreased activity of meta and para derivatives.[17]

Overall lipophilicity was shown to be a major predictive parameter for activity of propafenone type modulators.[35] Figure 3 shows a plot of calculated log *P* values of pyrazoles vs biological activity. Although a trend of increased activity with increased lipophilicity is apparent (eq 1), the whole set of compounds seems to fall into three different groups. The first contains the benzoyl derivatives **6** and **8**−**12**. A second group of compounds comprises the phenylpropionyl derivatives **16**−**19** and the thienyl compounds **13**−**15**, whereas the *N*-methyl derivatives **5** and **7** show markedly higher activity/lipophilicity ratios.

$$\log(1/EC_{50}) = 0.69(\pm 0.24) \log P - 2.72(\pm 0.60) \quad (1)$$

$$n = 15,\ r = 0.86,\ s = 0.39,\ F = 38.54;\ r^2_{cv} = 0.68$$

The decrease in the log *P*/log potency ratio for phenyl analogues might be due to steric hindrance. To test this hypothesis we introduced the STERIMOL parameter L[36] to describe the structural difference of the methyl and phenyl derivatives (eq 2). The regression coefficient is negative, which provides additional evidence for steric hindrance. Nevertheless, using an indicator variable for the *N*-methyl analogues **5** and **7** ($I = 1$, else $I = 0$) would lead to an equation with identical predictiveness, since only two different values for L are present in the data set.

$$\log(1/EC_{50}) = 0.74(\pm 0.18) \log P - 0.24(\pm 0.14)L - 1.43(\pm 0.87) \quad (2)$$

$$n = 15,\ r = 0.94,\ s = 0.28,\ F = 45.06;\ r^2_{cv} = 0.83$$

As shown previously for propafenones, hydrogen bond acceptor strength of the phenone carbonyl oxygen seems to be an additional and independent predictive parameter for activity.[17] Thus, we calculated the hydrogen bond acceptor strength (C_a) of the exocyclic acyl carbonyl oxygen using the software package HYBOT. Nevertheless, the calculation of C_a values for the carbonyl group in the benzoyl and thienoyl series leads to identical results, which seems rather unlikely. Since hydrogen bond acceptor strength is correlated with the charge of the corresponding atoms, the charges (Ch) of the acyl carbonyl oxygens were calculated using the Gasteiger−Hückel algorithm. By including this additional parameter into a multiple linear regression analysis, we obtained an improved equation with good predictive power (eq 3; Figure 4):

$$\log(1/EC_{50}) = 0.82(\pm 0.11) \log P - 50.24(\pm 24.12)Ch - 0.32(\pm 0.10)L - 21.52(\pm 9.66) \quad (3)$$

$$n = 15,\ r = 0.98,\ s = 0.17,\ F = 87.17;\ r^2_{cv} = 0.92$$

Scaling of the *x*- and *y*-parameters, which reflects the contribution of each regression coefficient in a normalized way, yielded eq 4:

$$\log(1/EC_{50}) = 1.02 \log P - 0.31Ch - 0.50L \quad (4)$$

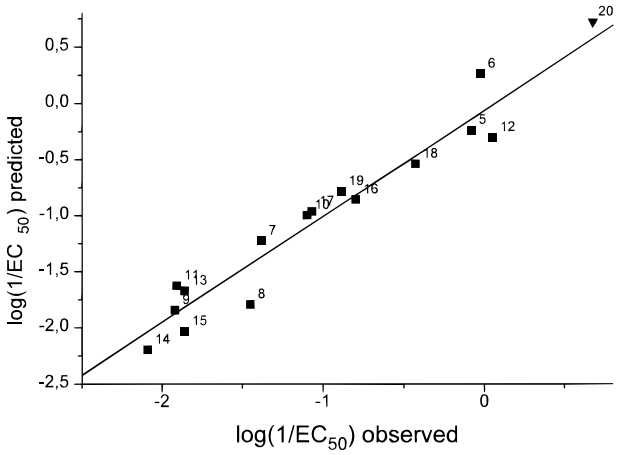


Figure 4. Plot of observed vs predicted MDR-modulating activity (expressed as $\log(1/EC_{50})$ values using the daunomycin efflux assay) for compounds **5**−**19** (■) using eq 6. Predicted values were obtained with a leave-one-out cross-validation procedure. Compound **20** also is shown (▼).

Table 2. Calculated log *P* Values and MDR-Modulating Activity of Byproducts **25**−**29**

no.	calcd log *P*	EC_{50} (μM; ±SD)[a]
25	3.95	11.03 (±2.65)
26	4.32	8.38 (±0.99)
27	1.73	272.50 (±37.50)
28	4.18	3.79 (±0.48)
29	5.27	0.68 (±0.05)

[a] Inhibition of daunomycin efflux from multidrug-resistant CEM vcr1000 human T-lymphoblasts.

To further test this hypothesis, the phenylpropionyl analogue (**20**) of compound **12**, which should show remarkably high MDR-modulating activity, was synthesized and tested in the daunomycin efflux assay (Table 1). Indeed, the observed EC_{50} value ($EC_{50} = 0.21$) is in excellent agreement to that calculated using eq 3 ($EC_{50} = 0.19$).

In addition, the PGP-inhibitory activity of the byproducts **25**−**29** was tested. The corresponding values are given in Table 2. All compounds showed lower log *P*/log potency ratios than those obtained for compounds **5**−**20**. This might be due to the fact, that the "basic" nitrogen atom in **25**−**29** has to be considered as a vinylogous amide nitrogen rather than an alkylamine.

Conclusions

Phenylpyrazoles of type **5**−**19** show a dependence of potency on lipophilicity of the compounds similar to the group of propafenones, whereby high lipophilicity is associated with high activity. Ortho substitution of the propanolamine side chain and the acyl moiety is favorable. The QSAR equation with the highest predictive power is obtained when hydrogen bond acceptor strength and steric parameters are included as descriptors. Using this equation, the activity of the additionally synthesized derivative **20** was predicted with excellent precision.

Materials and Methods

Chemistry. Melting points were determined on a Reichert-Kofler hot-stage microscope and are uncorrected. Elemental analyses were performed by Mikroanalytisches Laboratorium, Institute of Physical Chemistry, University of Vienna. Mass spectra were obtained on a Varian MAT 311A instrument (EI, 70 eV). NMR spectra were recorded on a Bruker AC80 spectrometer (80 MHz for ^{1}H, 20 MHz for ^{13}C) or a Varian UnityPlus spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C) from $CDCl_3$ solutions at 28 °C. The center of the solvent signal was used as an internal standard which was related to TMS with δ 7.26 ppm (^{1}H) and δ 77.0 ppm (^{13}C). The numbering of nuclei used for the assignments of ^{1}H and ^{13}C NMR spectra is given in Chart 2. Column chromatographic separations were performed on Merck Kieselgel 60 (70−230 mesh). Light petroleum refers to the fraction of bp 50−70 °C. Yields given below are not optimized and refer to analytically pure material.

General Procedure for the Preparation of 5-Alkoxy-1*H*-pyrazole Derivatives 5 and 6. Sodium (230 mg, 10 mmol) was dissolved in 10 mL of dry MeOH with stirring. After the evolution of hydrogen had ceased, 10 mmol of educt (**1**,[37] 2.16 g; **2**,[38] 2.78 g) was added, and the resulting solution was evaporated to dryness under reduced pressure. The residue was dissolved in 10 mL of dry DMF, then 925 mg (10 mmol) of epichlorohydrin was added, and the mixture was refluxed (110 °C) for 4 h. After filtration, the solvents were removed under reduced pressure, the residue was treated with 10 mL (71 mmol) of diisopropylamine, and the mixture was refluxed for 24 h. After filtering, the excessive amine was evaporated, and the residue was purified as described below.

[5-(3-(Diisopropylamino)-2-hydroxypropoxy)-1,3-dimethyl-1*H*-pyrazol-4-yl]phenylmethanone (5). Column chromatography (eluent: EtOAc−MeOH, 5:1) afforded a tan oil: yield 31%; MS *m*/*z* 373 (M^+, <1), 216 (78), 215 (74), 139 (52), 138 (63), 137 (14), 115 (33), 114 (100), 105 (61), 86 (22), 77 (52), 73 (10), 72 (69), 70 (29), 69 (18), 67 (29), 60 (27), 56 (25); ^{1}H NMR (300 MHz) δ 0.92 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 0.97 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 2.11 (m, 1H, H_c), 2.18 (s, 3H, 5-Me), 2.25 (m, 1H, H_c), 2.93 (m, 2H, CH of *i*-Pr), 3.48 (m, 1H, H_b), 3.64 (m, 1H, H_a), 3.69 (s, 3H, N-Me), 3.75 (m, 1H, H_a), 7.42 (m, 2H, H-3″,5″), 7.52 (m, 1H, H-4″), 7.74 (m, 2H, H-2″,6″); ^{13}C NMR (75 MHz) δ 14.8 (3-Me, ^1J = 128.5 Hz), 19.4 and 22.1 (Me of *i*-Pr), 33.8 (N-Me, ^1J = 140.8 Hz), 45.8 (C_c), 48.2 (CH of *i*-Pr), 65.3 (C_b), 78.3 (C_a), 105.5 (C-4, $^3J_{C-4,3-Me}$ = 2.5 Hz), 128.2 (C-3″,5″), 129.0 (C-2″,6″), 132.1 (C-4″), 139.6 (C-1″), 149.2 (C-3, $^2J_{C-3,3-Me}$ = 6.8 Hz), 154.6 (C-5), 190.3 (C=O). Anal. ($C_{21}H_{31}N_3O_3$) C, H, N.

[5-(3-(Diisopropylamino)-2-hydroxypropoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl]phenylmethanone (6). Column chromatography (eluent: EtOAc) afforded a yellowish oil: yield 22%; MS *m*/*z* 435 (M^+, <1), 322 (12), 279 (13), 278 (61), 277 (56), 200 (21), 115 (69), 114 (100), 105 (73), 100 (19), 91 (23), 77 (81), 72 (85), 70 (23), 67 (25), 57 (29), 56 (51); ^{1}H NMR (300 MHz) δ 0.88 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 0.92 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 2.11 (m, 1H, H_c), 2.26 (m, 1H, H_c), 2.27 (s, 3H, 3-Me), 2.88 (m, 2H, CH of *i*-Pr), 3.45 (m, 1H, H_b), 3.78 (m, 2H, H_a), 7.32 (m, 1H, H-4′), 7.45 (m, 2H, H-3′,5′), 7.48 (m, 2H, H-3″,5″), 7.57 (m, 1 H, H-4″), 7.75 (m, 2 H, H-2′,6′), 7.86 (m, 2 H, H-2″,6″); ^{13}C NMR (75 MHz) δ 14.9 (3-Me), 19.4 and 21.7 (Me of *i*-Pr), 46.8 (C_c), 48.6 (CH of *i*-Pr), 65.3 (C_b), 78.2 (C_a), 106.7 (C-4), 123.2 (C-2′,6′), 127.4 (C-4′), 128.4 (C-3″,5″), 129.0 (C-3′,5′), 129.2 (C-2″,6″), 132.5 (C-4″), 137.7 (C-1′), 139.3 (C-1″), 150.2 (C-3), 154.3 (C-5), 190.5 (C=O). Anal. ($C_{26}H_{33}N_3O_3$) C, H, N.

General Procedure for the Preparation of Pyrazolones 7−11 and 13−19. Sodium (230 mg, 10 mmol) was dissolved in 10 mL of dry MeOH with stirring. After the evolution of hydrogen had ceased, 10 mmol of educt (**1**,[37] 2.16 g; **2**,[38] 2.78 g; **3**,[39] 2.84 g; **4**,[40] 3.06 g) was added, and the resulting solution was evaporated to dryness under reduced pressure. To the residue was added 10 mL (128 mmol) of epichlorohydrin, and the mixture was refluxed (110 °C) for 4 h. Then the mixture was filtered and evaporated under reduced pressure. The residue was treated with 20 mL of the appropriate amine, and the resulting mixture was then refluxed for 4 h (reactions with *n*-propylamine) or 24 h (reactions with isopropylamine, *n*-butylamine, or *tert*-butylamine), respectively. After evaporation of excessive amine the obtained raw products were purified as described below.

4-Benzoyl-1-(3-(diisopropylamino)-2-hydroxypropyl)-2,5-dimethyl-1,2-dihydropyrazol-3-one (7). The raw material was taken up in 50 mL of dichloromethane and was washed five times with 40 mL of water. After evaporation of the solvent the residue was purified by column chromatography (eluent: EtOAc−MeOH, 5:1); subsequent recrystallization from diisopropyl ether−MeOH afforded colorless crystals of mp 140 °C: yield 32%; MS *m*/*z* 373 (M^+, <1), 144 (20), 114 (100), 105 (24), 77 (23), 72 (39), 56 (16); ^{1}H NMR (300 MHz) δ 1.00 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 1.02 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 2.30 (m, 1H, H_c), 2.53 (m, 1H, H_c), 2.53 (s, 3H, 5-Me), 3.00 (m, 2H, CH of *i*-Pr), 3.36 (s, 3H, N-Me), 3.69 (m, 1H, H_b), 3.79 (m, 1H, H_a), 3.92 (m, 1H, H_a), 7.38 (m, 2H, H-3″,5″), 7.46 (m, 1H, H-4″), 7.83 (m, 2H, H-2″,6″); ^{13}C NMR (75 MHz) δ 12.2 (5-Me), 20.0 and 21.6 (Me of *i*-Pr), 29.1 (N-Me), 47.9 (C_c), 48.4 (CH of *i*-Pr), 49.8 (C_a), 66.2 (C_b), 106.2 (C-4), 127.6 (C-3″,5″), 129.3 (C-2″,6″), 131.8 (C-4″), 138.9 (C-1″), 153.5 (C-5), 163.0 (C-3), 190.5 (C═O). Anal. ($C_{21}H_{31}N_3O_3$) C, H, N.

4-Benzoyl-1-(2-hydroxy-3-(propylamino)propyl)-5-methyl-2-phenyl-1,2-dihydropyrazol-3-one (8). Column chromatography (eluent: CH_2Cl_2−MeOH, 1:1) afforded a viscous, yellow-orange oil which solidified upon standing: yield 40%; MS *m*/*z* 393 (M^+, 2), 321 (12), 278 (22), 200 (24), 196 (15), 105 (75), 77 (34), 72 (100); ^{1}H NMR (80 MHz) δ 0.85 (t, ^3J = 6.7 Hz, 3H, Me of *n*-Pr), 1.34 (m, 2H, -C**H**$_2$−Me of *n*-Pr), 2.35−2.53 (m, 6H, NCH_2 of *n*-Pr, H_c, OH, NH), 2.64 (s, 3H, 5-Me), 3.61−3.99 (m, 3H, H_a, H_b), 7.26−7.56 (m, 8H, H-2′−6′ and H-3″−5″), 7.79−7.91 (m, 2H, H-2″,6″); ^{13}C NMR (20 MHz) δ 11.3 (Me of *n*-Pr), 12.9 (5-Me), 22.5 (**C**H_2−Me of *n*-Pr), 49.9 (C_a), 51.1 (NCH_2 of *n*-Pr), 52.1 (C_c), 66.8 (C_b), 105.9 (C-4), 126.4 (C-2′,6′), 127.5 (C-3″,5″), 128.2 (C-4′), 129.1 (C-2″,6″), 129.3 (C-3′,5′), 131.7 (C-4″), 133.9 (C-1′), 138.4 (C-1″), 158.6 (C-5), 163.8 (C-3), 190.3 (C═O). Anal. ($C_{23}H_{27}N_3O_3\cdot H_2O$) C, H, N.

4-Benzoyl-1-(2-hydroxy-3-(isopropylamino)propyl)-5-methyl-2-phenyl-1,2-dihydropyrazol-3-one (9). Column chromatography (eluent: EtOAc−NEt_3−EtOH, 8:1:1) and subsequent crystallization from EtOAc afforded yellowish crystals of mp 141−142 °C: yield 27%; MS *m*/*z* 393 (M^+, <1), 200 (10), 105 (39), 102 (18), 77 (21), 72 (100); ^{1}H NMR (80 MHz) δ 0.95 (d, ^3J = 6.7 Hz, 3H, Me of *i*-Pr), 0.96 (d, ^3J = 6.7 Hz, 3H, Me of *i*-Pr), 2.26−2.62 (m, 5H, CH of *i*-Pr, H_c,OH, NH), 2.66 (s, 3H, 5-Me), 3.56−3.79 (m, 3H, H_a, H_b), 7.23−7.48 (m, 8H, H-2′−6′ and H-3″−5″), 7.80−7.92 (m, 2H, H-2″,6″); ^{13}C NMR (20 MHz) δ 12.9 (5-Me), 22.2 and 22.4 (Me of *i*-Pr), 48.3 (CH of *i*-Pr), 49.7 (C_a), 49.9 (C_c), 67.1 (C_b), 105.8 (C-4), 126.3 (C-2′,6′), 127.4 (C-3″,5″), 128.2 (C-4′), 129.1 (C-2″,6″ and C-3′,5′), 131.6 (C-4″), 133.9 (C-1′), 138.6 (C-1″), 158.7 (C-5), 163.7 (C-3), 190.3 (C═O). Anal. ($C_{23}H_{27}N_3O_3\cdot H_2O$) C, H, N.

4-Benzoyl-1-(3-(diisopropylamino)-2-hydroxypropyl)-5-methyl-2-phenyl-1,2-dihydropyrazol-3-one (10). Column chromatography (eluent: EtOAc−MeOH, 4:1) and subsequent recrystallization from diisopropyl ether−MeOH afforded colorless crystals of mp 163 °C: yield 25%; MS *m*/*z* 435 (M^+, <1), 114 (100), 105 (23), 77 (21), 72 (23); ^{1}H NMR (300 MHz) δ 0.89 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 0.94 (d, ^3J = 6.6 Hz, 6H, Me of *i*-Pr), 2.11−2.28 (m, 2H, H_c), 2.69 (s, 3H, 5-Me), 2.89 (m, 2H, CH of *i*-Pr), 3.60 (m, 1H, H_b), 3.77 (m, 2H, H_a), 7.28 (m, 2H, H-2′,6′), 7.33 (m, 1H, H-4′), 7.38 (m, 2H, H-3″,5″), 7.44 (m, 2H, H-3′,5′), 7.46 (m, 1H, H-4″), 7.88 (m, 2H, H-2″,6″);^{13}C NMR (75 MHz) δ 13.0 (5-Me), 19.6 and 21.8 (Me of *i*-Pr), 47.7 (C_c), 48.3 (CH of *i*-Pr), 50.4 (C_a), 65.4 (C_b), 106.7 (C-4), 126.2 (C-2′,6′), 127.6 (C-3″,5″), 128.0 (C-4′), 129.3 (C-2″,6″ and C-3′,5′), 131.8 (C-4″), 134.1 (C-1′), 138.8 (C-1″), 159.7 (C-5), 164.1 (C-3), 190.6 (C═O). Anal. ($C_{26}H_{33}N_3O_3$) C, H, N.

4-Benzoyl-1-(2-hydroxy-3-(*tert*-butylamino)propyl)-5-methyl-2-phenyl-1,2-dihydropyrazol-3-one (11). The raw product was taken up in 25 mL of CH_2Cl_2 and washed several times with water. After drying (Na_2SO_4), the solvent was evaporated, and the residue was recrystallized from diisopropyl ether−EtOAc to give colorless crystals of mp 163−165 °C: yield 42%; MS *m*/*z* 407 (M^+, 1), 292 (21), 278 (12), 200 (17), 169 (13), 116 (47), 105 (55), 86 (100), 77 (20), 60 (40); ^{1}H NMR (300 MHz) δ 0.97 (s, 9H, *t*-Bu), 2.19 (m, 1H, H_c), 2.38 (m, 1H, H_c), 2.65 (s, 3H, 5-Me), 3.55 (m, 1H, H_b), 3.69 (m, 1H, H_a), 3.86 (m, 1H, H_a), 7.28 (m, 2H, H-2′,6′), 7.33 (m, 1H, H-4′), 7.36 (m, 2H, H-3″,5″), 7.41 (m, 1H, H-4″), 7.44 (m, 2H, H-3′,5′), 7.86 (m, 2H, H-2″,6″); ^{13}C NMR (75 MHz) δ 13.0 (5-Me, ^1J = 130.9 Hz), 29.0 (Me of *t*-Bu, ^1J = 125.0 Hz), 45.3 (C_c), 50.1 (C_a), 50.2 (**C**Me_3), 68.1 (C_b), 106.6 (C-4, $^3J_{C-4,5-Me}$ = 2.9 Hz), 126.3 (C-2′,6′), 127.6 (C-3″,5″), 128.1 (C-4′), 129.3 (C-2″,6″), 129.4 (C-3′,5′), 131.7 (C-4″), 134.6 (C-1′), 138.9 (C-1″), 159.5 (C-5, $^2J_{C-5,5-Me}$ = 6.7 Hz), 164.1 (C-3), 190.5 (C═O). Anal. ($C_{24}H_{29}N_3O_3$) C, H, N.

1-(2-Hydroxy-3-(propylamino)propyl)-5-methyl-2-phenyl-4-(2-thienoyl)-1,2-dihydropyrazol-3-one (13). Column chromatography (eluent: EtOAc−NEt_3−EtOH, 8:1:1) and subsequent recrystallization from EtOAc−light petroleum afforded nearly colorless crystals of mp 140−143 °C: yield 26%; MS *m*/*z* 399 (M^+, <1), 200 (12), 111 (26), 102 (12), 72 (100), 43 (36); ^{1}H NMR (80 MHz) δ 0.84 (t, ^3J = 6.7 Hz, 3H, Me of *n*-Pr), 1.33 (m, 2H, -C**H**$_2$−Me of *n*-Pr), 2.16−2.51 (m, 6H, NCH_2 of *n*-Pr, H_c, OH, NH), 2.68 (s, 3H, 5-Me), 3.61−4.07 (m, 3H, H_a, H_b), 7.06 (dd, ^3J = 3.9 and 4.9 Hz, 1H, H-4″), 7.23−7.50 (m, 5H, H-2′−6′), 7.56 (dd, ^3J = 4.9 Hz, ^4J = 1.1 Hz, 1H, H-5″), 8.40 (dd, ^3J = 3.9 Hz, ^4J = 1.1 Hz, 1H, H-3″); ^{13}C NMR (20 MHz) δ 11.4 (Me of *n*-Pr), 13.0 (5-Me), 22.7 (**C**H_2−Me of *n*-Pr), 50.0 (C_a), 51.1 (NCH_2 of *n*-Pr), 52.3 (C_c), 67.0 (C_b), 105.8 (C-4), 126.5 (C-2′,6′), 127.6 (C-4″), 128.3 (C-4′), 129.3 (C-3′,5′), 132.8 (C-5″), 133.9 (C-1′), 134.2 (C-3″), 145.1 (C-2″), 158.6 (C-5), 163.3 (C-3), 180.9 (C═O). Anal. ($C_{21}H_{25}N_3O_3S$) C, H, N.

1-(2-Hydroxy-3-(isopropylamino)propyl)-5-methyl-2-phenyl-4-(2-thienoyl)-1,2-dihydropyrazol-3-one (14). The raw product was taken up in 25 mL of CH_2Cl_2 and washed several times with water. After drying (Na_2SO_4) the solvent was evaporated, and the residue was recrystallized from diisopropyl ether−EtOH to give yellowish crystals of mp 127−134 °C: yield 20%; MS *m*/*z* 399 (M^+, <1), 200 (13), 111 (23), 72 (100), 60 (20), 45 (62), 43 (48); ^{1}H NMR (80 MHz) δ 0.94 (d, ^3J = 6.7 Hz, 3H, Me of *i*-Pr), 0.95 (d, ^3J = 6.7 Hz, 3H, Me of *i*-Pr), 2.12−2.63 (m, 3 H, CH of *i*-Pr, H_c), 2.68 (s, 3H, 5-Me), 3.60−3.97 (m, 3H, H_a, H_b), 7.06 (dd, ^3J = 3.9 and 4.9 Hz, 1H, H-4″), 7.23−7.51 (m, 5H, H-2′−6′), 7.57 (dd, ^3J = 4.9 Hz, ^4J = 1.1 Hz, 1H, H-5″), 8.40 (dd, ^3J = 3.9 Hz, ^4J = 1.1 Hz, 1H, H-3″); ^{13}C NMR (20 MHz) δ 13.0 (5-Me), 22.3 and 22.6 (Me of *i*-Pr), 48.3 (CH of *i*-Pr), 49.9 (C_a and C_c), 67.3 (C_b), 105.9 (4), 126.5 (C-2′,6′), 127.6 (C-4″), 128.3 (C-4′), 129.3 (C-3′,5′), 132.8 (C-5″), 133.9 (C-1′), 134.2 (C-3″), 145.1 (C-2″), 158.7 (C-5), 163.3 (C-3), 180.9 (C═O). Anal. ($C_{21}H_{25}N_3O_3S$) C, H, N.

1-(2-Hydroxy-3-(*tert*-butylamino)propyl)-5-methyl-2-phenyl-4-(2-thienoyl)-1,2-dihydropyrazol-3-one (15). The raw product was taken up in 25 mL of CH_2Cl_2 and washed several times with water. After drying (Na_2SO_4) the solvent was evaporated, and the residue was recrystallized from EtOAc−EtOH to give colorless crystals of mp 195−196 °C: yield 25%; MS *m*/*z* 413 (M^+, <1), 298 (10), 116 (46), 111 (28), 86 (100), 60 (57), 57 (42), 43 (25); ^{1}H NMR (300 MHz) δ 0.98 (s, 9H, *t*-Bu), 2.20 (m, 1H, H_c), 2.35 (m, 1H, H_c), 2.69 (s, 3H, 5-Me), 3.58 (m, 1H, H_b), 3.71 (m, 1H, H_a), 3.89 (m, 1H, H_a), 7.07 (dd, ^3J = 3.9 and 4.9 Hz, 1H, H-4″), 7.30 (m, 2H, H-2′,6′), 7.41 (m, 1H, H-4′), 7.45 (m, 2H, H-3′,5′), 7.57 (dd, ^3J = 4.9 Hz, ^4J = 1.1 Hz, 1H, H-5″), 8.41 (dd, ^3J = 3.9 Hz, ^4J = 1.1 Hz, 1H, H-3″); ^{13}C NMR (75 MHz) δ 13.2 (5-Me), 28.8 (Me of *t*-Bu), 45.3 (C_c), 50.1 (**C**Me_3 and C_a), 67.9 (C_b), 106.3 (C-4), 126.5 (C-2′,6′), 127.7 (C-4″), 128.3 (C-4′), 129.4 (C-3′,5′), 132.8 (C-5″), 134.2 (C-1′), 134.4 (C-3″), 145.2 (C-2″), 159.1 (C-5), 163.5 (C-3), 181.1 (C═O). Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N.

1-(2-Hydroxy-3-(propylamino)propyl)-5-methyl-2-phenyl-4-(3-phenylpropionyl)-1,2-dihydropyrazol-3-one (16). The raw product was taken up in 25 mL of CH_2Cl_2 and washed several times with water. After drying (Na_2SO_4) the solvent was evaporated, and the residue was recrystallized from EtOAc−light petroleum to give colorless crystals of mp 120−124 °C: yield 49%; MS *m*/*z* 421 (M^+, 9), 307 (12), 228 (10), 201 (12), 175 (14), 116 (16), 115 (18), 102 (17), 98 (21), 93 (23), 91 (25), 86 (20), 72 (100); ^{1}H NMR (300 MHz) δ 0.83 (t, ^3J = 6.7 Hz, 3H, Me of *n*-Pr), 1.36 (m, 2H, −C**H**$_2$−Me of *n*-Pr), 2.30 (m, 1H, H_c, NH*), 2.40 (m, 2H, NCH_2 of *n*-Pr), 2.41 (m, 1H,

H_c), 2.70 (s, 3H, 5-Me), 2.90 (br s, 1H, OH*), 2.96 (m, 2H, C**H**$_2$−Ph), 3.22 (m, 1H, CH_2−CO), 3.34 (m, 1H, CH_2−CO), 3.66 (m, 1H, H_b), 3.67 (m, 1H, H_a), 3.85 (m, 1H, H_a), 7.09−7.48 (m, 10H, H-2′−6′ and H-2″−6″); ^{13}C NMR (75 MHz) δ 11.4 (Me of *n*-Pr), 13.2 (5-Me), 22.7 (**C**H_2−Me of *n*-Pr), 30.0 (**C**H_2−Ph), 43.0 (**C**H_2−CO), 49.7 (C_a), 51.3 (NCH_2 of *n*-Pr), 52.2 (C_c), 67.2 (C_b), 105.9 (C-4, $^3J_{C-4,5-Me}$ = 2.5 Hz), 125.6 (C-4″), 126.4 (C-2′,6′), 128.1 (C-3″,5″), 128.5 (C-4′ and C-2″,6″), 129.5 (C-3′,5′), 134.4 (C-1′), 141.9 (C-1″), 158.5 (C-5), 165.1 (C-3), 196.0 (C=O). Anal. ($C_{25}H_{31}N_3O_3$) C, H, N.

1-(2-Hydroxy-3-(isopropylamino)propyl)-5-methyl-2-phenyl-4-(3-phenylpropionyl)-1,2-dihydropyrazol-3-one (17). The raw product was taken up in 25 mL of CH_2Cl_2 and washed several times with water. After drying (Na_2SO_4) the solvent was evaporated, and the residue was recrystallized from EtOAc−light petroleum to give colorless crystals of mp 146−149 °C: yield 42%; MS *m*/*z* 421 (M^+, 16), 307 (11), 228 (11), 116 (13), 115 (17), 102 (23), 93 (16), 91 (17), 72 (100); ^{1}H NMR (80 MHz) δ 0.92 (d, ^3J = 6.3 Hz, 3H, Me of *i*-Pr), 0.94 (d, ^3J = 6.3 Hz, 3H, Me of *i*-Pr), 2.25−2.68 (m, 3H, CH of *i*-Pr, H_c), 2.72 (s, 3H, 5-Me), 2.87−3.87 (m, 7H, H_a, H_b, CO−CH_2−CH_2−), 7.14−7.51 (m, 10H, H-2′−6′ and H-2″−6″);^{13}C NMR (20 MHz) δ 13.1 (5-Me), 22.4 and 22.7 (Me of *i*-Pr), 29.8 (**C**H_2−Ph), 42.9 (**C**H_2−CO), 48.4 (CH of *i*-Pr), 49.5 (C_c), 49.9 (C_a), 67.3 (C_b), 105.3 (C-4), 125.4 (C-4″), 126.3 (C-2′,6′), 128.0 (C-3″,5″), 128.3 (C-4′ and C-2″,6″), 129.3 (C-3′,5′), 133.9 (C-1′), 141.6 (C-1″), 158.1 (C-5), 164.8 (C-3), 195.8 (C=O). Anal. ($C_{25}H_{31}N_3O_3$) C, H, N.

1-(2-Hydroxy-3-(butylamino)propyl)-5-methyl-2-phenyl-4-(3-phenylpropionyl)-1,2-dihydropyrazol-3-one (18). The raw product was taken up in 25 mL of CH_2Cl_2 and washed several times with water. After drying (Na_2SO_4) the solvent was evaporated, and the residue was recrystallized from EtOAc−light petroleum to give colorless crystals of mp 95−97 °C: yield 21%; MS *m*/*z* 435 (M^+, 7), 349 (10), 307 (11), 229 (10), 228 (11), 201 (11), 175 (12), 130 (15), 116 (23), 93 (21), 91 (24), 86 (100), 44 (32); ^{1}H NMR (80 MHz) δ 0.87 (m, 3H, Me of *n*-Bu), 1.23−1.32 (m, 4H, Me−C**H**$_2$−C**H**$_2$− of *n*-Bu), 2.28−2.51 (m, 6H, N−CH_2 of *n*-Bu, H_c, OH, NH), 2.72 (s, 3H, 5-Me), 2.84−3.77 (m, 7H, H_a, H_b, C**H**$_2$−C**H**$_2$−Ph), 7.19−7.51 (m, 10H, H-2′−6′ and H-2″−6″); ^{13}C NMR (20 MHz) δ 13.1 (5-Me), 13.7 (Me of *n*-Bu), 20.0 (Me-**C**H_2 of *n*-Bu), 29.8 (**C**H_2−Ph), 31.8 (**C**H_2−CH_2−N of *n*-Bu), 42.9 (**C**H_2−CO), 49.1 (CH_2−N of *n*-Bu), 49.5 (C_a), 52.3 (C_c), 67.0 (C_b), 105.2 (C-4), 125.4 (C-4″), 126.3 (C-2′,6′), 128.0 (C-3″,5″), 128.3 (C-4′ and C-2″,6″), 129.3 (C-3′,5′), 133.8 (C-1′), 141.6 (C-1″), 158.0 (C-5), 164.8 (C-3), 195.8 (C=O). Anal. ($C_{26}H_{33}N_3O_3$) C, H, N.

1-(2-Hydroxy-3-(*tert*-butylamino)propyl)-5-methyl-2-phenyl-4-(3-phenylpropionyl)-1,2-dihydropyrazol-3-one (19). The raw product was recrystallized from diisopropyl ether−EtOH to afford colorless crystals of mp 178−180 °C: yield 52%; MS *m*/*z* 435 (M^+, 9), 129 (18), 116 (35), 114 (33), 86 (100), 72 (29), 60 (64), 57 (48), 56 (26), 45 (67), 43 (24); ^{1}H NMR (80 MHz) δ 0.99 (s, 9H, Me of *t*-Bu), 2.16−2.52 (m, 4H, H_c, OH, NH), 2.73 (s, 3H, 5-Me), 2.93−3.87 (m, 7H, H_a, H_b, C**H**$_2$−C**H**$_2$−Ph), 7.17−7.52 (m, 10H, H-2′−6′ and H-2″−6″); ^{13}C NMR (20 MHz) δ 13.1 (5-Me), 28.6 (Me of *t*-Bu), 29.8 (**C**H_2−Ph), 42.9 (**C**H_2−CO), 45.2 (C_c), 49.5 (C_a), 50.1 (**C**Me_3 of *t*-Bu), 67.6 (C_b), 105.3 (C-4), 125.4 (C-4″), 126.3 (C-2′,6′), 128.0 (C-3″,5″), 128.3 (C-4′ and C-2″,6″), 129.4 (C-3′,5′), 133.9 (C-1′), 141.7 (C-1″), 158.1 (C-5), 164.8 (C-3), 195.9 (C=O). Anal. ($C_{26}H_{33}N_3O_3$) C, H, N.

4-Benzoyl-1-[2-hydroxy-3-(4-*o*-tolylpiperazin-1-yl)propyl]-5-methyl-2-phenyl-1,2-dihydropyrazol-3-one (12). As described for the synthesis of compounds **7**−**11**, and **13**−**19**, crude epoxide product was prepared from pyrazolone **2** (2.78 g, 10 mmol) and epichlorohydrin (10 mL, 128 mmol). After evaporation of excessive epichlorohydrin, the residue was treated with 1.76 g (10 mmol) of *o*-tolylpiperazine in 75 mL of MeOH, and the mixture was heated to reflux for 8 h. After evaporation in vacuo the residue was subjected to column chromatography (eluent: EtOAc); subsequent recrystallization from 2-propanol−EtOAc gave colorless crystals of mp 175 °C: yield 15%; MS *m*/*z* 510 (M^+, 23), 417 (13), 364 (19), 278 (15), 277 (14), 219 (17), 189 (81), 187 (63), 156 (41), 118 (44), 105 (100), 91 (39), 77 (88), 71 (18), 70 (94), 67 (26), 57 (33), 56 (38), 55 (24); ^{1}H NMR (300 MHz) δ 2.18 (m, 2H, H_c), 2.27 (s, 3H, Me of tolyl), 2.30 and 2.53 (m, each 2H, H-2,6 of piperazine), 2.68 (s, 3H, 5-Me), 2.77 (m, 4H, H-3,5 of piperazine), 3.74−3.86 (m, 3H, H_a, H_b), 6.96 (m, 1H, H-6 of tolyl), 6.99 (m, 1H, H-4 of tolyl), 7.17 (m, 1H, H-5 of tolyl), 7.18 (m, 1H, H-3 of tolyl), 7.30 (m, 2H, H-2′,6′), 7.35 (m, 1H, H-4′), 7.40 (m, 2H, H-3″,5″), 7.47 (m, 2H, H-3′,5′), 7.48 (m, 1H, H-4″), 7.90 (m, 2H, H-2″,6″); ^{13}C NMR (75 MHz) δ 13.1 (5-Me), 17.7 (Me of tolyl), 50.4 (C_a), 51.6 (C-3,5 of piperazine), 53.6 (C-2,6 of piperazine), 61.1 (C_c), 65.0 (C_b), 106.6 (C-4), 118.9 (C-6 of tolyl), 123.2 (C-4 of tolyl), 126.3 (C-2′,6′), 126.5 (C-5 of tolyl), 127.7 (C-3″,5″), 128.1 (C-4′), 129.4 (C-2″,6″ and C-3′,5′), 131.0 (C-3 of tolyl), 131.9 (C-4″), 132.5 (C-2 of tolyl), 134.4 (C-1′), 138.7 (C-1″), 151.1 (C-1 of tolyl), 159.6 (C-5), 164.0 (C-3), 190.5 (C=O). Anal. ($C_{31}H_{34}N_4O_3$) C, H, N.

1-[2-Hydroxy-3-(4-*o*-tolylpiperazin-1-yl)propyl]-5-methyl-2-phenyl-4-(3-phenylpropionyl)-1,2-dihydropyrazol-3-one (20). Starting from pyrazolone **4**, compound **20** was prepared similarly as described for the preparation of **12**. Repeated column chromatography of the raw product (eluents: 1. gradient CH_2Cl_2−EtOAc−NH_3, 10:1:0.1, to CH_2Cl_2−MeOH−NH_3, 10:1:0.1; 2. EtOAc) and subsequent recrystallization from EtOAc gave colorless crystals of mp 188 °C: yield 14%; MS *m*/*z* 538 (M^+, 41), 445 (12), 392 (16), 201 (21), 189 (99), 187 (61), 174 (20), 146 (38), 131 (32), 118 (46), 105 (26), 91 (100), 77 (52), 70 (61), 67 (37), 65 (24), 57 (26), 56 (26), 42 (35); ^{1}H NMR (300 MHz) δ 2.10 (m, 2H, H_c), 2.17 (s, 3H, Me of tolyl), 2.27 and 2.49 (m, each 2H, H-2,6 of piperazine), 2.67 (s, 3H, 5-Me), 2.70 (m, 4H, H-3,5 of piperazine), 2.90 (m, 2H, C**H**$_2$−Ph), 3.12−3.41 (m, 2H, CH_2−CO), 3.65−3.80 (m, 3H, H_a, H_b), 6.87 (m, 1H, H-6 of tolyl), 6.92 (m, 1H, H-4 of tolyl), 7.05−7.45 (m, 12H, H-2′−6′, H-2″−6″, H-3 and H-5 of tolyl); ^{13}C NMR (75 MHz) δ 13.3 (5-Me), 17.7 (Me of tolyl), 29.9 (**C**H_2−Ph), 43.1 (**C**H_2−CO), 49.8 (C_a), 51.5 (C-3,5 of piperazine), 53.5 (C-2,6 of piperazine), 61.0 (C_c), 64.9 (C_b), 106.0 (C-4), 118.8 (C-6 of tolyl), 123.3 (C-4 of tolyl), 125.6 (C-4″), 126.2 (C-2′,6′), 126.5 (C-5 of tolyl), 128.1 (C-3″,5″), 128.4 (C-4′), 128.5 (C-2″,6″), 129.6 (C-3′,5′), 131.0 (C-3 of tolyl), 132.5 (C-2 of tolyl), 134.2 (C-1′), 141.8 (C-1″), 151.0 (C-1 of tolyl), 158.7 (C-5), 164.9 (C-3), 196.2 (C=O). Anal. ($C_{33}H_{38}N_4O_3$) C, H, N.

1-[5-(2,3-Epoxypropoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl]-3-phenylpropan-1-one (22). To a solution of 306 mg (1 mmol) of pyrazolone **4**, 393 mg (1.5 mmol) of Ph_3P, and 93 mg (1.25 mmol) of 2,3-epoxypropanol in 20 mL of THF was added slowly 261 mg (1.5 mmol) of diethyl azodicarboxylate, and the mixture was stirred for 20 h at room temperature. Then 1 mL of MeOH was added; the mixture was poured onto 20 mL of water and was then exhaustively extracted with ether. The combined organic phases were washed with 2 M NaOH, water (several times), and brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. Column chromatography (eluent: CH_2Cl_2−EtOAc−light petroleum, 3:1:1) gave a nearly colorless oil: yield 50%; MS *m*/*z* 362 (M^+, 78), 279 (15), 277 (20), 257 (27), 231 (21), 201 (90), 200 (21), 174 (29), 167 (34149 (82), 117 (43), 105 (29), 100 (13), 92 (20), 91 (89), 83 (24), 77 (70), 71 (37), 70 (24), 69 (32), 67 (26), 65 (21), 60 (21), 57 (100), 56 (18), 55 (41), 51 (16); ^{1}H NMR (300 MHz) δ 2.43 (m, 1H, CH_2 of oxirane), 2.50 (s, 3H, 3-Me), 2.70 (m, 1H, CH_2 of oxirane), 3.06 (m, 2H, C**H**$_2$−Ph), 3.08 (m, 1H, CH of oxirane), 3.18 (m, 2H, CH_2−CO), 3.75 (m, 1H, OCH_2), 4.13 (m, 1H, OCH_2), 7.19 (m, 1H, H-4″), 7.27 (m, 4H, H-2″,3″,5″,6″), 7.37 (m, 1H, H-4′), 7.48 (m, 2H, H-3′,5′), 7.65 (m, 2H, H-2′,6′); ^{13}C NMR (75 MHz) δ 15.9 (3-Me), 29.9 (**C**H_2−Ph), 43.0 (**C**H_2−CO), 44.3 (CH_2 of oxirane), 49.3 (CH of oxirane), 76.5 (OCH_2), 109.0 (C-4), 123.3 (C-2′,6′), 126.0 (C-4″), 127.9 (C-4′), 128.4 (C-2″,3″,5″,6″), 129.3 (C-3′,5′), 137.5 (C-1′), 141.5 (C-1″), 150.3 (C-3), 153.9 (C-5), 194.2 (C=O). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

(3-Methyl-1-phenyl-5-(propylamino)-1*H*-pyrazol-4-yl)-phenylmethanone (25). Epoxide **21**[22] (334 mg, 1 mmol) was treated with 414 mg (7 mmol) of *n*-propylamine, and the mixture was stirred for 2 h at 50−60 °C. Then the excessive amine was evaporated, and the residue was taken up in

CH_2Cl_2. The organic phase was washed several times with water, dried, and evaporated. Column chromatography (eluent: EtOAc−MeOH, 9:1) afforded nearly colorless crystals of mp 65−66 °C: yield 30%; MS *m*/*z* 320 ($M^+ + 1$, 42), 319 (M^+, 100), 318 ($M^+ - 1$, 55), 291 (17), 290 (72), 200 (28), 105 (61), 91 (18), 77 (58); ^{1}H NMR (300 MHz) δ 0.79 (t, ^3J = 7.4 Hz, 3H, Me of *n*-Pr), 1.44 (m, 2H, C**H**$_2$−Me), 1.86 (s, 3H, 3-Me), 2.79 (q, J = 6.7 Hz, 2H, N−CH_2), 7.36−7. 58 (m, 10H, H-2′−6′ and H-2″−6″), 7.61 (t, ^3J = 6.7 Hz, 1H, NH);^{13}C NMR (75 MHz) δ 11.1 (Me of *n*-Pr), 15.3 (3-Me), 23.4 (CH_2−Me), 46.7 (NCH_2), 105.9 (C-4), 125.6 (C-2′,6′), 127.5 (C-3″,5″), 128.2 (C-2″,6″), 128.3 (C-4′), 129.1 (C-3′,5′), 130.5 (C-4″), 139.6 (C-1′), 141.3 (C-1″), 149.6 (C-3), 153.7 (C-5), 192.2 (C=O). Anal. ($C_{20}H_{21}N_3O$) C, H, N.

1-(3-Methyl-1-phenyl-5-(propylamino)-1*H*-pyrazol-4-yl)-3-phenylpropan-1-one (26). Compound **26** was prepared from raw epoxide **22** (achieved from 306 mg (1 mmol) of pyrazolone **4**) in a similar fashion as described for **25**. Column chromatography (eluent: light petroleum−EtOAc, 3:1) afforded—besides 12% of **28**—compound **26**, which after crystallization from EtOH afforded tan crystals of mp 97 °C: yield 45% (regarding **4**); MS *m*/*z* 348 ($M^+ + 1$, 27), 347 (M^+, 100), 319 (18), 318 (20), 242 (90), 226 (21), 217 (19), 216 (29), 215 (48), 200 (18), 186 (22), 173 (19), 105 (17), 92 (15), 91 (71), 77 (51), 66 (16), 65 (16); ^{1}H NMR (300 MHz) δ 0.77 (t, ^3J = 7.4 Hz, 3H, Me of *n*-Pr), 1.41 (m, 2H, C**H**$_2$−Me), 2.44 (s, 3H, 3-Me), 2.73 (q, ^3J = 6.6 Hz, 2H, N−CH_2), 3.06 ("s", 4H, C**H**$_2$−C**H**$_2$−Ph), 7.18−7. 52 (m, 10H, H-2′−6′ and H-2″−6″), 7.91 (t, $^3J \sim$ 6.0 Hz, 1H, NH); ^{13}C NMR (75 MHz) δ 11.0 (Me of *n*-Pr), 16.3 (3-Me), 23.4 (CH_2−Me), 30.4 (**C**H_2−Ph), 42.4 (**C**H_2−CO), 46.5 (NCH_2), 105.5 (C-4), 125.6 (C-2′,6′), 126.0 (C-4″), 128.2 (C-4′), 128.3 (C-2″,6″), 128.4 (C-3″,5″), 129.0 (C-3′,5′), 139.6 (C-1′), 141.5 (C-1″), 148.6 (C-3, $^2J_{C-3,3-Me}$ = 6.6 Hz), 153.6 (C-5, $^3J_{C-5,NCH2}$ = 3.2 Hz), 194.5 (C=O). Anal. ($C_{22}H_{25}N_3O$) C, H, N.

2,5-Dimethyl-4-[(*Z*)-phenyl(propylamino)methylene]-2,4-dihydropyrazol-3-one (27). Educt **1** (216 mg, 1 mmol) was taken up in 5 mL of *n*-propylamine, and the mixture was refluxed for 4 h. Excessive amine was removed in vacuo, and the residue was subjected to column chromatography (eluent: EtOAc−MeOH, 5:1). Recrystallization from diisopropyl ether gave a tan powder of mp 67−68 °C: yield 38%; MS *m*/*z* 258 ($M^+ + 1$, 24), 257 (M^+, 100), 256 ($M^+ - 1$, 36), 214 (33), 199 (34), 125 (28), 104 (27), 77 (28), 66 (25), 58 (18); ^{1}H NMR (300 MHz) δ 0.88 (t, ^3J = 7.4 Hz, 3H, Me of *n*-Pr), 1.32 (s, 3H, 5-Me), 1.54 (m, 2H, C**H**$_2$−Me), 3.04 (m, 2H, N−CH_2), 3.39 (s, 1H, 2-Me), 7.24 (m, 2H, H-2″,6″), 7.47 (m, 1H, H-4″), 7.48 (m, 2H, H-3″,5″), 11.17 (s, 1H, NH); ^{13}C NMR (75 MHz) δ 11.0 (Me of *n*-Pr, ^1J = 126.0 Hz), 15.0 (5-Me, ^1J = 128.2 Hz), 23.4 (**C**H_2−Me, ^1J = 125.4 Hz), 30.6 (2-Me, ^1J = 138.7 Hz), 45.8 (N−CH_2), 98.5 (C-4, $^3J_{C-4,5-Me}$ = 2.3 Hz), 127.2 (C-2″,6″), 128.8 (C-3″,5″), 130.0 (C-4″), 131.5 (C-1″), 145.9 (C-5, $^2J_{C-5,5-Me}$ = 7.0 Hz), 165.4 (C=**C**-N, $^3J_{C=C-N,NCH2}$ = 3.7 Hz), 166.0 (C-3, $^3J_{C-3,2-Me}$ = 2.1 Hz). Anal. ($C_{15}H_{19}N_3O$) C, H, N.

5-Methyl-2-phenyl-4-[(*Z*)-3-phenyl-1-(propylamino)propylidene]-2,4-dihydropyrazol-3-one (28). Educt **4** (306 mg, 1 mmol) was taken up in 4 mL (49 mmol) of *n*-propylamine, and the mixture was refluxed for 5 h. Then excessive *n*-propylamine was removed in vacuo, the residue was taken up in CH_2Cl_2, and the organic layer was washed with 2 N NaOH and water, dried, and evaporated. Recrystallization from EtOH afforded 24% of colorless crystals of mp 106 °C: MS *m*/*z* 348 ($M^+ + 1$, 24), 347 (M^+, 100), 330 (11), 200 (18), 199 (30), 97 (16), 91 (47), 77 (19), 69 (22), 58 (31), 57 (38), 55 (28); ^{1}H NMR (300 MHz) δ 1.01 (t, ^3J = 7.4 Hz, 3H, Me of *n*-Pr), 1.68 (m, 2H, C**H**$_2$−Me), 2.43 (s, 3H, 5-Me), 2.95 ("s", 4H, C**H**$_2$−C**H**$_2$−Ph), 3.22 (m, 2H, N−CH_2), 7.13 (m, 1H, H-4′), 7.21 (m, 2H, H-2″,6″), 7.29 (m, 1H, H-4″), 7.31 (m, 2H, H-3″,5″), 7.39 (m, 2H, H-3′,5′), 8.02 (m, 2H, H-2′,6′), 11.60 (s, 1H, NH); ^{13}C NMR (75 MHz) δ 11.3 (Me of *n*-Pr), 17.0 (5-Me, ^1J = 127.8 Hz), 22.8 (**C**H_2−Me), 30.4 (**C**H_2−CH_2−Ph), 34.4 (CH_2-**C**H_2−Ph), 44.7 (N−CH_2), 97.9 (C-4), 119.2 (C-2′,6′), 124.1 (C-4′), 127.0 (C-4″), 128.1 (C-2″,6″), 128.6 (C-3′,5′), 128.9 (C-3″,5″), 139.0 (C-1″), 139.2 (C-1′), 145.9 (C-5, $^3J_{C-5,5-Me}$ = 6.7 Hz), 166.1 (C-3), 167.4 (C=**C**−N). Anal. ($C_{22}H_{25}N_3O$) C, H, N.

5-Methyl-2-phenyl-4-[(*Z*)-phenyl(4-*o*-tolylpiperazin-1-yl)methylene]-2,4-dihydropyrazol-3-one (29). Educt **2** (2.78 g, 10 mmol) was successively treated with 1 equiv of NaOMe in MeOH and 1 equiv of epichlorohydrin as described for the preaparation of **6**. Then 1.76 g (10 mmol) of *o*-tolylpiperazine was added, and the mixture was refluxed for 30 h. After filtration, the excessive amine was removed in vacuo, and the residue was taken up in CH_2Cl_2. The organic layer was washed several times with water, dried, and evaporated. The residue was subjected to column chromatography (eluent: EtOAc) to afford—besides 3% of **12**—compound **29**, which was crystallized from MeOH-EtOAc, 1:1, to afford 20% of yellow crystals of mp 230 °C: MS *m*/*z* 437 ($M^+ + 1$, 13), 436 (M^+, 40), 277 (30), 146 (100), 144 (15), 132 (34), 131 (19), 130 (15), 118 (44), 91 (50), 77 (33), 65 (15), 56 (18); ^{1}H NMR (300 MHz) δ 1.35 (s, 3H, 5-Me), 2.34 (s, 3H, Me of tolyl), 3.07 and 3.21 (each m, 2H, piperazine H-3 and H-5), 3.62 and 4.19 (each m, 2H, piperazine H-2 and H-6), 7.03 (m, 1H, H-4 of tolyl), 7.06 (m, 1H, H-6 of tolyl), 7.11 (m, 1H, H-4′), 7.20 (m, 1H, H-5 of tolyl), 7.21 (m, 1H, H-3 of tolyl), 7.38 (m, 2H, H-3′,5′), 7.48 (m, 2H, H-2″,6″), 7.54 (m, 2H, H-3″,5″), 7.61 (m, 1H, H-4″), 8.05 (m, 2H, H-2′,6′); ^{13}C NMR (75 MHz) δ 15.5 (5-Me, ^1J = 128.2 Hz), 17.8 (Me of tolyl), 51.6 and 55.7 (piperazine C-2 and C-6), 52.8 (piperazine C-3,5), 103.0 (C-4, $^3J_{C-4,5-Me}$ = 2.5 Hz), 119.1 (C-6 of tolyl), 119.1 (C-2′,6′), 123.7 (C-4′), 124.0 (C-4 of tolyl), 126.6 (C-5 of tolyl), 128.5 (C-3′,5′), 129.0 (C-3″,5″), 130.5 (C-2″,6″), 131.3 (C-3 of tolyl), 131.8 (C-4″), 132.8 (C-2 of tolyl), 135.2 (C-1″), 139.6 (C-1′), 149.4 (C-5, $^2J_{C-5,5-Me}$ = 6.9 Hz), 150.2 (C-1 of tolyl), 161.9 (C-3), 166.2 (C=**C**−N). Anal. ($C_{28}H_{28}N_4O$) C, H, N.

Pharmacology. 1. Cell Lines. The CCRF-CEM T-lymphoblast cell line, as well as the resistant lines, were obtained as described previously.[17,34] 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. 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.[34] This cell line has been chosen on grounds of distinct PGP expression and does not show the mutation at codon 185.[41] In addition, no significant contribution of other factors to MDR was observed.

The mouse lymphoma cell line L5178Y was infected by a retroviral vector carrying the human *mdr1* gene. The vector was grown in, and encapsidated by, the packaging cell line PA12MDR1/$_{A-1}$. The viral RNA is from a replication-defective, amphotropic virus that is extruded by the cells into surrounding medium.[42] Polybrene was added to a final concentration of 2 μg/mL to this filtered supernatant which was then diluted 1:5 with growth medium. Here, too, Polybrene was added to a final concentration of 2 μg/mL. The cells to be infected were plated in medium containing the virus and Polybrene. After 2 days, this medium was replaced with McCoy's 5A medium containing 10% horse serum, to which 60 ng/mL colchicine was added to select resistant cells.[43] These resistant cells were termed L5178Y VMDR C.06. The line was maintained in colchicine-containing culture medium.

2. Rhodamine 123 and Daunomycin Efflux Studies. Rhodamine efflux studies were performed using modified published methods.[17] 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 rhodamine 123 (Sigma Chemical Co., St. Louis, MO) at a final concentration of 0.2 μg/mL (0.53 μmol/L). Cell suspensions were incubated at 37 °C for 15 min. Tubes were chilled on ice and pelleted at 500*g* in an Eppendorf 5403 centrifuge (Eppendorf, Germany). Supernatants were removed, and the cell pellet was resuspended in medium which was prewarmed to 37 °C and contained either no modulator or chemosensitizer at various concentrations ranging from 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 30, 60, 90, and 120 s, aliquots of the incubation mixture were transferred to tubes containing an equal volume of ice-cold stop solution (RPMI1640 medium containing verapamil at a final concentration of 10 μg/mL). Zero time points were done by immediately pipetting rhodamine 123-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 rhodamine 123 fluorescence levels were adjusted to be equal to initial levels observed in resistant cells. Samples drawn at the respective time points were kept in an ice water bath and measured within 1 h on a Becton Dickinson FACSCALIBUR flow cytometer (Becton Dickinson, Vienna, Austria). Viable cells were gated on the basis of forward and side scatter. The excitation wavelength was 488 nm, and the emission was measured in the FL1 channel (520−550 nm); 5000 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.

For daunomycin efflux an analogous experimental protocol was used, whereby the daunomycin concentration was 3.2 μmol/L and the preloading time was 30 min. Time points were 60, 120, 180, and 240 s. The excitation wavelength was 488 nm, and the emission was measured in the FL3 channel (650−780 nm). Otherwise, conditions were identical to those outlined for the rhodamine 123 efflux experiments.

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References

(1) De Vita, V. T., Jr. The Problem of Resistance; Keynote Address. In *Drug Resistance: Mechanisms and Reversal*; Mihich, E., Ed.; John Libbey CIC: Roma, Milano, London, Paris, New York, 1990.

(2) Dano, K. Active Outward Transport of Daunomycin in Resistant Ehrlich Ascites Tumour Cells. *Biochim. Biophys. Acta* **1973**, *323*, 466−483.

(3) Bosch, I.; Croop, J. P-Glycoprotein Multidrug Resistance and Cancer. *Biochim. Biophys. Acta* **1996**, *1288*, F37−F54.

(4) Bradley, G.; Juranka, P. F.; Ling, V. Mechanism of Multidrug Resistance. *Biochim. Biophys. Acta* **1988**, *948*, 87−128.

(5) Ford, J. M. Experimental Reversal of P-glycoprotein Mediated Multidrug Resistance by Pharmacological Chemosensitizers. *Eur. J. Cancer* **1996**, *32A*, 991−1001.

(6) Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. Overcoming of Vincristine Resistance in P 388 Leukemia In Vivo and In Vitro through Enhanced Cytotoxicity of Vincristine and Vinblastine by Verapamil. *Cancer Res.* **1981**, *41*, 1967−1972.

(7) Boer, R.; Gekeler, V. Chemosensitizers in Tumor Therapy: New Compounds Promise Better Efficacy. *Drugs Future* **1995**, *20*, 499−509.

(8) Chauffert, B.; Martin, M.; Hamman, A.; Michel, M. F.; Martin, F. Amiodaron-Induced Enhancement of Doxorubicin and 4′-Deoxydoxorubicin Cytotoxicity to Rat Colon Cancer Cells In Vitro and In Vivo. *Cancer Res.* **1986**, *46*, 825−830.

(9) Yang, C. P.; DePinho, S. H.; Greenberger, L. M.; Arceci, R. J.; Horwitz, S. B. Progesterone Interacts With P-glycoprotein in Multidrug-Resistant Cells and in the Endometrium of Gravid Uterus. *J. Biol. Chem.* **1989**, *264*, 782−788.

(10) Twentyman, P. R. Cyclosporins as Drug Resistance Modifiers. *Biochem. Pharmacol.* **1992**, *43*, 109−117.

(11) Ford, J. M.; Prozialeck, W. C.; Hait, W. N. Structural Features Determining Activity of Phenothiazines and Related Drugs for Inhibition of Cell Growth and Reversal of Multidrug Resistance. *Mol. Pharmacol.* **1989**, *35*, 105−115.

(12) Ford, J. M.; Bruggemann, E. P.; Pastan, I.; Gottesman, M. M.; Hait, W. N. Cellular and Biochemical Characterization of Thioxanthenes for Reversal of Multidrug Resistance in Human and Murine Cell Lines. *Cancer Res.* **1990**, *50*, 1748−1756.

(13) Dhainaut, A.; Regnier, G.; Atassi, G.; Pierre, A.; Leonce, S.; Kraus-Berthier, L.; Prost, J. F. New Triazine Derivatives as Potent Modulators of Multi-Drug Resistance. *J. Med. Chem.* **1992**, *35*, 2481−2496.

(14) Dodic, N.; Dumaitre, B.; Daugan, A.; Pianetti, P. Synthesis and Activity Against Multidrug Resistance in Chinese Hamster Ovary Cells of New Acridone-4-carboxamides. *J. Med. Chem.* **1995**, *38*, 2418−2426.

(15) Ecker, G.; Chiba, P. Recent Developments in Overcoming Tumour Cell Multi-Drug Resistance. *Exp. Opin. Ther. Patents* **1997**, *7*, 589−599.

(16) Chiba, P.; Burghofer, S.; Richter, E.; Tell, B.; Moser, A.; Ecker, G. Synthesis, Pharmacologic Activity and Structure−Activity-Relationships of a Series of Propafenone-Related Modulators of Multi-Drug-Resistance. *J. Med. Chem.* **1995**, *38*, 2789−2793.

(17) Chiba, P.; Ecker, G.; Schmid, D.; Drach, J.; Tell, B.; Gekeler, V. Structural Requirements for Activity of Propafenone Type Modulators in PGP-Mediated Multidrug Resistance. *Mol. Pharmacol.* **1996**, *49*, 1122−1130.

(18) Elguero, J.; Marzin, C.; Katritzky, A. R.; Linda, P. The Tautomerism of Heterocycles. In *Advances in Heterocyclic Chemistry, Supplement 1*; Katritzky, A. R., Boulten, A. J., Eds.; Academic Press: New York, San Francisco, London, 1976; pp 313−336 and references therein.

(19) Schofield, K.; Grimmett, M. R.; Keene, B. T. R. *Heteroaromatic Nitrogen Compounds: The Azoles;* Cambridge University Press: Cambridge, 1976; pp 85−91 and references therein.

(20) Holzer, W.; Plagens, B. On the Methylation of Pyrazolones. *Sci. Pharm.* **1996**, *64*, 455−462.

(21) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909. AMPAC 5.0, 1994; Semichem, 12715 W. 66th Terrace, Shawnee, KS 66216.

(22) Holzer, W.; Plagens, B.; Lorenz, K. Alkylation of Pyrazolones via the Mitsunobu Reaction. *Heterocycles* **1997**, *45*, 309−314.

(23) Patt, S. L.; Shoolery, J. N. Attached Proton Test for Carbon-13 NMR. *J. Magn. Reson.* **1982**, *46*, 535−539.

(24) Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis;* VCH Publishers: New York, Weinheim, Cambridge, 1989; pp 211−252.

(25) Bax, A.; Freeman, R. Investigation of Complex Networks of Spin−Spin Coupling by Two-Dimensional NMR. *J. Magn. Reson.* **1981**, *44*, 542−561.

(26) Bax, A.; Subramanian, S. Sensitivity-Enhanced Two-Dimensional Heteronuclear Shift Correlation NMR Spectroscopy. *J. Magn. Reson.* **1986**, *67*, 565−569.

(27) Davis, D. G.; Bax, A. Simplification of ^{1}H NMR Spectra by Selective Excitation of Experimental Subspectra. *J. Am. Chem. Soc.* **1985**, *107*, 7197−7198.

(28) Sarkar, S. K.; Bax, A. A Simple and Sensitive One-Dimensional NMR Technique for Correlation of Proton and Carbon Chemical Shifts. *J. Magn. Reson.* **1985**, *62*, 109−112.

(29) Bax, A. Structure Determination and Spectral Assignment by Pulsed Polarisation Transfer Via Long-Range ^{1}H-^{13}C couplings. *J. Magn. Reson.* **1984**, *57*, 314−318.

(30) Ghose, A. K.; Pritchett, A.; Crippen, G. M. Atomic Physicochemical Parameters for Three-Dimensional Structure Directed Quantitative Structure−Activity Relationships III: Modeling Hydrophobic Interactions. *J. Comput. Chem.* **1988**, *9*, 80−90.

(31) P. Baricic, M. Mackov, distributed by Milan Hudecek, P. Horova 18, 841 07 Bratislava, Slowakia.

(32) O. A. Raevsky, V. Y. Grigor′ev, Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russia.

(33) Tripos GmbH, Munich, Germany; 1997.

(34) Gekeler, V.; Frese, G.; Noller, A.; Handgretinger, R.; Wilisch, A.; Schmidt, H.; Muller, D.; Dopfer, R.; Klingbiel, T.; Diddens, H.; Probst, H.; Niethammer, D. Mdr1/P-glycoprotein, Topoisomerase and Glutathione S-transferase Gene Expression in Primary and Relapsed State Adult and Childhood Leukemias. *Br. J. Cancer* **1992**, *66*, 507−517.

(35) Ecker, G.; Chiba, P.; Hitzler, M.; Schmid, D.; Visser, K.; Cordes, H. P.; Csöllei, J.; Seydel, J. K.; Schaper, K.-J. Structure−Activity Relationship Studies on Benzofurane Analogues of Propafenone-Type Modulators of Tumor Cell Multidrug Resistance. *J. Med. Chem.* **1996**, *39*, 4767−4774.

(36) Kubinyi, H. QSAR: Hansch Analysis and Related Approaches. In *Methods and Principles in Medicinal Chemistry*; Mannhold, R., Krogsgaard-Larsen, P., Timmermann, H., Eds.; Verlag Chemie: Weinheim, 1993; Vol. 1, pp 21−53.

(37) Sarenko, A. S.; Kvitko, I. Y.; Efros, L. S. Heterocyclic Analogues of Xanthones II. C-Acylation of 5-Pyrazolones and Synthesis of Chromono[3,2-d]pyrazoles. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1972**, *8*, 722−727.

(38) Jensen, B. S. The Synthesis of 1-Phenyl-3-methyl-4-acyl-pyrazolones-5. *Acta Chem. Scand.* **1959**, *13*, 1668−1670.

(39) Avar, L.; Hofer, K.; Preiswerk, M. Stabilizing Organic Materials Employing Pyrazole Compounds and Stabilized Compositions Thereof. U.S. Patent 4,008,200, Feb 15, 1977.

(40) Lakkis, M.; Lakkis, Z.; Goetz-Grandmont, G. J.; Brunette, J.-P. Extraction of Cobalt(II) With Mixtures of 4-Acyl-pyrazol-5-ols and Aliquat 336 in Toluene: Effect of the Pyrazolol Substituents. *Monatsh. Chem.* **1991**, *122*, 9−15.
(41) Gekeler, V.; Weger, S.; Probst, H. Mdr1/P-glycoprotein Gene Segments Analyzed from Various Human Leukemic Cell Lines Exhibiting Different Multidrug Resistance Profiles. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 796−802.
(42) Pastan, I.; Gottesman, M. M.; Ueda, K.; Lovelace, E.; Rutherford, A. V.; Willington, M. C. A Retrovirus Carrying an *mdr1* cDNA Confers Multidrug Resistance and Polarized Expression of P-glycoprotein in MDCK Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4486-4490.
(43) Gilboa, E.; Eglitis, M. A.; Kantoff, P. W.; Anderson, F. Transfer and Expression of Cloned Genes Using Retroviral Vectors. *Biotechniques* **1986**, *4*, 504−512.

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