g) was dissolved in acetic acid (400 mL) and hydrogenated at 50 psi/30 °C by using Adams' catalyst. The catalyst was removed, the filtrate evaporated, and the residue basified to about pH 12 (Na_2CO_3) and then extracted with chloroform. The combined organic extracts were dried (Na_2SO_4) and evaporated to leave *N*-butyl-4-piperidinecarboxamide (18.7 g, 14%), mp 75–79 °C. A sample of the product was converted to the oxalate salt, which was recrystallized from 2-propanol/ethyl acetate, mp 143–144 °C. Anal. ($C_{10}H_{20}N_2O \cdot C_2H_2O_4$) C, H, N. *N*-Butyl-3-piperidinecarboxamide [oxalate salt, mp 145–146 °C; anal. ($C_{10}H_{20}N_2O \cdot C_2H_2O_4$) C, H, N] and *N*-phenyl-4-piperidinecarboxamide [hydrochloride salt, mp 231–233 °C; anal. ($C_{12}H_{16}N_2O \cdot HCl$) C, H, N] were prepared by hydrogenation of *N*-butylnicotinamide and *N*-phenyl-4-pyridinecarboxamide by the method above.

***N*-Propyl-4-piperidineacetamide.** Methyl 4-pyridineacetate (10 g), propylamine (21.5 g), and 3A molecular sieves were heated at 100 °C for 48 h in a stainless steel bomb. Excess amine and molecular sieves were removed, and the crude product in glacial acetic acid (100 mL) was hydrogenated over Adams' catalyst at 30 °C/50 psi. The reaction was filtered and evaporated and the residue basified ($NaHCO_3$ solution) and extracted with ethyl acetate (100 mL). The extract was dried ($MgSO_4$) and evaporated to leave *N*-propyl-4-piperidineacetamide as a crude oil, which was characterized spectroscopically.

Route E. *N*-Methyl-*N*-(phenylmethyl)-4-piperidinecarboxamide. A solution of 1-[(phenylmethoxy)carbonyl]-4-piperidinecarbonyl chloride (7.5 g) in toluene (50 mL) was added dropwise to a cooled (ice/water) solution of *N*-methylbenzylamine (10.0 g) in toluene (100 mL). After the addition was complete, the solution was stirred at room temperature for 7 h and then allowed to stand at room temperature overnight. The mixture was washed with water, dried (Na_2SO_4), and evaporated. A cooled (ice/water), stirred solution of the residue (7.19 g) in acetic acid (25 mL) was slowly treated with a saturated solution of HBr in acetic acid (50 mL) and then the mixture stirred at 0 °C for 1 h and at room temperature for a further 3 h. Ether (250 mL) was added, the mixture evaporated, and the residue basified (Na_2CO_3), extracted with chloroform, dried ($MgSO_4$), and evaporated. The residue was taken up in chloroform and treated with ethereal HCl to give *N*-methyl-*N*-(phenylmethyl)-4-piperidinecarboxamide hydrochloride, mp 175–176 °C. Anal. ($C_{14}H_{20}N_2O \cdot HCl$) C, H, N.

***N*-(2-Methoxyethyl)-4-piperidinecarboxamide.** *N*-(2-Methoxyethyl)-1-[(phenylmethoxy)carbonyl]-4-piperidinecarboxamide (5.76 g) [mp 85–86 °C; Anal. ($C_{17}H_{24}N_2O_4$) C, H, N], prepared as above but with chloroform as solvent, was hydrogenated over 5% Pd/C at 50 psi/50 °C. The catalyst was removed and the filtrate evaporated to leave 4-[*N*-(2-methoxyethyl)carbamoyl]piperidine as an oil, which was characterized spectroscopically. *N*-(2-Hydroxyethyl)-4-piperidinecarboxamide was prepared and characterized similarly.

Route F. *N*-Butyl(4-piperidinyloxy)acetamide Hydrochloride. (a) 1-(Phenylmethyl)-4-hydroxypiperidine (10 g, 52 mmol) in dry DMF (50 mL) was added dropwise to a stirred suspension of NaH (5 g, 50% dispersion in mineral oil) in dry DMF (50 mL) at 20 °C under an atmosphere of nitrogen. The suspension was stirred at 20 °C for 4 h, and then chloroacetic acid (4.95 g, 52 mmol) in DMF (50 mL) was added slowly in two equal portions with a 2-h interval between. The resulting thick slurry was stirred at 20 °C for 24 h, 2-propanol (75 mL) added, and the mixture acidified to pH 6 (2 N HCl) and then concentrated. The aqueous residue was adjusted to pH 10 (NaOH) and extracted with chloroform (3 × 100 mL) and then the aqueous layer acidified to pH 3 (2 N HCl) and extracted with chloroform (3 × 100 mL). The residual aqueous phase was concentrated to half-volume and filtered and the filtrate evaporated to give [[1-(phenylmethyl)-4-piperidinyl]oxy]acetic acid hydrochloride (3 g, 20%), characterized by NMR.

(b) A solution of [[1-(phenylmethyl)-4-piperidinyl]oxy]acetyl chloride [prepared from the corresponding acid hydrochloride salt (7.0 g, 24 mmol) with thionyl chloride] in chloroform (50 mL) was added dropwise to a stirred solution of *n*-butylamine (3.70 g, 50 mmol) in chloroform (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 4 h and then left at room temperature overnight. The chloroform solution was washed with water (3 × 50 mL), sodium hydroxide solution (2 N, 3 × 50 mL), and HCl solution (2 N, 3 × 50 mL). The combined acidic extracts were basified to pH 12 and extracted with chloroform (3 × 100 mL), and then the organic extracts were dried (Na_2SO_4) and evaporated. The residue was taken up in ether and then treated with ethereal HCl to give *N*-butyl[[1-(phenylmethyl)-4-piperidinyl]oxy]acetamide hydrochloride (3.5 g, 42%), characterized by NMR.

(c) The above product (3.0 g, 8.8 mmol) in ethanol (100 mL) was hydrogenated over 5% Pd/C at 50 psi/50 °C, then the catalyst removed, and the filtrate evaporated. The residue was triturated with ether and *N*-butyl(4-piperidinyloxy)acetamide hydrochloride (1.7 g, 77%) collected. A sample was recrystallized from 2-propanol/diethyl ether and then ethyl acetate, mp 145–146 °C. Anal. ($C_{11}H_{22}N_2O_2 \cdot HCl$) C, H, N.

Biology. Experimental details for evaluation of binding, functional, and antihypertensive activities have been detailed previously.[1]

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Synthesis of a Series of Compounds Related to Betaxolol, a New β_1-Adrenoceptor Antagonist with a Pharmacological and Pharmacokinetic Profile Optimized for the Treatment of Chronic Cardiovascular Diseases

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A series of para-substituted phenoxypropanolamines has been synthesized and tested for β-adrenoceptor blocking activity. Some derivatives (**8**, 11, **12**, **20**, 21) exhibited greater in vitro potency than the reference drugs metoprolol and propranolol. This series, in contrast to propranolol but similar to metoprolol, possesses cardioselectivity. The 3-[*p*-[(cycloalkylmethoxy)ethyl]phenoxy]-1-substituted-amino-2-propanol derivatives **8** (cyclopropylmethoxyethyl: betaxolol) and 11 (cyclobutylmethoxyethyl) produced antihypertensive effects in spontaneously hypertensive rats. Betaxolol (Kerlon, **8**) was found to exhibit an appropriate preclinical pharmacological and human pharmacokinetic profile (elevated oral bioavailability and prolonged plasma half-life) for the treatment of chronic cardiovascular diseases such as hypertension and angina.

Certain chronic cardiovascular diseases such as hypertension and angina are treated efficaciously with β-adrenoceptor antagonists. Within this therapeutic class there are drugs that have variable degrees of potency associated with ancillary properties such as membrane stabilizing activity, relative selectivity toward the β_1-adrenoceptor

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subtype, and partial agonism. Furthermore, β-adrenoceptor antagonists can be distinguished on the basis of their pharmacokinetic profile, which is of great importance for determining the most appropriate regimen to relieve chronic diseases.

At the beginning of our research program on new β-adrenoceptor antagonists aimed at the therapy of hypertension and angina, we established some acceptance criteria for a compound that would enter the clinical utilization market in the early 1980s. The desirable properties we wished to combine in a single molecule were present, although separately, in the various known β_1-adrenoceptor antagonists; they were as follows: a relatively high potency (not less than that of propranolol), elevated β_1-adrenoceptor selectivity, excellent oral absorption accompanied by a low first pass metabolism, a plasma half-life above 12–13 h, and the absence of active and particularly noncardioselective metabolites. The latter three properties would be essential in order to reduce interindividual variations in drug plasma concentration and allow a "once-a-day" dosage without the need of a special galenic formulation. This pharmacokinetic profile would also facilitate the practitioners task to determine the appropriate dose regimen for optimal therapeutic benefit and at the same time improve patients compliance to the prescribed therapy.

The literature available at the time suggested that cardioselectivity could be conferred on a phenoxypropanolamine skeleton by appropriate substitution in the para position of the aromatic ring (e.g., practolol[1] (**1**), atenolol[2] (**2**), and metoprolol[3] (**3**)) or on the amino group of the propanolamine chain (e.g., tolamolol[4]).

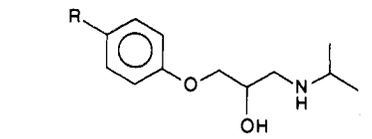
Metoprolol (**3**) was known to be a cardioselective β_1-adrenoceptor antagonist; however, it possessed a relatively short duration of action and an elevated "first pass" hepatic deactivation that was responsible for its low oral bioavailability.[5] We advanced the hypothesis that the replacement of a methyl group of the methoxyethyl chain of metoprolol (**3**) by bulky stable substituents at the extremity of the chain in the para position would result in compounds that would be rather resistant to hepatic microsomal deactivation.

Thus, certain substituents were chosen to afford substantial modifications in physicochemical properties of the parent molecule (**3**).

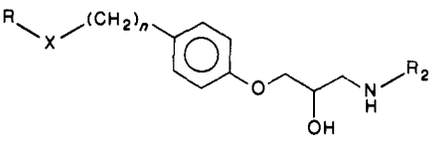
This paper describes the synthesis and some pharmacological properties of a small goal-oriented chemical series (compounds **4–27**). Furthermore, the criteria that were followed in the choice of the selective β_1-adrenoceptor antagonist betaxolol (Kerlon, **8**) for clinical development will also be briefly exposed.

Chemistry

Most of the derivatives (**4–27**) were prepared by the alkylation of 4-substituted phenols with epichlorhydrin followed by reaction with various amines (method A) to afford the final products as racemates. The stereospecific synthesis of the enantiomers of **8** was carried out as indicated in Scheme I. The synthesis of **9**, the *S* enantiomer of **8**, was achieved by alkylation of phenol **33** with (*S*)-(−)-2-phenyl-3-isopropyl-5-(hydroxymethyl)oxazolidinyl tosylate[6] followed by acid-catalyzed hydrolysis. Compound **10** was prepared by reaction of **33** with (2*R*)-3-(tosyloxy)-1,2-propanediol acetonide.[7] Hydrolysis of the product obtained, tosylation, epoxidation, and treatment with isopropylamine afforded **10**, the *R* enantiomer of **8**.



1 (practolol), R = H_3CCONH
2 (atenolol), R = H_2NCOCH_2
3 (metoprolol), R = $H_3CO(CH_2)_2$



4–27

R = $(CH_2)_m$—$(CH_2)_p$; m = 2–5; p = 0, 1; n = 1–3
X = O, S, SO, SO_2
R_2 = *i*-Pr, other substituents (see Table I)

The phenolic intermediates were obtained by the routes indicated in Scheme II. Ethyl 4-methoxy(or 4-(benzyloxy))phenylalkanecarboxylates were reduced by $LiAlH_4$ or by $NaBH_4$ in the presence of lithium chloride, to afford the corresponding alcohols. The compounds were etherified in the presence of a base[8] (method B) or converted into halides or mesylates, which were then used to alkylate various alkoxides (method C). Reaction of activated alcohols with thiourea gave thiols that were alkylated and then oxidized into sulfones or sulfoxides (method D). Phenols were obtained by demethylation of methyl ethers by means of pyridine hydrochloride (method E) or catalytic hydrogenation of the benzyl ethers (method F). An alternative method involving etherification of a diazo ketone with an alcohol,[9] followed by hydrogenation of the keto ether, gave the corresponding phenols (method G).

Biological Results

The β_1-adrenoceptor blocking potency in vitro of all compounds was determined in spontaneously beating atria isolated from rats that were stimulated with the β-adrenoceptor agonist isoproterenol.[10]

The β_2-adrenoceptor potency in vitro of some derivatives was evaluated in the rat tracheal chain contracted with carbachol and then relaxed with isoproterenol.[11] The degree of cardioselectivity of these compounds was defined as the ratio of the antagonist potency toward β_1- over β_2-adrenoceptors.

The local anesthetic activity of some derivatives was measured in the frog sciatic nerve preparation[12] and oral antihypertensive effects of the compounds (5 mg/kg) determined in adult spontaneously hypertensive rats (SHR).

Some homologues of **8** were submitted to metabolic studies using rat microsomal enzymes to analyze the in-

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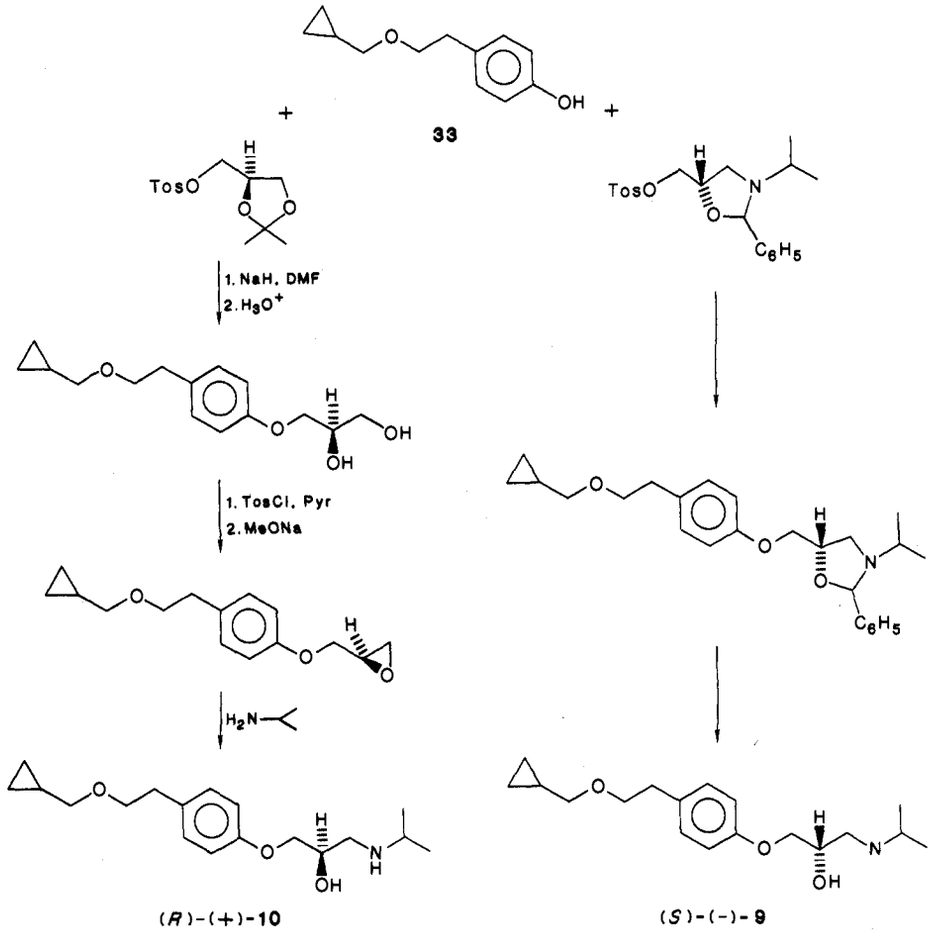
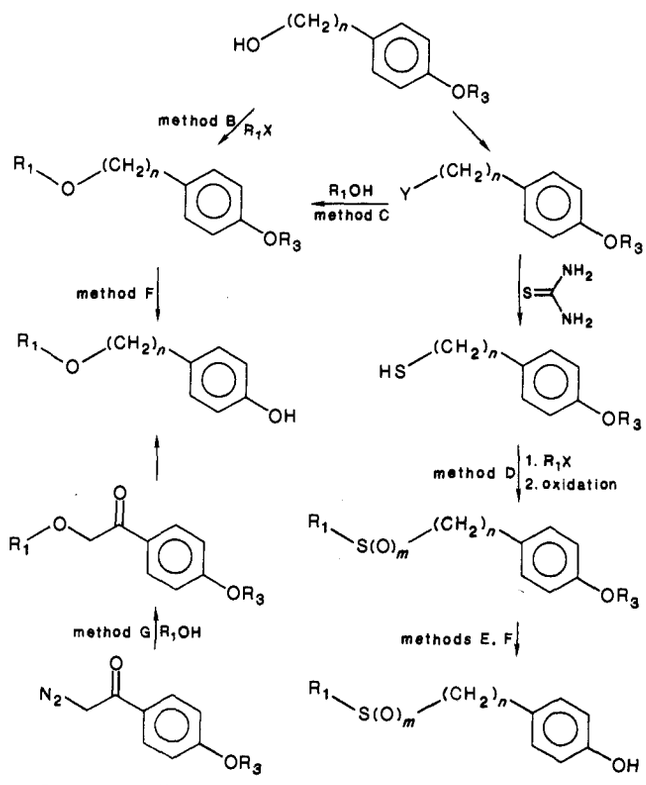
Scheme I



Scheme II[a]



[a] R_1 = cycloalkyl(C_3–C_6)methyl, cycloalkyl(C_5, C6); R_3 = CH_3, CH_2Ph; X, Y = leaving groups; n = 1–3; m = 0–2.

fluence of structural modifications on the rate of metabolic changes.

Table I shows the high β_1-adrenoceptor antagonist potency of derivatives **8**, **11**, **20**, **21** when compared to that of metoprolol (**3**) or propranolol. In metabolism studies, the selected compounds **8**, **11**, **18** showed a relative resistance to microsomal enzyme deactivation, while metoprolol was more rapidly metabolized.

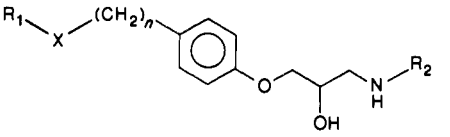
Structure-Activity Relationships

(1) In Vitro β_1-Adrenoceptor Blocking Activity. The derivatives **8**, **11**, **20**, **21**, which possess two carbon atoms (n = 2) between the substituent X (Table I) and the aromatic ring, were found to be the most potent compounds of this chemical series. For instance, **11** was more active than the corresponding derivatives **5** and **27**, which had either a shorter (n = 1) or a longer alkylene chain (n = 3), respectively. The same observation holds for **8**, a compound that was 5 times more potent than the derivative **4** in which n = 1.

Replacement of the *N*-isopropyl group on the nitrogen of **11** by groups conferring higher hydrophilicity (**13**, **14**) or lipophilicity (**15**–**17**) afforded less potent β-adrenoceptor antagonists. The derivative in which the oxygen atom of **11** had been replaced by a sulfur (**23**) was poorly active while **24**, which has a sulfonyl group, retained a satisfactory β-adrenoceptor antagonist potency. Replacement of cyclobutyl or cyclohexyl ring of **24** and **25** with a tetrahydro-2-furyl moiety in **26** resulted in a weak β-adrenoceptor activity.

Compound **20**, in which the cyclopentyl group was directly attached to the oxygen atom, exhibited a considerable potency that was of similar magnitude to that of the cyclobutylmethyl analogue (**11**). Interestingly, these two derivatives possessed equivalent lipophilicity. However, the isolipophilic cyclohexyl (**22**) and cyclopentyl-

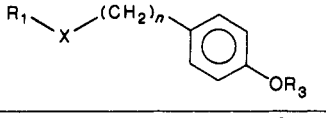
Table I. 1-(4-Substituted phenoxy)propanolamines



no.	*n*	X	R_1	R_2	formula[a]	salt	mp, °C	yield, %	$pA_2(\beta_1)$[b]	SHR[c] 2 h	SHR[c] 4 h	SHR[c] 24 h
4	1	O	c-PrCH$_2$	*i*-Pr	$C_{17}H_{27}NO_3$	base	48	50	8.01	−4	−3	−6
5	1	O	c-BuCH$_2$	*i*-Pr	$C_{18}H_{30}ClNO_3$	HCl	98	40	7.44	−9	−6	−1
6	1	SO	c-PrCH$_2$	*i*-Pr	$C_{21}H_{31}NO_7S$	mal[h]	131	68	7.38	−4	−8	−2
7	1	SO	c-Pent-CH$_2$	*i*-Pr	$C_{19}H_{31}NO_3S$	base	90	44	inact	−5	−7	−1
8	2	O	c-PrCH$_2$	*i*-Pr	$C_{18}H_{30}ClNO_3$	HCl	116	80	8.53	−22*[d]	−19*	−6
9[e]	2	O	c-PrCH$_2$	*i*-Pr	$C_{22}H_{33}NO_7$	mal	96	42	8.30	−10	−12	−13
10[f]	2	O	c-PrCH$_2$	*i*-Pr	$C_{22}H_{33}NO_7$	mal	95–97	83	6.90	3	5	3
11	2	O	c-BuCH$_2$	*i*-Pr	$C_{19}H_{32}ClNO_3$	HCl	104	71	9.55	−16*	−16*	−4
12	2	O	c-BuCH$_2$	c-Pr	$C_{19}H_{30}ClNO_3$	HCl	130	45	8.53	0	−5	0
13	2	O	c-BuCH$_2$	CH(CH$_3$)CH$_2$CN	$C_{20}H_{31}ClN_2O_3$	HCl	126	50	6.47	−2	−4	−2
14	2	O	c-BuCH$_2$	(CH$_2$)$_2$NHCOCH$_3$	$C_{20}H_{33}ClN_2O_4$	HCl	150	42	7.21	−6	−8	−7
15	2	O	c-BuCH$_2$	(CH$_2$)$_2$C$_6$H$_3$(3,4-(OMe)$_2$)	$C_{26}H_{38}ClNO_5$	HCl	152	67	7.75	−7	−5	−6
16	2	O	c-BuCH$_2$	CH(CH$_3$)(CH$_2$)$_2$C$_6$H$_5$	$C_{26}H_{37}NO_3$	base	56	25	5.11	−1	1	2
17	2	O	c-BuCH$_2$	CH(CH$_3$)(CH$_2$)$_2$C$_6$H$_4$-4-F	$C_{26}H_{36}FNO_3$	base	56	42	5.54	−4	−10	−2
18	2	O	c-PentCH$_2$	*i*-Pr	$C_{22}H_{35}NO_5$	fum[i]	139	50	6.89	−21*	−16*	−8
19	2	O	c-HexCH$_2$	*i*-Pr	$C_{21}H_{36}ClNO_3$	HCl	122	60	6.84	−14	−18*	−7
20	2	O	c-Pent	*i*-Pr	$C_{19}H_{31}NO_3$	base	66	48	9.30	−17*	−11	−4
21[g]	2	O	c-Pent	*i*-Pr	$C_{19}H_{32}ClNO_3$	HCl	97	17	8.70	−8	−16*	−7
22	2	O	c-Hex	*i*-Pr	$C_{22}H_{35}NO_5$	fum	98	63	8.23	4	−2	−12
23	2	S	c-BuCH$_2$	*i*-Pr	$C_{19}H_{31}NO_2S$	base	52	32	7.11	−2	−6	−3
24	2	SO$_2$	c-BuCH$_2$	*i*-Pr	$C_{19}H_{32}ClNO_4S$	HCl	143	27	7.94	−4	−13	1
25	2	SO$_2$	c-HexCH$_2$	*i*-Pr	$C_{21}H_{36}ClNO_4S$	HCl	148	30	8.13	−6	−2	−2
26	2	SO$_2$	THfur-CH$_2$[k]	*i*-Pr	$C_{19}H_{32}ClNO_5S$	HCl	156	40	6.80	−22*	−16*	−3
27	3	O	c-BuCH$_2$	*i*-Pr	$C_{22}H_{35}NO_7$	oxa[j]	117	55	7.02	−11*	−11	−1
3	2	O	CH$_3$[l]						7.64	−18*	−9	−1
propranolol									8.36	0	0	−9

[a] All compounds exhibited satisfactory (±0.4% of the calculated values) elemental (C, H, Cl, N, S) analysis. [b] β_1-Adrenoceptor antagonist activity in spontaneously beating rat atria (n = 5/group); the standard error of the pA_2 value was generally less than 5% of the reported mean value. [c] Antihypertensive activity expressed as percentages of base-line systolic blood pressure of base-line values, in spontaneously hypertensive rats (SHR, n = 5/group). [d] Significant response ($p < 0.05$, paired t test). [e] *S*-(−) isomer of **8**. [f] *R*-(+) isomer of **8**. [g] *S*-(−) isomer of **20**. [h] Maleate. [i] Fumarate. [j] Oxalate. [k] Tetrahydrofuryl-2-methyl. [l] Metoprolol.

Table II. 4-Substituted Ethers and Phenols



no.	*n*	X	R_1	R_3	formula[a]	yield, %	meth	mp[b] or bp,[c] °C
28	1	O	c-PrCH$_2$	CH$_2$C$_6$H$_5$	$C_{18}H_{20}O_2$	75	C	190/0.01
29	1	O	c-PrCH$_2$	H	$C_{11}H_{14}O_2$	85	F	125/0.05
30	1	O	c-BuCH$_2$	CH$_2$C$_6$H$_5$	$C_{19}H_{22}O_2$	85	C	160/0.02
31	1	O	c-BuCH$_2$	H	$C_{12}H_{16}O_2$	90	F	130/0.05
32	2	O	c-PrCH$_2$	CH$_2$C$_6$H$_5$	$C_{19}H_{22}O_2$	74	B	182/0.07
33	2	O	c-PrCH$_2$	H	$C_{12}H_{16}O_2$	84	F	138/0.07
34	2	O	c-BuCH$_2$	CH$_2$C$_6$H$_5$	$C_{20}H_{24}O_2$	80	B	175/0.07
35	2	O	c-BuCH$_2$	H	$C_{13}H_{18}O_2$	80	F	134/0.05
36	2	O	c-PentCH$_2$	CH$_2$C$_6$H$_5$	$C_{21}H_{26}O_2$	31	B	183/0.03
37	2	O	c-PentCH$_2$	H	$C_{14}H_{20}O_2$	85	F	135/0.05
38	2	O	c-HexCH$_2$	CH$_2$C$_6$H$_5$	$C_{22}H_{28}O_2$	71	B	185/0.01
39	2	O	c-HexCH$_2$	H	$C_{15}H_{22}O_2$	90	F	140/0.05
40	2	S	c-BuCH$_2$	CH$_3$	$C_{14}H_{20}OS$	72	D	52
41	2	S	c-BuCH$_2$	H	$C_{13}H_{18}OS$	65	E	145/0.05
42	2	SO$_2$	c-BuCH$_2$	CH$_3$	$C_{14}H_{20}O_3S$	81	D	52
43	2	SO$_2$	c-BuCH$_2$	H	$C_{13}H_{18}O_3S$	92	E	112
44	2	SO$_2$	THfur-CH$_2$[d]	CH$_2$C$_6$H$_5$	$C_{20}H_{24}O_4S$	71	D	118
45	2	SO$_2$	THfur-CH$_2$[d]	H	$C_{13}H_{18}O_4S$	90	F	54
46	2	O	c-Pent	CH$_2$C$_6$H$_5$	$C_{20}H_{24}O_2$	80	C	<30
47	2	O	c-Pent	H	$C_{13}H_{18}O_2$	97	F	77
48	2	O	c-Hex	H	$C_{14}H_{20}O_2$	96	G	130/0.1
49	3	O	c-BuCH$_2$	CH$_2$C$_6$H$_5$	$C_{21}H_{26}O_2$	75	B	180/0.03
50	3	O	c-BuCH$_2$	H	$C_{14}H_{20}O_2$	85	F	140/0.05

[a] All compounds exhibited satisfactory (±0.4% of the calculated values) elemental (C, H, Cl, N, S) analysis. [b] Melting point for **40**, **42–47**. [c] Boiling point (temperature pressure in millimeters) for **28–39**, **41**, **48–50**. [d] Tetrahydrofuryl-2-methyl.

methyl (**18**) derivatives did not exhibit the same level of β_1-adrenoceptor antagonist activity.

It seems, therefore, that the potency of the compounds as β_1-adrenoceptor antagonists depends simultaneously on

Table III. Cardioselectivity of Some Derivatives

compound	$pA_2 \pm$ SEM (against isoprenaline) chronotropic effects in rat atria (n = 5/group)	guinea pig tracheal chain (n = 5/group)	antilog (pA_2 atria − pA_2 trachea), degree of selectivity[a]
4	8.01 ± 0.17	5.54 ± 0.10	295.12
8	8.53 ± 0.18	5.40 ± 0.16	1348.96
11	9.56 ± 0.12	6.02 ± 0.05	3467.37
19	6.84 ± 0.20	5.42 ± 0.08	26.30
metoprolol	7.64 ± 0.10	6.14 ± 0.06	31.62
propranolol	8.36 ± 0.08	7.90 ± 0.08	2.88
practolol	6.73 ± 0.19	5.08 ± 0.10	44.67

[a] Difference between the pA_2 values of the antagonists obtained in the isolated rat atria and guinea pig tracheal chain against isoprenaline providing an indication of selectivity for cardiac β_1-adrenoceptors.

the size of R, the nature of X, the length of the alkylene chain between the aromatic ring and X, and the nature of the substituent on the amine nitrogen. The best compounds (**8**, **11**, **20**, **21**) of our chemical series exhibited a greater in vitro potency than either metoprolol or propranolol as β_1-adrenoceptor antagonists.

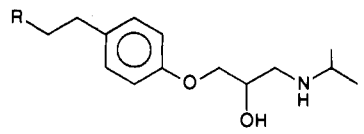
(2) β_2-Adrenoceptor Blocking Activity. The compounds of this series exhibit low β_2-adrenoceptor activity. This confirms the earlier observation[13] that para substitution on the phenyl ring leads to cardioselective β-blockers. However, the degree of cardioselectivity depends on the nature and the size of the substituents introduced in the chain on the para position of the phenoxypropanolamine moiety. Table III shows that all the derivatives tested possessed a high degree of cardioselectivity.

(3) Antihypertensive Activity. Some derivatives with n = 2 and X = O (**8**, **11**, **18**, **20**) exhibited oral antihypertensive activity in the SHR, while **24**, which has a sulfonyl group and was more potent than metoprolol as a β_1-adrenoceptor blocker, was found inactive in the SHR. Thus, structural requirements for antihypertensive efficacy seem to differ from those for the β_1-adrenoceptor blocking activity.

(4) Local Anesthetic Activity. The membrane stabilizing property of β_1-adrenoceptor blocking agents has been suggested as being responsible for the myocardial depressant action of these compounds[14] and not suitable for the use of these agents to treat glaucoma. Table IV shows that lipophilicity (π_R) correlates to a certain extent with local anesthetic potency.[15] The low value found for betaxolol (**8**) was judged to be an advantage over **11**, which was, however, a more potent β_1-adrenoceptor antagonist. The small local anesthetic effect of betaxolol allowed the development of this compound as an antiglaucoma agent (Betoptic).

(5) Studies on the Biotransformation Rate of Some Compounds (8, 11, 18) under in Vitro and in Vivo Conditions in the Rat. Under in vivo conditions, the derivatives **8**, **11**, **18**, which were the object of advanced studies, possessed a β_1-adrenoceptor antagonist activity longer than that of metoprolol (**3**) and propranolol. Moreover, their potencies did not substantially differ whether determined by the intravenous or oral administration.[17] These results indicate that the oral bioavailability of these compounds is very high. Further analysis of the role played by the size of the cycloalkyl moiety in the para position of the aromatic ring was done by determining in vitro the apparent Michaelis–Menten constants[18] (K_M in μmol/L and V_{max} in nM min^{-1} (mg of protein)$^{-1}$) with the S_9 microsomal fraction of the rat liver. This study demonstrated a 6 times faster rate of metabolism for **3** (metoprolol) (K_M = 3.6, V_{max} = 0.46) than for **8** (betaxolol)[19] (K_M = 6.3, V_{max} = 0.07). The correlation coefficients of the Lineweaver–Burk plots for all the studied compounds were 0.99 and the degrees of freedom of the correlation analysis were 14 (for metoprolol) and 28 (for betaxolol). The K_M and V_{max} of **11** and **18**, which have bulkier substituents, could not be precisely calculated because their biotransformation was even slower than that of **8**. These data indicate that increasing the size of the substituent on the *p*-ethoxy side chain slowed greatly the rate of hepatic metabolism.

Table IV. Local Anesthetic Activity of Some β_1-Adrenoceptor Blockers



no.	R	π_R[a]	AAL/Prop[b]
3[c]	H_3CO	−1.07	0.01
24	c-$BuCH_2SO_2$	−0.35	0.01
8[d]	c-$PrCH_2O$	0.23	0.23
5	c-$BuCH_2O$	0.75	0.55
20	c-PentO	0.76	0.63
18	c-$PentCH_2O$	1.27	1.07
19	c-$HexCH_2O$	1.79	1.02
23	c-$BuCH_2S$	1.83	1.35

[a] Calculated hydrophobic parameter of the substituent R (from ref 16). [b] Local anesthetic activity in the frog sciatic nerve preparation compared with propranolol (activity = 1). [c] Metoprolol. [d] Betaxolol.

Pharmacokinetic studies in vivo in the rat confirmed these in vitro results.[19] In humans the bioavailability and the half-life of betaxolol (**8**) were respectively more elevated (approximately twice) and prolonged (approximately 4 times) than those of metoprolol (**3**), confirming therefore the described in vitro studies using the S_9 microsomal fraction.[20]

Discussion

The structural modifications introduced on the metoprolol molecule (**3**) to yield compounds **4**–**27** were initially designed to achieve improvements in certain pharmacokinetics properties (e.g., biological half-life) as mentioned earlier. However, unexpectedly some derivatives exhibited significantly higher β_1-adrenoceptor potency than metoprolol.

Increasing the cycloalkyl ring size enhanced lipophilicity and the value of the steric parameter. It seems, therefore, difficult to determine which of these two factors plays the more critical role. For derivatives in which n = 2, β_1-adrenoceptor potency increased with the lipophilicity of the substituent R_1 chain (as measured by the value of the

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hydrophobic parameter) up to a value of approximately 1.8.

The observation, however, that compounds **5** and **7**, which are isolipophilic with **8** and **24**, respectively, are less active than the latter ones suggests that the length of the chain plays an important role. Hence, both the steric parameter and the lipophilicity determine the degree of β-adrenoceptor potency as well as other pharmacological properties of the present chemical series. An attempt to correlate activity with these factors by regression analysis did not reveal any significant relationship. Comparison of the structure of metoprolol with that of our derivatives suggests that a lipophilic cycloalkyl moiety at the end of the chain improves the interaction between the antagonist and the β-adrenoceptor, as well as the presence of a two-carbon chain between the oxygen and the aromatic ring.

The pharmacokinetic profile of β-adrenoceptor antagonists is strongly dependent upon their relative hydrophilicity and lipophilicity. In general, hydrophilic drugs (e.g., nadolol, atenolol, practolol) are poorly absorbed from the gastrointestinal tract, undergo a minor liver deactivation, and are cleared from the body unchanged, generally via the kidney. In contrast, lipophilic drugs (e.g., propranolol) are well absorbed from the gut, but they may undergo substantial first pass hepatic metabolism, thus becoming poorly bioavailable as parent compounds, and are cleared from the body as hydrosoluble metabolites often completely inactive.[21]

One of the main metabolites of metoprolol has a phenylacetic skeleton that is probably formed by the rapid, microsomal enzyme cleavage of the methoxyethyl chain to afford a 2-phenylethanol derivative, which subsequently undergoes an additional oxidation process to yield the corresponding acid.[22] In humans[20] and rats,[19] the main metabolic pathways of **8** were found to be the same as those of metoprolol, but the rate of cleavage of the cyclopropylmethyl group was slower.

Pharmacokinetic studies in humans have demonstrated the interesting properties of **8** and its superiority over metoprolol (**3**) and propanolol.[20] The first pass biotransformation of the latter compounds amounts to 60%. In contrast, as expected on the basis of the small value of V_{max} (0.07 nM/min) found in rat microsomal enzyme experiments, **8** showed in humans a first pass of only 15%, while for metoprolol this value was 50% with a V_{max} in rats of 0.46 nM/min. Similarly, the half-life of **8** in humans was 16–22 h and thus significantly longer than that of either metoprolol (**3**) (3–6 h) or propanolol (3.5–6 h).[20,23]

Conclusion

At the beginning of our chemical program, it was considered that a relatively high β_1-adrenoceptor potency was a necessary, but not sufficient, criterion to justify undertaking full clinical development of a new β-adrenoceptor antagonist.

Potent oral antihypertensive activity in SHR, high cardioselectivity, low membrane stabilizing activity, elevated bioavailability, and a long duration of action were postulated as desirable attributes of a candidate compound. Derivative **8** (betaxolol, Kerlon) satisfied these criteria[20,24] and also exhibited a weaker myocardial depressant activity than **11** and **20** in the pentobarbital-anesthetized dogs.[17] Thus, betaxolol (**8**) can be considered a molecule with pharmacokinetics properties very desirable for a drug aimed to treat chronic cardiovascular diseases.[22] The overall preclinical and toxicological safe profiles of betaxolol[25] (**8**) also permitted its development as an antiglaucoma agent.[26]

Experimental Section

Melting points were determined on a Büchi melting point apparatus and are uncorrected. IR spectra were measured on a Perkin-Elmer 399 spectrophotometer. NMR spectra were obtained on a Bruker WP 80 or WP 200 SY spectrometer using tetramethylsilane as internal reference. Structural assignments for all the new compounds were consistent with spectra. Where analyses are reported by elemental symbols, results were within ±0.4% of the calculated values.

Cyclobutanemethanol,[27] cyclopentanemethanol,[28] cyclohexanemethanol,[29] and tetrahydrofurylmethanol[30] tosylates, 2-aminopropanitrile,[31] *N*-acetylethanediamine,[32] and 1-phenyl-3-butanamine[33] were prepared according to literature methods.

Method A. General Method for the Preparation of 4-(Substituted phenoxy)-3-(substituted amino)-2-propanols (Table I, 4–27). 1-[4-[(Cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol (8). To a stirred solution of 38 g (0.2 mol) of 4-[(cyclopropylmethoxy)ethyl]phenol (**33**) and 10 g of NaOH (0.25 mol) pellets in 300 mL of H_2O was added 23 mL of epichlorhydrin (0.29 mol) at ambient temperature. The mixture was stirred 12 h and then extracted with ether. The extract was washed with water, dried, and then concentrated in vacuo to afford an oily residue, which was used without further purification.

A 48-g mixture of the crude 1-[4-[(cyclopropylmethoxy)ethyl]phenoxy]-2,3-epoxypropane obtained above and 250 mL of isopropylamine was heated under reflux for 48 h. The mixture was then evaporated under reduced pressure to afford **8** as a base (80%), mp 70–72 °C (from ligroin), which was converted into its hydrochloride and crystallized from acetone to give 50 g (73%) of **8**, mp 116 °C. Anal. ($C_{18}H_{30}ClNO_3$) C, H, N, Cl.

General Methods (B–G) for the Preparation of 4-Substituted Phenol Ethers and Phenols 28–51. Method B. 2-[4-(Phenylmethoxy)phenyl]ethanol. A 80-g (0.3 mol) sample of 2-[4-(phenylmethoxy)phenyl]acetic acid ethyl ester[34] was added dropwise to a mixture of 6.6 g of $LiAlH_4$ in 200 mL of THF. During the addition, the temperature of the mixture was kept below 0 °C; after completion of the addition, the mixture was stirred for 3 h at room temperature and then was carefully treated with cold water. The precipitate obtained was filtered off. The filtrate was concentrated under reduced pressure to yield 58.4 g (86%), mp 86–88 °C, of 2-[4-(phenylmethoxy)phenyl]ethanol. Anal. ($C_{15}H_{16}O_2$) C, H, N.

4-[(Cyclobutylmethoxy)ethyl]-1-(phenylmethoxy)benzene (34). A stirred suspension of 50% NaH in mineral oil (2.2 g, 0.045 mol) in 50 mL of DMF was treated under argon with 10 g (0.043 mol) of 2-[4-(phenylmethoxy)phenyl]ethanol. The mixture was stirred until H_2 evolution ceased and then treated with 11.3 g (0.047 mol) of cyclobutanemethanol tosylate[25] and heated at 60 °C for 18 h. The mixture was cooled, poured on to ice, and extracted with ether. The ether extract was washed with water, dried over $MgSO_4$, filtered, and concentrated. The residue was treated with ligroin. A solid separated, which was filtered off. The filtrate was concentrated to dryness under reduced pressure

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to afford 10 g (80%) of **34** as an oil, bp 115 °C (0.07 mm).

Method C. 4-[(Cyclobutyloxy)methyl]-1-(phenylmethoxy)benzene (30). This compound was prepared from 8.6 g (0.1 mol) of cyclobutanemethanol and 25.5 g (0.11 mol) of 4-(phenylmethoxy)benzyl chloride[35] as described above for **34** to yield 38 g (85%) of **30**, bp 160 °C (0.02 mm). Anal. ($C_{19}H_{22}O_2$) C, H.

Method D. 4-[[(Cyclobutylmethyl)sulfonyl]ethyl]-1-methoxybenzene (42). A solution of 28 g (0.13 mol) of 2-(4-methoxyphenyl)-1-bromoethane[36] and 9.9 g (0.13 mol) of thiourea in 250 mL of ethanol was refluxed for 2 h. The solution was evaporated after acidification with concentrated HCl and the residue was extracted with ether. The extract was washed with water and aqueous $NaHCO_3$ and then dried. The ethereal phase was concentrated to afford 19.2 g (88%) of 2-(4-methoxyphenyl)ethyl mercaptan as an oil, bp 115 °C (0.1 mm). Anal. ($C_9H_{12}OS$) C, H, S.

A solution of 16.3 g (0.068 mol) of cyclobutanemethanol tosylate[27] in 20 mL of methanol was added dropwise to a solution of 11 g (0.065 mol) of 2-(4-methoxyphenyl)ethyl mercaptan and 4.2 g (0.065 mol) of KOH pellets in 70 mL of methanol. The mixture was refluxed for 2 h, cooled, and filtered. The filtrate was evaporated and the residue dissolved in ether followed by washing and drying. After evaporation 14.8 g (72%) of **40** was obtained, mp 52 °C, bp 135 °C (0.1 mm). Anal. ($C_{14}H_{20}OS$) C, H, S.

A solution of 12 g (0.05 mol) of **40** in 50 mL of CH_2Cl_2 was added dropwise to a solution of 20.6 g (0.1 mol) of 85% *m*-chloroperbenzoic acid in 100 mL of CH_2Cl_2 at −10 °C. During the addition the internal temperature was kept at −10 °C and then the mixture was stirred for 1 h at 0 °C and 2 h at 20 °C. The solid was filtered off and the filtrate evaporated. The solid residue was treated with ether and aqueous $NaHCO_3$. The ethereal phase was washed with water, dried, and evaporated to dryness to afford 10.9 g (81%) of **42**, mp 52 °C. Anal. ($C_{14}H_{20}O_3S$) C, H, S.

Method E. 4-[2-[(Cyclobutylmethyl)sulfonyl]ethyl]phenol (43). A mixture of 10 g (0.037 mol) of **42** and 6.4 g (0.055 mol) of pyridine hydrochloride was heated at 180–190 °C for 1 h. The mixture was cooled and treated with water and ether. The ethereal solution was washed with water, dried over $MgSO_4$, and evaporated to give 8.7 g (92%) of **43**, mp 112 °C. Anal. ($C_{13}H_{18}O_3S$) C, H, S.

Method F. 4-[2-[(Tetrahydrofurylmethyl)sulfonyl]ethyl]phenol (45). A mixture of 11.9 g (0.033 mol) of 44, 2 g of 5% Pd/C, and 250 mL of THF was hydrogenated in a Parr apparatus at 50–60 °C at 50 psi until uptake of H_2 ceased. The mixture was filtered and the filtrate concentrated in vacuo to give a residue, which crystallized from pentane to yield 8 g (90%) of **45**, mp 54 °C. Anal. ($C_{13}H_{18}O_4S$) C, H, S.

Method G. 4-[2-(Cyclohexyloxy)ethyl]phenol (48). Four milliliters of BF_3·Et_2O was added to a mixture of 100 g (1 mol) of cyclohexanol and 33 g (0.13 mol) of 4-(phenylmethoxy)-2-diazoacetophenone[9] heated at 35 °C with stirring. After 4 h the solution was evaporated under reduced pressure. The residue was purified by column chromatography on silica with cyclohexane–ethyl acetate (7:3) as eluent to give 21 g (50%) of [(cyclohexyloxy)methyl][4-(phenylmethoxy)phenyl] ketone, mp 65 °C. Anal. ($C_{21}H_{24}O_3$) C, H.

A mixture of 16 g (0.05 mol) of the above product, 1 g of 5% Pd/C, and 0.5 mL of 70% perchloric acid in 150 mL of methanol was hydrogenated at 50 psi at 40 °C. After complete uptake of H_2, the mixture was filtered and the filtrate evaporated under vacuo. The residue was treated with ether and water. The ether extract was washed with aqueous $NaHCO_3$ and then with water and was dried and evaporated to dryness in vacuo. The residue was chromatographed on silica with cyclohexane–ethyl acetate (8:2) as eluent to give 6.5 g (96%) of **48**, bp 130 °C (0.1 mm). Anal. ($C_{14}H_{20}O_2$) C, H.

(*S*)-(−)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-(isopropylamino)-2-propanol (9). A stirred suspension of 4.8 g (0.1 mol) of 50% NaH in mineral oil in 50 mL of DMF was treated with 17.3 g (0.09 mol) of phenol (**33**) until H_2 evolution ceased. The mixture was cooled to 0 °C and 33.9 g (0.09 mol) of (*S*)-(−)-2-phenyl-3-isopropyl-5-(hydroxymethyl)oxazolidinyl tosylate[6] in 25 mL of DMF was added dropwise and then heated at 50–60 °C for 6 h. The mixture was cooled, poured on to ice, and extracted with ether. The extract was washed with water, dried, and concentrated under reduced pressure. The oily residue was treated with 10 mL of water and 50 mL of concentrated HCl and the mixture was stirred overnight. The mixture was extracted with ether and the aqueous phase was basified with NaOH and then extracted with ether. The ether extract was washed with water, dried over $MgSO_4$, and then evaporated under reduced pressure to give an oil, which was treated with maleic acid in ether. The salt was recrystallized in a mixture of acetone and ether to yield 24.8 g (65%), mp 96–97 °C, of **9**, $[\alpha]^{22}_D$ −14.9° (*c* 2.4, CH_3OH). Anal. ($C_{22}H_{33}NO_7$) C, H, N.

(*R*)-(−)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1,2-propanediol. A mixture of 38.4 g (0.2 mol) of phenol **33**, 8 g (0.2 mol) of NaOH, and 28.6 g (0.1 mol) of (2*R*)-(−)-3-(tosyloxy)-1,2-propanediol acetonide[7] in 200 mL of EtOH was refluxed for 24 h. The solution was concentrated under reduced pressure and the residue treated with Et_2O and 5% NaOH. The ether extract was washed with water, dried, and concentrated to afford 45.9 g (75%) of (*R*)-(+)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-propanediol acetonide, bp 145 °C (0.01 mm), $[\alpha]^{20}_D$ +6.3° (*c* 7.5, CH_3OH), which was hydrolyzed with 2.5 N HCl to give (*R*)-(−)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-propanediol (91% yield), $[\alpha]^{25}_D$ −5.3° (*c* 4.9, CH_3OH). Anal. ($C_{15}H_{22}O_4$) C, H.

(*R*)-4-[2-(Cyclopropylmethoxy)ethyl]phenyl 2,3-Epoxypropyl Ether. Four grams (0.021 mol) of *p*-toluenesulfonyl chloride in 50 mL of benzene was added dropwise to a solution of 5.3 g (0.02 mol) of (*R*)-(−)-3-[4-[2-(cyclopropylmethoxy)-ethyl]phenoxy]-1,2-propanediol in 12 mL of pyridine while the temperature was kept at 0 °C. The mixture was stirred for 5 h and then poured into 200 mL of 5 N HCl. The resulting solution was extracted with ether and the ether layer washed with water and dried over $MgSO_4$ and concentrated under reduced pressure to afford a viscous oil. This was purified by chromatography on silica gel with a mixture of CH_2Cl_2–acetone (95:5) as eluent to yield 6 g (71%) of tosylate, which was used in the next step without further purification.

A solution of 4.6 g (0.011 mol) of the above product was dissolved in a mixture of 30 mL of CH_3OH–Et_2O (50:50) and then cooled at 0 °C and treated with 0.25 g (0.011 mol) of Na added portionwise while the temperature was kept at 0 °C for 12 h. Dry ice was then introduced into the mixture. After addition of water and extraction with CH_2Cl_2, followed by washing, drying, and evaporation under reduced pressure, 2.7 g of the crude (*R*)-4-[2-(cyclopropylmethyl)methoxy]ethyl]phenyl 2,3-epoxypropyl ether was obtained (100%), which was used without purification in the next step.

(*R*)-(+)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-(isopropylamino)-2-propanol (10). A 7.9-g (0.032 mol) sample of the above epoxide and 60 mL of isopropylamine were heated at reflux for 4 days and then evaporated under reduced pressure. The residue was treated with maleic acid in Et_2O to give 11.2 g (83%) of **10**, mp 95–97 °C, $[\alpha]_D$ +14.5° (*c* 2.93, CH_3OH). Anal. ($C_{22}H_{33}NO_7$) C, H, N.

Pharmacological Assays. (1) Determination of β_1-Adrenoceptor Blocking Potency.[10] Male rats (Sprague–Dawley, Charles River Laboratories) weighing 250–300 g were decapitated. The atria were dissected and suspended in an isolated tissue bath and immersed in Moran's solution (composition in mM: NaCl 120, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 2.1, $NaHCO_3$ 25.0, glucose 10) gassed with 95% O_2 and 5% CO_2. The preparation was fixed to a holder immersed in the bath and the other side connected through a thread to a force-displacement transducer and then subjected to a 0.5-g tension. The atrial rate of the spontaneously beating preparation was measured with a tachometer triggered by the contractile force signals and recorded on a polygraph (Grass Model 79B).

The preparations were allowed to stabilize for at least 60 min. The atrial rate concentration–response curves to isoproterenol were constructed as described by Van Rossum[10] before (control) and after 30-min exposure of the preparation to a Moran's solution containing one of the newly synthethized compounds (0.1–10 μM), metoprolol (0.1–10.0 μM), or propranolol (0.07–0.1 μM).

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Increasing (log intervals) molar concentrations of isoproterenol were added to the atria in a cumulative manner. Each successive concentration was added when the response to the previous one had reached a maximum. Upon completion of the first concentration–response curve, the preparation was washed several times until the measured parameters returned to base-line levels or attained equilibrium. This required approximately 45 min. The β-adrenoceptor blocking agents were then added to the atria before a second concentration–response curve was determined.

Analysis of Results. The changes in atrial rate produced by each concentration of isoproterenol were transformed into percentages of the response evoked by a supramaximal concentration of isoproterenol. The potencies of compounds metoprolol and propranolol as competitive β-adrenoceptor blocking agents were assessed by calculating the pA_2 values.[10]

The pA_2 is defined as the negative logarithm of the concentration of an antagonist that requires the doubling of the concentration of isoproterenol in order to get the same response obtained under control conditions.

(2) β_2-Adrenoceptor Blocking Activity.[11] Guinea pigs (Hartley) weighing 400–500 g were sacrificed by cervical dislocation. The trachea was removed and cut into 10 rings of similar width, which were subsequently joined together with cotton thread. The resulting chain was mounted in a 30-mL bath containing van Dyke–Hastings solution (composition in mM: NaCl 120.0, KCl 3.0, $CaCl_2$ 0.6, $MgCl_2 \cdot 6H_2O$ 0.5, KH_2PO_4 1.0, K_2HPO_4 1.0, $NaHCO_3$ 29.8, and glucose 2.8) maintained at 37 °C and aerated with a 5% CO_2 and 95% O_2 mixture. One side of the chain was fixed to a holder immersed in the isolated organ bath and the opposite side connected through a thread to a force-displacement transducer (Grass Model FTO3 C). A resting tension of 0.5 g was applied to the chain and continuously adjusted at this level for a 60-min equilibration period. At the end of this time the trachea was contracted with carbachol (0.3 M) and a concentration–response curve to isoproterenol (relaxant agent) was constructed. This concentration–response curve was recorded a second time before exposing the preparation for 30 min to a solution containing one of the new derivatives (**4–27**), metoprolol, or propranolol. At the end of this period the tracheal chain was again contracted with carbachol and a second concentration–response curve to isoproterenol constructed.

The pA_2 values of the three β-adrenoceptor blocking agents against isoproterenol were calculated as explained above for the β_1-adrenoceptor blocking activity.

(3) Determination of Local Anesthetic Activity.[12] Female frogs (Rana esculenta) were pithed and their sciatic nerves carefully dissected from the spinal cord origin to the knee. The nerves were placed in a Plexiglas chamber (50 × 20 × 20 mm) consisting of three compartments separated by a thin wall.

The cranial portion of the nerve was kept moist in the first chamber, which contained two pairs of electrodes 7 mm apart: one for electrical stimulation and one for recording. The central portion of the nerve was immersed in frog Ringer solution (composition in mM: NaCl 105, KCl 1.8, $CaCl_2$ 1.0, NaH_2PO_4 0.16, $NaHCO_3$ 3.6) of pH 7 left at room temperature (20 °C). The caudal portion was laid on the third chamber, which contained a pair of recording electrodes.

After a 20-min stabilization period, the central portion of the nerve was exposed for 20 min to control Ringer solution.

Nerve action potentials were evoked by electrical stimulation (F. Haer stimulator, Model Pulsar 6) of the cranial end of the sciatic nerve with single rectangular pulses of 0.01-ms duration and supramaximal voltage (2.8 V) and displayed on a dual-beam storage oscilloscope (Tektronix, Model 5103N). During the whole experiment, action potentials were evoked each 5 min from the segments of the nerve lying in the first (V_1) and third compartment (V_3) of the chamber. In this way, the action potential in the first compartment acted as a control for the portion of nerve exposed to the treatment.

The following formula was employed to assess at any given time ($t = T$) the effect of the drug, as compared to no drug condition ($t = 0$):

$$\% V = [(V_1/V_3)_{t=0}/(V_1/V_3)_{t=T}] \times 100$$

% V is an estimation of the local anesthetic activity of the compound since it gives the percentage change from control in the action potential height after the treatment. Therefore, possible modifications in the nerve responsiveness occurring during the time $t = T$ are not expected to influence the effects of the compounds on nerve conduction due to the use of the ratios V_1/V_3.

Propranolol, metoprolol, and our compounds were dissolved in Ringer solution. The effects of several concentrations of these compounds were tested in independent groups of preparations.

The mean percentage decreases (weighted by the inverse of the mean variance) in the height of the action potential obtained 10 min after exposure of the nerve to a compound and the corresponding concentrations were fitted to a logistic (sigmoid curve) equation. The concentration producing a 50% decrease in the height of action potential (EC_{50}) and its standard deviation were calculated. The calculated EC_{50} values were used to assess the possible existence of a significant difference between treatments by means of an unpaired t test. Results given in Table IV compare the potency of the new derivatives to propranolol, which was attributed an activity of 1.

(4) Determination of Antihypertensive Activity in Spontaneously Hypertensive Rats. Spontaneously hypertensive rats (SHR) (Okamoto strain aged over 20 weeks) were placed for approximately 30 min in a temperature (28 °C) and humidity (60%) controlled cabinet before an inflatable cuff and a piezoelectric transducer were applied to the tail to pick up arterial pulse waves (W & W BP Recorder, Model 8005).

Heart rate was measured by counting automatically the arterial pulses occurring over 10-s periods. The pressure within the cuff required to produce the disappearance of the arterial pulse was considered the value of systolic blood pressure.

Three successive systolic blood pressure measurements were taken before administering any treatment, and the lowest one was chosen as a base-line value (212 ± 2 mmHg, $n = 150$). Changes in blood pressure were expressed as percentages of this value and were used to perform statistical analysis (paired t test).

Groups of five animals were dosed orally with either placebo (water) or 5.0 mg/kg propranolol, metoprolol, and our compounds.

Systolic blood pressure and heart rate were measured 2, 4, and 24 h after administration.

(5) Determination of Enzymatic Parameters (K_M and V_{max}) in Rat Liver Microsomal Fraction. A microsomal fraction was prepared from rat liver according to a already described method.[37] Briefly, male Sprague–Dawley rats (Charles River) weighing 220–240 g were starved overnight before their sacrifice by decapitation. The liver was removed, washed with pH 7.4 phosphate buffered saline (1000-3, Sigma), cut in pieces and homogenized at 4 °C (Potter). The homogenate was centrifugated at 10000*g* for 30 min at 4 °C, the supernatant was separated, and its protein content (adjusted for the assay to 30 mg/mL) was routinely determined.[38]

The rate of metabolism of the substrates **8**, **11**, **18**, and metoprolol by the microsomal fraction was determined by measuring their rate of disappearance. For technical reasons, we did not follow the appearance of their common metabolite (the phenylacetic acid moiety). The reaction medium (1 mL) was prepared by mixing in a tube (1) 700 μL of pH 7.4 phosphate buffer containing NADP (1.08 mM), glucose 6-phosphate (17.5 mM), and magnesium sulfate (25.7 mM); (2) 200 μL of a microsomal preparation; (3) 100 μL of an aqueous solution of **8**, **11**, **18**, or metoprolol (three to four concentrations ranging from 1 to 150 μM).

The assay tubes were incubated for 10 min at 37 °C, and then 0.5 mL NaOH (1 N) was added to stop the biological reactions. Then the tubes were transferred in an ice bath and fractions of 5–800 μL of their content were transferred to extraction vials. The internal standard was added (1000 μL of a 2 mg/mL aqueous solution of **11** for the determination of **8**, **18**, and metoprolol or 100 μL of a 1 mg/mL solution of **8** for the determination of **11**). The pH of each vial was adjusted to pH 12 (200 μL of 1 N NaOH). The extraction of the compounds was carried out with 7 mL of diethyl ether (20 min of shaking). After centrifugation, the organic

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phase was separated and evaporated at 37 °C under a light current of nitrogen. To the dry extract was added 200 μL of a solution of heptafluorobutyric anhydride (HFBA) in ethyl acetate (1:10, v/v). The mixture was heated for 20 min at 60 °C and then evaporated to eliminate any excess of HFBA. The residue was washed twice with 300 μL of ethyl acetate, and then 200 μL of *n*-hexane was added. Two microliters of this organic solution was injected by means of an automatic injector (HP 7672 A) into a gas chromatograph (HP 5880) fitted with a capillary quartz column (25 m and 0.22 mm i.d.). The phase film used was OV1 (0.11 μM). Vector and make-up gases was argon–methane (95:5, v/v) delivered at 0.5 and 40 mL/min, respectively. The split ratio was 1:10. The temperatures of the column, injector, and detector were 215, 300, and 300 °C, respectively. The detection was achieved by electron capture (^{63}Ni source).

Under these conditions, the retention times for **8**, **11**, **16**, and metoprolol were 12.3, 15.6, 18.9, and 6.5 min, respectively. The calibration curves of these compounds were linear for reaction mixtures containing from 0 to 200 ng/mL of substrate and their minimal detectable quantities by this method were 10 ng/mL.

The initial rates of disappearance within the 10-min reaction time were determined for **8**, **11**, **16**, and metoprolol. These values were corrected for the spontaneous degradation of these compounds. The apparent constant of Michaelis–Menten (K_M) and the maximal rate of disappearance of the substrate (V_{max}) were calculated according to the procedure described by Mazel.[39] It should be mentioned that the chosen incubation time (10 min) permitted to obtain, for all the compound tested, a linear relationship between the logarithm of the rate concentration and the reciprocal of the rate of the reaction, allowing a correct determination of V_{max} and K_M.

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Conformationally Defined Adrenergic Agents. 5. Resolution, Absolute Configuration, and Pharmacological Characterization of the Enantiomers of 2-(5,6-Dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline: A Potent Agonist at α-Adrenoceptors†

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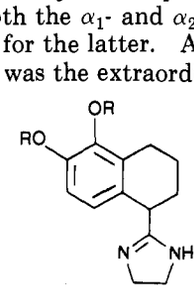
(±)-2-(5,6-Dimethoxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline has been resolved into its (+) and (−) enantiomers, and the absolute configuration was established by single-crystal X-ray diffraction studies. The more active isomer has been assigned the *R* absolute configuration. Cleavage of the respective (+)- and (−)-dimethyl ethers with boron tribromide provided the corresponding (+)- and (−)-2-(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline hydrobromides and these were pharmacologically characterized. In various preparations, the *R* enantiomer has been shown to be an extremely potent α agonist with preferential activity at the α_2-adrenergic receptor.

Imidazoline derivatives are well recognized as an important class of drugs that interact with α-adrenergic receptors.[1-5] Studies have even appeared in the literature on optically active derivatives of various imidazolines.[6-11] Generally speaking, these optical isomers were derivatives of the parent achiral molecule in which an asymmetric center was generated through modifications on the imidazoline ring[6,7] or incorporation of a benzylic hydroxyl atom.[8] The biological activity of the antipodes of tetrahydrazoline has also been reported.[9-11]

Recently,[12] we reported that the compound (±)-2-(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline (**1a**) is an agonist at both the α_1- and α_2-adrenoceptors with modest selectivity for the latter. A remarkable feature associated with **1a** was the extraordinary potency it displayed in vitro for both the α_1- as well as the α_2-adrenergic receptors, being 13 and 184 times, respectively, more potent than norepinephrine (NE).[12] Our interest in the relationship between stereochemical drug–receptor interactions and the resulting biological effects led us to resolve **1a** into its respective enantiomers. Furthermore, we



1a, R = H
b, R = CH_3

† A preliminary account of this work has been presented to the Division of Medicinal Chemistry, 192nd National Meeting of the American Chemical Society, Anaheim, CA, 1986; DeBernardis, J. F.; Kerkman, D. J., paper MEDI-70.

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