Synthesis and Adrenergic Activity of a New Series of *N*-Aryl Dicyclopropyl Ketone Oxime Ethers: SAR and Stereochemical Aspects

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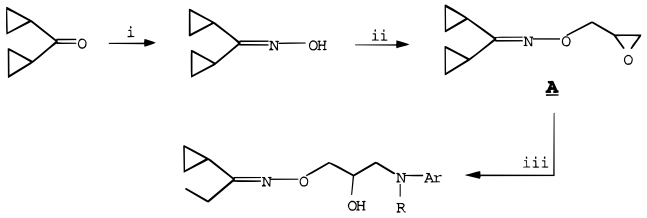
A novel series of 31 *N*-aryl dicyclopropyl ketone oxime ethers were synthesized and tested for their activity at α- and *β*-adrenergic receptors. All of the compounds showed greater affinity for *β*-than for α_1-receptor sites. Some compounds had pure antagonist effects whereas some were partial agonists. Several compounds had an antagonist effect matching that of propranolol in in vitro (binding data and pA_2 values on rat heart ventricle homogenates and guinea pig spontaneously beating right and electrically driven left atrial isolated preparations, respectively) and in in vivo tests (measurement of antagonism toward isoprenaline-induced tachycardia in anesthetized rats). Furthermore, all of the compounds showed a β_1-adrenergic selectivity (β_2-affinity > 1500 nM).

Introduction

β-Adrenoreceptor antagonists are used clinically for the treatment of various cardiovascular diseases. It is well-known that *β*-adrenergic blockers are very homogeneous in their chemical structures which stem either from arylethanolamines or from (aryloxy)propanolamines.[1] However, we have shown previously that insertion of a C,N double bond into the side chain of *β*-blockers did not abolish *β*-adrenoreceptor activity[2] and, in some cases, led to potent β_2-selective antagonists.[3] We have also shown that contrary to what is generally accepted, the aromatic nucleus of these compounds was not a requisite feature for potent activity.[4] This was exemplified by several cyclopropylketoxime propanolamines which displayed potent activities.[5] In particular, the dicyclopropyl analogue (falintolol) has been found to be useful in the clinical treatment of glaucoma.[6] It is also well-known that most of the initial *β*-blockers contained an isopropyl- or *tert*-butylamine. Substitution of the nitrogen present within catecholamines by aralkylamines was found to produce *β*-adrenergic agonists having additional α-adrenolytic properties.[7,8] Similarly, it was found that *N*-aralkyl substitution could also confer α-adrenolytic properties to *β*-antagonists.[9,10] However, to the best of our knowledge, the influence of a direct aromatic substitution on the terminal amine upon adrenergic activity has scarcely been reported excepted in a few articles which described some *N*-aryl *β*-blockers which were found to be very weakly potent.[11,12] In the present article we describe the synthesis of a series of 31 new *N*-aryl dicyclopropyl ketone oxime ether derivatives and discuss the results from their examination in in vitro and in vivo *β*-adrenergic receptor assays.

Scheme 1



[a] Reagents: (i) NH_2OH; (ii) epibromohydrin; (iii) RNHAr.

Chemistry

Compounds **1**−**31** were obtained by opening the epoxide ring of A with various arylamines as outlined in Scheme 1. The alkylation of dicyclopropyl ketone oxime by epibromohydrin was most conveniently achieved under phase transfer conditions.[13] This procedure significantly increased the yield while reducing the time required for each reaction. When the arylamine also contained another amino group, the target compound **22** was accompanied by byproducts **30** and **31** (Scheme 2). ^{1}H NMR data allowed the assignment of these structures by comparison with **1** and **19** (Table 1). The methylene group attached to an aniline function appears at 3.32 ppm for **22** (3.28 and 3.22 ppm for **1** and **19**), whereas a methylene group attached to an aliphatic amine appears at higher field, e.g. 2.70 ppm for **30** and **31**. Chemical shifts for the ^{13}C NMR spectra are also in agreement with these data. Finally, a chemical proof of structure **22** was obtained by catalytic reduction (H_2, Pd/C, 1 atm) of the nitro group present within **21** which gave a mixture of compounds from which **22** was isolated by column chromatography (EtOAc/Hex, 2/8). ^{1}H NMR, TLC, and IR were identical for both derivatives. The high yield of **30** (46%) when compared to that of **22** (27%) can be explained by the higher nucleophilicity of a an aliphatic amine function versus an aromatic amine. The high yield of the product resulting from disubstitution **31** is probably related to employing

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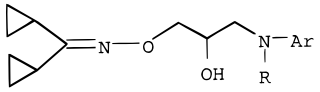
Table 1. Physicochemical Data of *N*-Aryl Dicyclopropyl Ketone Oxime Ethers



compd	Ar	R	R_f (eluent)[b]	mp, °C (salt or base)	recrystn solv	yield, %	formula[c]
1	C_6H_5	H	0.20 (B)	122−124 (oxalate)	EtOAc/MeOH	36	$C_{18}H_{24}N_2O_6$
2	C_6H_4-2-Me	H	0.24 (B)	181−183 (oxalate)	EtOAc/hexane	43	$C_{19}H_{26}N_2O_6$
3	C_6H_4-2-I	H	0.19 (B)	162−164 (HCl)	EtOAc/hexane	31	$C_{16}H_{21}ClIN_2O_2$
4	C_6H_4-2-C_6H_5	H	0.31 (A)	oil (HCl)	MeOH	53	$C_{22}H_{27}ClN_2O_2$
5	2-pyridyl	H	0.23 (F)	hygroscopic (base)	EtOAc	46	$C_{15}H_{21}N_3O_2$
6	2-pyridyl-6-Me	H	0.21 (F)	138−140 (HCl)	Et_2O/MeOH	11	$C_{16}H_{23}N_3O_2$
7	C_6H_4-3-Me	H	0.26 (B)	186−188 (HCl)	EtOH	49	$C_{17}H_{25}ClN_2O_2$
8	C_6H_4-3-I	H	0.20 (B)	178−180 (base)	Et_2O/MeOH	43	$C_{16}H_{21}IN_2O_2$
9	2-pyridyl-5-Me	H	0.42 (C)	hygroscopic (HCl)	EtOAc	21	$C_{16}H_{23}N_3O_2$
10	C_6H_4-4-Me	H	0.36 (A)	128−130 (maleate)	EtOAc	36	$C_{21}H_{28}N_2O_6$
11	C_6H_4-4-F	H	0.26 (B)	126−128 (maleate)	Et_2O/EtOAc	15	$C_{20}H_{25}FN_2O_6$
12	C_6H_4-4-I	H	0.23 (A)	95−97 (maleate)	Et_2O	31	$C_{20}H_{25}IN_2O_6$
13	C_6H_4-4-NO_2	H	0.22 (C)	162−164 (maleate)	MeOH	11	$C_{20}H_{25}N_3O_8$
14	C_6H_4-4-OH	H	0.18 (H)	oil (HCl)	Et_2O/MeOH	39	$C_{16}H_{23}ClN_2O_3$
15	C_6H_4-4-COOH	H	0.25 (H)	202−204 (maleate)	EtOAc/MeOH	36	$C_{21}H_{26}N_2O_8$
16	C_6H_4-4-Br	H	0.29 (B)	108−110 (maleate)	Et_2O/EtOAc	15	$C_{20}H_{25}BrN_2O_6$
17	C_6H_4-4-Cl	H	0.28 (B)	118−120 (maleate)	Et_2O/EtOAc	19	$C_{20}H_{25}ClN_2O_6$
18	C_6H_4-4-*t*-Bu	H	0.28 (B)	158−160 (HCl)	Et_2O/hexane	41	$C_{20}H_{31}ClN_2O_2$
19	C_6H_4-4-*n*-Bu	H	0.51 (B)	168−170 (HCl)	Et_2O/MeOH	47	$C_{20}H_{31}ClN_2O_2$
20	C_6H_4-4-C_6H_5	H	0.39 (B)	124−126 (maleate)	Et_2O	24	$C_{26}H_{30}N_2O_6$
21	C_6H_4-4-$CH_2C(CH_3)_2NO_2$	H	0.25 (D)	168−170 (HCl)	Et_2O	59	$C_{20}H_{30}ClN_3O_4$
22	C_6H_4-4-$CH_2C(CH_3)_2NH_2$	H	0.21 (D)	oil (HCl)	Et_2O/hexane	27	$C_{20}H_{32}ClN_3O_2$
23	C_6H_4-4-COOEt	H	0.61 (C)	184−186 (HCl)	EtOAc/MeOH	43	$C_{19}H_{27}ClN_2O_4$
24	C_6H_4-4-CF_3	H	0.52 (E)	84−86 (HCl)	Et_2O/MeOH	33	$C_{17}H_{22}ClF_3N_2O_2$
25	4-pyridyl	H	0.25 (F)	hygroscopic (base)	EtOAc	36	$C_{15}H_{21}N_3O_2$
26	2-pyridyl-4-Me	H	0.45 (C)	140−142 (maleate)	EtOAc/hexane	14	$C_{20}H_{27}N_3O_6$
27	2-benzothiazole	H	0.10 (G)	182−184 (base)	EtOAc	31	$C_{17}H_{21}N_3O_2S$
28	2-benzimidazole	H	0.59 (G)	132−134 (oxalate)	Et_2O/MeOH	61	$C_{21}H_{26}N_4O_6$
29	C_6H_5	CH_3	0.21 (D)	oil (base)		52	$C_{17}H_{24}N_2O_2$
30	Scheme 2		0.30 (D)	oil (base)		46	$C_{20}H_{30}N_2O_2$
31	Scheme 2		0.45 (D)	oil (base)		27	$C_{30}H_{46}N_4O_4$

[a] See the Experimental Section. [b] A: EtOAc/hexane (2:8). B: EtOAc/hexane (1:9). C: EtOAc/hexane (4:6). D: EtOAc/cyclohexane (3:7). F: methanol. G: EtOAc/cyclohexane (9:1). H: EtOAc/hexane (6:4). [c] C, H, N were analyzed: the values are at ±0.4% of theoretical values.

a 2-fold excess of amine and to the long duration of the reaction (72 h). Enantiomers of **10**, **18**, and **28** were prepared from (*R*)- and (*S*)-glycidyl tosylates and the sodium salt of dicyclopropyl oxime according to a previously described procedure.[5] The physicochemical properties of compounds **1**−**31** are recorded in Table 1.

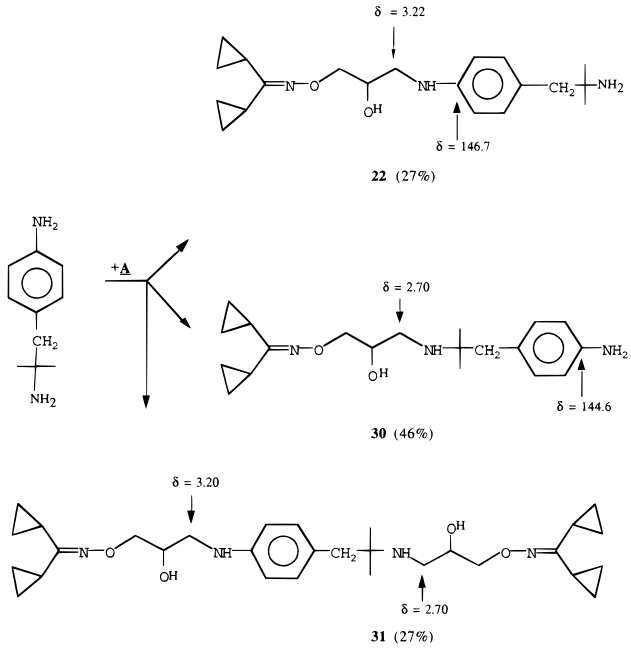
Pharmacology

The affinities of compounds **1**−**31** were investigated in receptor-binding assays using [^{3}H]prazosin and [^{3}H]-dihydroalprenolol as ligands for α- and *β*-adrenoceptors, respectively, and [^{3}H]CGP 12177 to assess β_1-adrenoceptor selectivity (Table 2). The in vitro efficacy of these compounds was evaluated from pA_2 values[14] on guinea pig isolated spontaneously beating right (chronotropic effect) and electrically driven left (inotropic effect) atrial preparations. The in vivo efficacy was assessed by the measurement of the antagonistic effect against isoprenaline-induced tachycardia within anesthetized rats.

Discussion

Isolated Guinea Pig Left and Right Atria. As can be seen, several of the compounds displayed pA_2 values comparable to that of propranolol which was employed as a standard. Compounds substituted in the para position of the aromatic nucleus were generally the most potent. Indeed, the *β*-adrenergic activity increased according to the rank order: ortho as in **2** ≤ meta position as in **7** < para position as in **10**. The sequence

Scheme 2



for the pyridyl derivatives was meta as in **9** ≤ ortho as in **6** < para as in **26**. Similarly the 4-iodo derivative **12** was more active than the 3-iodo derivative **8** or the 2-iodo derivative **3** and the 4-phenyl derivative **20** was more active than the 2-phenyl derivative **4.** Interestingly, the nature of the para substituent has no marked

Table 2. In Vitro α- and β-Blocking Properties and Specificity

compd	pA_2[a] right atria chronotropy	pA_2[a] left atria inotropy	$IC_{50}\beta$,[b] nM	$IC_{50}\alpha$,[b] nM	β-specificity[c]	$IC_{50}\beta_2$,[d] nM
1	7.4 ± 0.1	6.9 ± 0.2	3.3	13000	3940	>10000
2	6.9 ± 0.2	6.6 ± 0.1	13.0	22000	1690	>10000
3	5.9 ± 0.1	5.6 ± 0.1	108	53000	490	>10000
4	4.0 ± 0.1	4.6 ± 0.5	998	>100000	>100	>10000
5	7.1 ± 0.1	7.2 ± 0.2	35.6	36400	1020	>10000
6	5.0 ± 0.1	ND	ND	ND	ND	>10000
7	7.0 ± 0.1	6.9 ± 0.1	501	>100000	>200	>10000
8	5.3 ± 0.1	5.5 ± 0.1	630	>100000	>160	>10000
9	4.6 ± 0.3	ND	ND	ND	ND	>10000
10	7.9 ± 0.1	8.0 ± 0.1	7.6	15000	1970	>10000
11	6.2 ± 0.2	5.9 ± 0.2	12.4	26200	2120	>10000
12	7.8 ± 0.2	7.2 ± 0.1	4.1	27500	6700	>10000
13	7.0 ± 0.1	6.6 ± 0.2	7.9	19000	2400	>10000
14	7.1 ± 0.1	6.7 ± 0.2	10.4	28300	2730	>10000
15	7.0 ± 0.2	6.2 ± 0.2	10.0	37300	3570	>10000
16	7.7 ± 0.3	7.0 ± 0.1	4.1	19000	4630	>10000
17	7.4 ± 0.2	6.9 ± 0.2	11.4	18700	1640	>10000
18	7.9 ± 0.1	7.3 ± 0.2	3.4	70800	20800	>10000
19	7.1 ± 0.1	6.6 ± 0.2	7.0	30700	4390	>10000
20	8.0 ± 0.1	7.4 ± 0.2	4.5	3100	690	>10000
21	7.7 ± 0.1	7.1 ± 0.2	3.4	12600	3700	>10000
22	6.3 ± 0.1	ND	ND	ND	ND	>10000
23	7.1 ± 0.1	6.6 ± 0.2	12.8	95100	7430	>10000
24	7.2 ± 0.2	6.9 ± 0.2	13.8	>100000	>7250	>10000
25	7.2 ± 0.1	6.6 ± 0.3	32.3	72300	2240	>10000
26	7.5 ± 0.1	7.1 ± 0.1	23.9	15300	640	>10000
27	7.8 ± 0.1	7.6 ± 0.3	3.4	>100000	>29400	>10000
28	7.2 ± 0.1	7.0 ± 0.2	13.2	>100000	>7580	>10000
29	3.1 ± 0.1	ND	ND	ND	ND	>10000
30	ND	ND	ND	ND	ND	2000
31	ND	ND	ND	ND	ND	1500
propranolol	8.3 ± 0.3	8.1 ± 0.5	3.0	>100000	>33300	ND
phentolamine	ND	ND	ND	2.7	ND	ND
ICI 118 551						2

[a] Mean ± SEM on isolated atria (from five to seven assays), chronotropic effect studied in right spontaneously beating inotropic and inotropic effect in electrically driven left atrial preparation, respectively. [b] Mean values on rat ventricle homogenates (from three to five assays with six concentrations in duplicate); SEM values smaller than 10% of mean values. [c] $IC_{50}\alpha$ (vs [^{3}H]prazosin)/$IC_{50}\beta$ (vs [^{3}H]dihydroalprenolol). [d] Displacement experiments in rat lung homogenates in the presence of 100 nM CGP 20712 A; SEM values smaller than 10% of mean values. ND: not determined.

effect upon activity. Electron-donating or electron-withdrawing groups behave similarly. Thus **10**, **12, 16**, **18**, and **21** substituted by CH_3, I, Br, *t*-Bu, and $CH_2C(CH_3)_2NO_2$, respectively, are practically equipotent. *N*-Methylation of the potent secondary amine derivative **1** led to **29** which displayed a dramatic drop in potency (ca. 10 000 times), thus confirming the crucial importance of the secondary amine function. In view of the potent β-adrenergic activities of **10**, **18**, and **28** and in order to shed further light into the stereochemical requirements for this new class of molecules, we have determinate the pA_2 values of their six corresponding enantiomers (Table 3). The (*R*)- and (*S*)-enantiomers of **10** and **18** are equipotent, confirming our previous observations.[5,15] Very surprisingly the (*R*)- and (*S*)-benzimidazole enantiomers differ markedly. For this pair only the (*S*)-enantiomer was found to be active, as is generally observed among β-blockers of the (aryloxy)propanolamine type. Undoubtedly this result is related to a lack of symmetry between the two enantiomers, but a satisfactory explanation remains to be found.

Inhibition of [^{3}H]Dihydroalprenolol, [^{3}H]CGP 12 177, and [^{3}H]prazosin Binding (Table 2). There was a weak but significant correlation between IC_{50} and pA_2i or pA_2c. So only compounds **12**, **18**, **20**, **21**, and **27** comprised the groups of the most potent eight compounds in the two series (pA_2 and IC_{50}). The compound showing the greatest affinity for the α_1-adrenergic receptor **20** had an IC_{50} of 3100 nM, which is about 1150 times less potent than that of phentolamine. The specificity of each compound for β-receptors was estimated as the $IC_{50}\alpha/IC_{50}\beta$ ratio (increasing β-specificity reflected by an increasing ratio). The eight compounds possessing the greatest affinity for the β-receptor had specificity ranging from 690 for **20** to more than 29 400 for **27**. Compounds **18** and **27** showed both a high affinity for β-receptor sites and very high specificity.

All of the compounds tested in β_2-receptors displacement experiments showed a very weak affinity since, for the majority of them, IC_{50} values were higher than

Table 3. Optical Rotation ($[\alpha]^{25}_D$) and pA_2 ± SD Values of Chiral Ether Oxime Derivatives

compd	$[\alpha]^{25}_D$, deg	apparent pA_2[a] atrium (β_1)
(*R*)-(−)-**10**	−11.5 (c = 2.5, $CHCl_3$)	7.90 ± 0.21 (5)
(*S*)-(+)-**10**	+11.5 (c = 2.5, $CHCl_3$)	7.86 ± 0.25 (4)
(*R*)-(−)-**18**	−13.6 (c = 2.5, $CHCl_3$)	7.96 ± 0.13 (5)
(*S*)-(+)-**18**	+13.8 (c = 2.5, $CHCl_3$)	7.99 ± 0.19 (5)
(*R*)-(−)-**28**	−9.3 (c = 2.5, $CHCl_3$)	inactive up to 10^{-4} M
(*S*)-(+)-**28**	+9.4 (c = 2.5, $CHCl_3$)	7.31 ± 0.18 (4)
(*S*)-(−)-propranolol	−25.2 (c = 1.2, EtOH)	8.62 ± 0.30 (4)
(*R*)-(+)-propranolol	+24.3 (c = 1.1, EtOH)	5.58 ± 0.25 (3)

[a] β_1-Antagonism was expressed as pA_2 ± standard error with the number of experimental values in parentheses.

Table 4. In Vivo β-Mimetic and Blocking Properties

compd	MED,[a] mmol/kg iv	β-mimetic[b] activity	β-blocking[c] effect
1	2.7	10	100
2	5.3	37[d]	65
3	5.7	10	100
4	>12.9	0	8
5	4.4	0	100
7	9.2	42[d]	91
8	7.8	42[d]	75
10	2.5	64[d]	60
11	2.4	74[d]	58
12	1.6	64[d]	76
13	1.8	10	100
14	3.1	10	100
15	2.3	65[d]	54
16	2.1	10	100
17	2.1	47[d]	100
18	2.0	44[d]	100
19	2.7	10	100
20	1.0	70[d]	74
21	2.4	10	100
23	2.4	10	100
24	2.2	47[d]	91
25	3.7	0	100
26	2.6	0	100
27	4.1	10	100
28	2.0	10	100
propranolol	3.0	0	100

[a] Minimum efficient dose (smallest dose giving maximum effect), evaluated by dose−response curve, from 0.1 to 5 mg/kg, in 5−10 animals. [b] Percent of increase in heart rate induced by the studied compound with respect to that measured after isoprenaline (increase from 307 ± 3 to 394 ± 6 mmHg), in 5−10 animals. [c] β-Blocking effect estimated as percent of inhibition of increase in heart frequency induced by isoprenaline, in 5−10 animals. [d] Significant increase ($p \leq 0.05$) with respect to baseline values (293 ± 7 to 310 ± 5 mmHg according to the different groups of animals (in 5−10 animals).

10 μM and respectively 2 and 1.5 μM for derivatives **30** and **31**. Conversely, in the same experiments, ICI 118 551 (kindly supplied by Imperial Chemical Industries, UK) had a IC_{50} value of 2 nM.

Comparison Between in Vitro and in Vivo β-Blocking Activities. As shown in Table 4, we can divide the 28 most potent compounds into two groups, e.g. pure antagonists (**1**, **3**, **5**, **13**, **14**, **16**, **19**, **21**, **23**, **25**, **26**, **27**, and **28**) and partial agonists. The latter can be further divided into two groups, those able to inhibit 75% or more of the increase in heart rate induced by isoprenaline (**7**, **8**, **12**, **17**, **18**, and **24**) and those unable to induce this inhibition (**2**, **10**, **11**, **15**, and **20**) at the dose used. Compound **4** did not show any activity at the higher dose used. If β_1- and β_2-adrenoreceptors coexist in both left and right atria of rat heart in approximately a 2/1 ratio, only β_1-adrenoreceptors seem to mediate the chronotropic and inotropic effects of β-adrenoreceptor agonists, so it was hypothesized that the compounds studied bind preferentially to β_1-receptor sites,[16] and this was corroborated by binding experiments. Figure 1 illustrates the inhibition of isoprenaline-induced tachycardia in anesthetized rats by compounds **24**, **20**, and **26**.

Conclusion

In summary, many of the new *N*-aryl dicyclopropyl ketone oxime ethers described in this article have potent β-adrenergic activities which match that of propranolol in in vitro and in vivo tests. Some products have partial agonist properties, and all have poor affinity for α_1-adrenergic sites. Furthermore, it was demonstrated that all of the compounds possess a β_1-adrenergic specificity (β_2-affinity > 1500 nM). Thus, the presence of a terminal *N*-alkyl or *N*-aralkyl group previously thought to be compulsory for β-adrenergic activity does not seem to be essential. The nature of a given aromatic substituent does not account for the observed preferred potency for the para position on the phenyl ring. When these finding are coupled with our previous results which indicate that compounds lacking an aromatic ring can also exhibit potent adrenergic activities, the classic views as originally expressed by Belleau[17] and Bloom and Goldman[18] about the adrenergic receptor may need to be further considered.

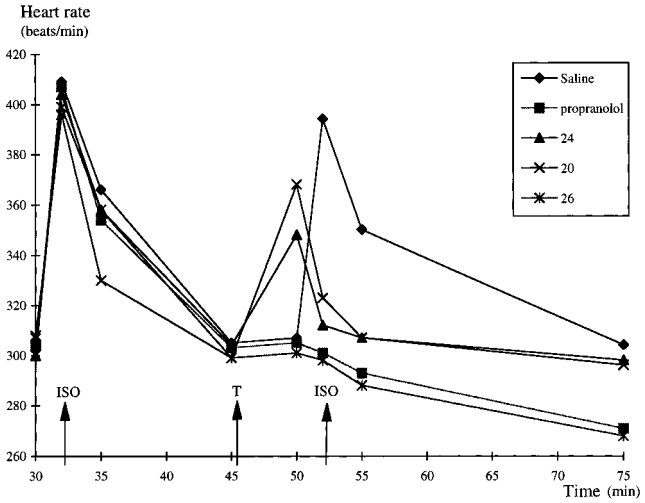


Figure 1. Inhibition of isoprenaline-induced tachycardia in anesthetized rats. ISO: isoprenaline (0.5 mg/kg; iv) at time 32 and at time 52 min. T: saline or compound on study at minimum efficient dose (lowest dose inducing maximum effect) at time 45 min.

Experimental Section

Chemistry. Thin-layer chromatography (TLC) was performed on plates of silica gel 60F254 (Merck art. 5735). Visualization was achieved by UV. Merck silica gel 60 (70−230 mesh) was used in column chromatography. ^1H and ^{13}C NMR spectra were recorded at 200 MHz with a Bruker AC 200 F.T. spectrometer. Melting points were determined on a Kofler block and are uncorrected. Elemental analyses (C, H, N) were performed by the Analytical Central Section, CNRS, 69390 Vernaison.

***O*-[3-(4-Butylaminophenyl)-2-hydroxypropyl] Dicyclopropyl Ketone Oxime (19). Method A.** Dicyclopropyl ketone oxime prepared according to ref 19 (1.5 g, 12 mmol) was stirred magnetically during 50 min at room temperature in 50% aqueous NaOH (16 mL). To this mixture were added toluene (15 mL), epibromohydrin (1.7 g, 12 mmol), and tetrabutylammonium bromide (3 mmol, 0.25 equiv). The reaction was monitored by TLC. After 3 h water (75 mL) was added, the mixture was extracted with Et_2O (3 × 30 mL), and the organic extracts were washed with water (3 × 30 mL), dried over $MgSO_4$, and evaporated under reduced pressure to give 95% of epoxide homogeneous on TLC. The crude epoxide (1.8 g, 10 mmol) was dissolved in dry ethanol (20 mL) containing 4-butylaniline (3.0 g, 20 mmol), and the mixture was stirred magnetically at room temperature for 24 h. The solvent was removed under reduced pressure, and the crude product was chromatographed on silica gel eluting with EtOAc/hexane (2:8): ^1H NMR ($CDCl_3$) δ 0.92 (t, J = 7.17 Hz, 3H, CH_3); 1.35 (m, 2H, CH_2); 1.55 (m, 2H, CH_2); 2.50 (t, J = 7.8 Hz, 2H, CH_2); 3.22 (m, 2H, CH_2); 3.78 (m, 2H, CH_2); 4.15 (m,

1H, CH); 6.69 (d, $J = 8.5$ Hz, 2H, 2 CH ar); 7.00 (d, $J = 8.5$ Hz, 2H, 2 CH ar.).

Method B. (*R*)- and (*S*)-**10**, (*R*)- and (*S*)-**18**, and (*R*)- and (*S*)-**28** were prepared similarly from (*R*)- and (*S*)-glycidyl tosylate according to ref 5 (Table 3).

Pharmacology. Isolated Guinea Pig Left and Right Atria. Male Dunkin−Hartley guinea pigs, weighing about 400 g, were killed by cervical dislocation. Left and right (which retained spontaneous rhythm) atria were dissected out and mounted in a 20 mL organ bath containing Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 24.9, $MgSO_4$ 1.6, KH_2PO_4 1.2, and glucose 12) maintained at 32 ± 1 °C, pH = 7.4, and bubbled with a mixture of 95% O_2 and 5% CO_2 according to previously described method.[20] Phentolamine (3×10^{-7} M) was added to the solution in order to avoid any receptor influence.[16] Both atria were fasten to a force displacement transducer (Polygraph HSE, WR 3001, 0.5 g resting tension). Left atria was placed between two platinum electrodes for electrical stimulation (2.5 Hz, 5 ms, 3 times threshold voltage), and the right atria had a spontaneous beating rate ranging from 150 to 170 bpm. After a 60 min stabilization period with a wash every 15 min, a dose−response curve for isoprenaline was generated with increasing cumulative concentrations (3×10^{-11} to 3×10^{-8} M) (total volume added less than 0.3 mL) until a maximum effect was reached, either in the untreated preparation or after a pretreatment after a 30 min equilibration period with the product studied, and at least three response curves were repeated and pA_2 values determinated.[13]

β-Adrenergic Binding Assay Procedure. Rat heart ventricle and lung membranes were prepared as previously described[21] with some slight modifications. Male Wistar rats weighing about 250 g were killed by decapitation and the heart ventricles and lungs quickly removed, dissected, and then washed in isotonic saline. They were homogenized in 40 vol (v/w) of ice cold Tris-buffer (50 mM Tris-HCl, pH 7.4 at 4 °C) with a polytron (Kinematica, Lucern, Switzerland) at setting 1.5 for 15 s. The homogenate was centrifuged at 1000*g* during 10 min at 4 °C and the supernatant centrifuged at 40000*g* for 10 min at 4 °C, the pellet resuspended in 50 vol of ice-cold Tris-buffer and recentrifuged as before. The resultant pellet was suspended at a protein concentration of 0.3−0.8 mg/mL in Tris buffer. Binding assays were run by incubating 500 μL of rat ventricle and lungs membrane suspensions containing 10 mM $MgCl_2$ at 37 °C for 30 min with 0.7 nM [^{3}H]dihydroalprenolol (NEN, France, specific act. 1780 GBq mmol^{-1}) in a total volume of 1 mL of buffer for heart ventricle homogenates and with 0.8 nM [^{3}H]CGP 12 177 (Amersham, France, specific act. 1780 GBq mmol^{-1}) in the presence of 100 nM CGP 20712A (kindly supplied by Ciba-Geigy Corp., Switzerland), for blocking β_1-adrenergic receptors, in 750 μL for lung homogenates, respectively. The inhibition of the specific binding was determined in the presence of various concentrations of unlabeled competing drugs. Incubations were terminated by rapid filtration through Whatman GF/B glass fiber filters (Whatman, Maidstone, UK) in vacuo (Manifold 1125, Millipore, France). The filters were rinsed three times with 5 mL of ice-cold Tris buffer, transferred to vials containing 10 mL of Instagel (Packard, France) and counted for radioactivity (Tri-Carb 300, Packard, 55% efficiency), and specific binding was defined as the difference between total and nonspecific binding, in the presence of 10 μM propranolol. In displacement experiments, the IC_{50} (concentration of competitor which displaced 50% of the maximum specific binding) and the slope factor of the displacement curve were calculated using a modified[22] ligand program[23] for Macintosh device (Biosoft, Cambridge, UK). Each result is given as the mean of three to five experiments, each performed in duplicate, with six different concentrations of the competing ligand. Homogenates protein concentrations were measured using the method of Bradford.[24]

Effects on Heart Frequency in Anesthetized Rats. Male Wistar rats, weighing about 280 g, were anesthetized with sodium pentobarbital (40 mg kg^{-1} ip or more, if requested), and after tracheotomy and intubation, right jugular vein was catheterized for iv drug administration. Heart rate was measured with a HSE polygraph WR 3001 connected to subcutaneous electrodes. Groups of five or six animals were used for each drug studied according to a previously described method.[25] After a 30 min stabilization period, 0.5 mg kg^{-1} isoprenaline was administered iv and the increase in heart rate measured (as control values); 15 min later, the test compound was administered at minimum efficient dose, that is the smallest dose giving maximum effect, as previously determined. The β-mimetic effect was assessed as the ratio of drug-induced tachycardia/isoprenaline-induced tachycardia. Then 5 min later, a second isoprenaline injection at the same dose as the previous one was added for measuring the β-lytic effect, which was estimated as the reduction in magnitude of the effect with respect to that obtained after the control isoprenaline injection.

α_1-Adrenergic Binding Assay Procedure. Rat heart ventricle homogenates were prepared as described above. Binding assays were run with 0.4 nM [^{3}H]prazosin (NEN, France, specific act. 3000 GBq mmol^{-1}) and incubated 30 min at 37 °C, in the presence of six different (each in duplicate) concentrations of each competing ligand. Nonspecific binding was determined in the presence of 10 μM phentolamine. The reaction was terminated by a rapid filtration on Whatman GF/B filters and three 5 mL ice-cold Tris buffer washes. Filter radioactivity was measured, and the IC_{50} values were calculated as previously described. Each result is given as the mean of three to five experiments, each performed in duplicate, with six different concentrations of the competing ligand.

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