Eur J Med Chem (1996) 31, 199–206

New chiral methyloxyiminomethyl (MOIM) β-adrenergic antagonists. (*S*)- and (*R*)-*N*-[3-(alkylamino)-2-hydroxypropylidene](*p*-chlorophenylmethyloxy)amines as probes for determining enantiomeric specificity in the class of MOIM-type β-adrenergic blocking agents

A Balsamo[1], MC Breschi[2], G Chiellini[2], B Macchia[1], M Macchia[1], C Manera[1], P Saccà[1], R Scatizzi[2]

[1]*Dipartimento di Scienze Farmaceutiche, Università di Pisa;*
[2]*Istituto Policattedra di Discipline Biologiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy*

(Received 22 September 1995; accepted 21 November 1995)

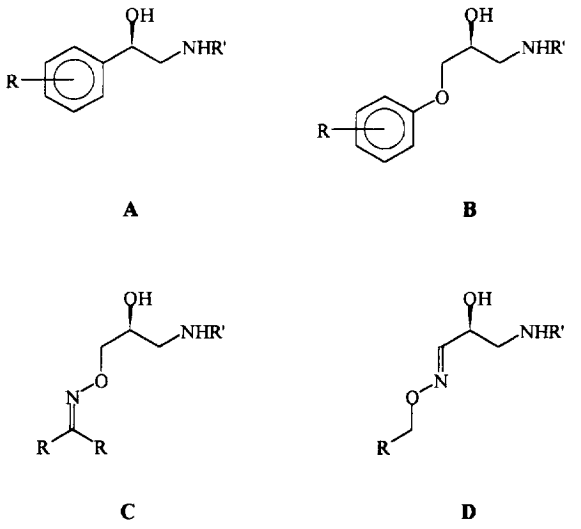
Summary — The chiral (*S*)- and (*R*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene](*p*-chlorophenylmethyloxy)amines ((*S*)-**1a** and (*R*)-**1a**) and their corresponding *N*-*tert*-butyl-substituted analogs ((*S*)-**1b** and (*R*)-**1b**) were synthesized from optically active precursors of known absolute configuration by procedures which had no effect on the configuration of the asymmetric carbon. Compounds (*S*)-**1a,b** and (*R*)-**1a,b** were tested for their β-adrenergic properties by radioligand binding experiments and functional tests on isolated preparations. The biopharmacological results show that compounds (*S*)-**1a,b,** in which the geometry of the chiral carbon adjacent to the hydroxyl group resembles that of natural catecholamines with the *R* configuration, interacted better with β-receptors, even if the stereochemical selectivity among enantiomeric pairs is not particularly marked.

adrenergic drug / β-blocking agent / chiral *N*-[3-(alkylamino)-2-hydroxypropylidene](*p*-chlorophenylmethyloxy)amine

Introduction

Most adrenergic β-blocking agents belong to the structural classes **A** or **B**, and are made up of a common ethanolaminic portion linked to an aryl (Ar) or aryloxymethyl group ($ArOCH_2$), respectively. The similar β-blocking properties of type **A** and **B** drugs have been explained on the basis of the existence both of spatial correspondences [1, 2], shown by crystallographic studies, and chemical reactivity analogies [3–5], found by means of theoretical calculations, between the Ar of type **A** compounds and a portion of the $ArOCH_2$ moiety of type **B** compounds, consisting of part of the aromatic system and the OCH_2 group (see fig 1). Among type **A** and **B** drugs, a separation in potency exists between stereoisomers, with the activity residing prevalently in the isomer in which the geometry of the carbon adjacent to the hydroxyl group resembles that of the same carbon in (*R*)-(–)-catecholamines [6–8]. The capacity of the β-adrenergic receptor to recognize one of the two enantiomers of type **A** and **B** drugs is commonly explained [7] on the basis of the Easson–Stedman hypothesis [9] of a three-point attachment for active chiral molecules, assuming the Ar-hydroxy-amino or $ArOCH_2$-hydroxy-amino triads as pharmacophores.

A third class of β-adrenergic antagonists is made up of type **C** compounds, in which the ethanolaminic portion of type **A** and **B** drugs is linked to a methyleneaminoxymethyl moiety (MAOMM), substituted either by completely aliphatic [10–13] or aromatic groups



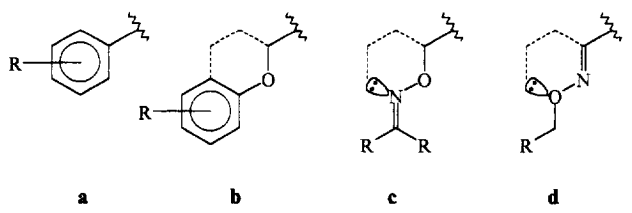
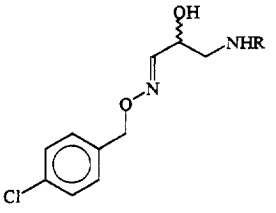


Fig 1. Representation of the spatial correspondence existing between molecular portions of Ar (**a**), $ArOCH_2$ (**b**), MAOMM (**c**) and MOIMM (**d**) which may account for their bioisosteric relationship.

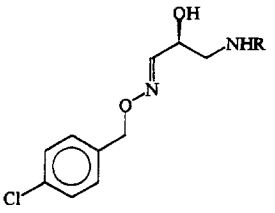
[14–16]. The activity of these types of drugs has been attributed to the existence of a bioisosteric relationship between their MAOMM and the Ar or $ArOCH_2$ of type **A** or **B** drugs (see fig 1) [12, 13]. The enantiomeric forms of type **C** drugs do not display any appreciable differences in β-antagonistic activity [8, 17, 18]. This unusual absence of stereochemical selectivity for chiral active molecules has been attributed to the considerable flexibility of type **C** compounds, in particular around the OCH_2 bond, which causes the amino group, the hydroxyl and a part of the MAOMM and its substituents to be practically superimposable for the enantiomeric forms [17, 18].

Type **D** compounds, in which the ethanolaminic chain is linked to the methyloxyiminomethyl moiety (MOIMM), represent a new class of β-adrenergic blocking agents recently designed by our group [19, 20]. The β-adrenergic properties of the compounds of this type were attributed to the existence of a bioisosterism between the MOIMM and the MAOMM, and therefore the Ar or $ArOCH_2$, in these different classes of β-antagonists (see fig 1) [19, 20]. Among the new type **D** compounds synthesized and tested as racemates, the *p*-chlorophenyl-substituted species, in which the substituent on the amino nitrogen is the *t*-butyl group ((*R,S*)-**1b**), proved to possess the best β-adrenergic properties [20].

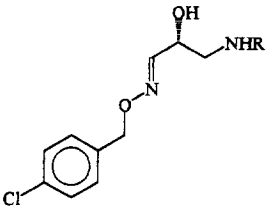
In view of the possibility of developing type **D** compounds as new β-blocking agents, it appeared to be of interest to verify whether the chirality of drugs of this new class influences the biopharmacological β-adrenergic characteristics, as in the case of compounds of types **A** and **B**, or is devoid of any influence, as in the case of compounds of type **C**. Consequently, the enantiomers of (*R,S*)-**1b** ((*S*)-**1b** and (*R*)-**1b**) and the corresponding *N*-isopropyl-substituted analog (*R,S*)-**1a** ((*S*)-**1a** and (*R*)-**1a**) were synthesized and subjected to in vitro binding and functional tests on β-adrenoceptors. This report describes the results of this study.



(*R,S*)-**1**



(*S*)-**1**



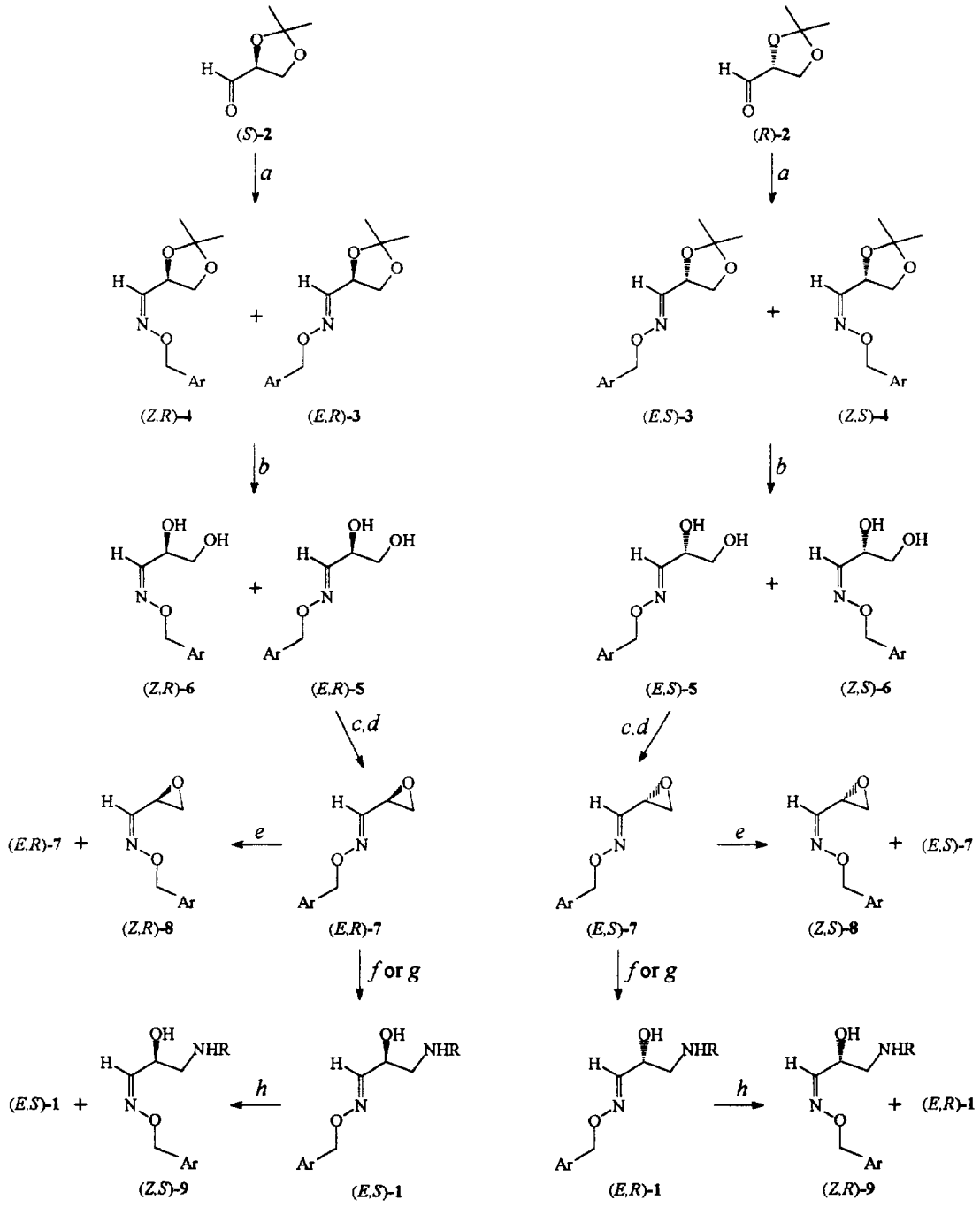
(*R*)-**1**

a, R = *i*-Pr; **b**, R = *t*-Bu

Chemistry

The optically active aminoalcohols (*S*)-**1a,b** and (*R*)-**1a,b** were prepared as reported in scheme 1, starting from (*S*)- [21] and (*R*)-isopropylideneglyceraldehyde [22] ((*S*)-**2** and (*R*)-**2**) as chiral precursors with known absolute configuration.

Enantiomerically pure (*S*)-**2** and (*R*)-**2**, obtained by the methods reported in literature [21, 22], were treated with the hydrochloride salt of *O*-*p*-chlorophenylmethylhydroxylamine [23] in the presence of Et_3N to yield, after purification by flash chromatography of the crude product, mixtures of the corresponding (*E*)-**3** and (*Z*)-**4** oxime ethers with *R* and *S* configurations, respectively, in a ratio of approximately 2:1. Hydrolysis of the *E*/*Z* mixtures of **3** and **4** with *R* or *S* configurations, afforded *E*/*Z* mixtures of the corresponding diols **5** and **6** with *R* or *S* configurations, respectively, from which the *E* compounds ((*E,R*)-**5** and (*E,S*)-**5**) were obtained by fractional crystallization, without any detectable quantities (^1H NMR) of the corresponding *Z* isomers ((*Z,R*)-**6** and (*Z,S*)-**6**). Reaction of (*E,R*)-**5** with *p*-toluenesulfonylchloride in pyridine, followed by the treatment of the crude product with *t*-BuOK in *t*-BuOH, afforded the *E* epoxide with the *R* configuration ((*E,R*)-**7**). The analogous reaction sequence carried out on the diol (*E,S*)-**5** yielded the *E* epoxide with the *S* configuration ((*E,S*)-**7**). Aminolysis of the epoxide (*E,R*)-**7** with *i*-$PrNH_2$ or *t*-$BuNH_2$ and subsequent treatment of the



Ar = *p*-Cl-C$_6$H$_4$; a, R = *i*-Pr ; b, R = *t*-Bu

Scheme 1. Reaction conditions: (a) *p*-ClC$_6$H$_4$CH$_2$ONH$_2$·HCl, Et$_3$N, MeOH, 4 h, 10 °C; (b) CF$_3$COOH, THF/H$_2$O, rt; (c) TsCl, pyridine, 12 h, 4 °C; (d) *t*-BuOK, *t*-BuOH, 6 h, rt; (e) CF$_3$COOD, CDCl$_3$; (f) *i*-PrNH$_2$, EtOH/benzene, 96 h, rt; (g) *t*-BuNH$_2$, EtOH/benzene, 144 h, rt; (h) H$^+$, D$_2$O.

crude products with oxalic acid afforded mixtures of the oxalate salts of the aminoalcohols (*E,S*)-**1a** and (*Z,S*)-**9a** or (*E,S*)-**1b** and (*Z,S*)-**9b**, respectively, in which the *E* and *Z* isomers are in a ratio of about 5:1, and from which the *E* isomers (*E,S*)-**1a** and (*E,S*)-**1b** were isolated by crystallization. Ring-opening reaction of the epoxide (*E,S*)-**7** with the same amines (*i*-$PrNH_2$ or *t*-$BuNH_2$) followed by treatment with oxalic acid yielded mixtures of the oxalate salts of the *E* and *Z* aminoalcohols with the *R* configuration (*E,R*)-**1a** and (*Z,R*)-**9a** or (*E,R*)-**1b** and (*Z,R*)-**9b**, respectively, in which the *E* and *Z* isomers are in a ratio of approximately 5:1, and from which the *E* isomers (*E,R*)-**1a** and (*E,R*)-**1b** were obtained by crystallization.

The partial $E \rightarrow Z$ isomerization that takes place in the step leading from **7** to **1** and **9** should be attributable to the treatment with oxalic acid during the salification process. No isomerization was detected in $CDCl_3$ solution for epoxides **7**, or for the free bases of the final products **1**, in the absence of acid catalysis. On the contrary, compounds **7** in $CDCl_3$ solution in the presence of trifluoroacetic acid and the oxalate salts of **1** in D_2O yielded isomeric mixtures of the corresponding *E* and *Z* isomers in a ratio of about 5:1, after 30–45 min. This ratio was also found to be practically unchanged for the oxalate salts of **1** in D_2O after 4 h. Analogous acid-catalyzed $E \rightarrow Z$ isomerizations have previously been reported for compounds with similar structures [18, 24].

The configuration of epoxides (**7**, **8**) and *N*-isopropyl- (**1a**, **9a**) and *N-tert*-butyl-substituted (**1b**, **9b**) aminoalcohols around the N=C double bond was assigned on the basis of a comparison of their ^1H NMR spectral characteristics with those of the corresponding previously described racemic compounds [20]. The configuration around the N=C double bond of the dioxolanic (**3**, **4**) and dihydroxy derivatives (**5**, **6**) was assigned on the basis of findings for the chemical shift values of the signal of the proton linked to the iminic carbon for analogous oxime ethers with *E* and *Z* configurations. In the compounds with the *E* configuration, such as **3** and **5**, this proton resonates at chemical shift values which are markedly lower than those found for the same proton in the compounds with the *Z* configuration, such as **4** and **6** [19, 20, 25].

The absolute configuration of the chiral carbon of all intermediate (**3–8**) and final products (**1a,b** and **9a,b**) was attributed on the basis of the knowledge of the configuration of the starting chiral isopropylidene glyceraldehydes (*S*)-**2** [21] and (*R*)-**2** [22], bearing in mind that in the synthetic sequences that lead from (*S*)-**2** and (*R*)-**2** to the aminoalcohols (*S*)-**1** and (*S*)-**9** and (*R*)-**1** and (*R*)-**9**, respectively, the chiral carbon is not involved. The optical purity of (*S*)-**1a,b** and (*R*)-**1a,b** was checked by HPLC analysis on a chiral phase, using a Chiracel OD-H column and eluting with a 97:3:0.1 hexane/*i*-PrOH/Et_2NH mixture. For all compounds, the enantiomeric impurities were lower than 2%.

Results

Radioligand binding assays

The β-adrenergic affinity of MOIM enantiomers (*S*)-**1a,b** and (*R*)-**1a,b** and dichloroisoproterenol and propranolol, taken as reference drugs (see table I), was checked by binding tests on rat brain and bovine lung membrane preparations for β_1- and β_2-adrenoceptors, respectively. 1-[[2-(3-Carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]phenoxy]-2-propanol (^3H-CGP 26505) [26] was used as a specific tritiated ligand for rat brain β_1-adrenoceptors. ^3H-Dihydroalprenolol (^3H-DHA) [27] was used to label bovine lung β_2-adrenoceptors in the presence of 50 nM CGP 26505, which displaced ^3H-DHA binding from the bovine lung β_1-adrenoceptor subpopulation (17%) [28]. Table I also shows the results previously obtained by us in the same types of tests with the MOIM derivatives (*R,S*)-**1a,b** as racemic mixtures [20].

Rat brain β_1-adrenoceptors

The *N*-isopropyl- and *N-tert*-butyl-substituted compounds with the *S* configuration, (*S*)-**1a** and (*S*)-**1b**, showed affinities 3.3- and 22-fold higher than those of the corresponding enantiomers with the *R* configuration, (*R*)-**1a** and (*R*)-**1b**. The affinities of (*S*)-**1a** and (*S*)-**1b** were also 2.1 and 2.6 times higher than those of the corresponding racemates, (*R,S*)-**1a** and (*R,S*)-**1b**, while those of (*R*)-**1a** and (*R*)-**1b** were 1.6 and 8.5 times lower than those of (*R,S*)-**1a** and (*R,S*)-**1b**, respectively.

Bovine lung β_2-adrenoceptors

The MOIM derivatives with the S configuration, (*S*)-**1a** and (*S*)-**1b**, showed affinities for this receptor 7.0- and 4.7-fold higher than those of the corresponding enantiomers (*R*)-**1a** and (*R*)-**1b**, and slightly higher than those of the corresponding racemates (*R,S*)-**1a** and (*R,S*)-**1b**. The compounds with the *R* configuration, (*R*)-**1a** and (*R*)-**1b**, exhibited affinities 5.9 and 3.6 times lower than those of (*R,S*)-**1a** and (*R,S*)-**1b**, respectively.

Functional tests

The optically active MOIM compounds (*S*)-**1a,b** and (*R*)-**1a,b**, the racemate (*R,S*)-**1a** and the reference drugs dichloroisoproterenol and propranolol were tested on isolated guinea-pig atria and on isolated

Table I. β-Adrenergic activity and radioligand binding affinity of MOIM enantiomers **1a,b**.

Compound[a]	*R*	*β-Adrenergic binding affinity*[b] *Ki (nM)*		*β-Adrenergic activity*[c] *pIC_{50}*[d]	
		Rat brain (β_1)	*Bovine lung (β_2)*	*Isolated guinea-pig atria (β_1)*	*Isolated guinea-pig tracheal strips (β_2)*
(*S*)-**1a**	*i*-Pr	1100 (950–1250)	263 (249–277)	4.81 ± 0.16	6.10 ± 0.22
(*R*)-**1a**	*i*-Pr	3660 (3410–3910)	1840 (1640–2040)	4.23 ± 0.08	5.97 ± 0.24
(*R,S*)-**1a**	*i*-Pr	2300 (1910–2690)[e]	310 (270–350)[e]	4.62 ± 0.04	6.05 ± 0.32
(*S*)-**1b**	*t*-Bu	119 (109–129)	75 (70–80)	5.42 ± 0.01	7.27 ± 0.09
(*R*)-**1b**	*t*-Bu	2620 (2420–2820)	350 (320–380)	4.54 ± 0.07	6.30 ± 0.15
(*R,S*)-**1b**	*t*-Bu	310 (270–350)[e]	98 (85–110)[e]	5.13 ± 0.08[e]	6.77 ± 0.31[e]
Dichloroisoproterenol		58 (50–66)	144 (124–168)	6.80 ± 0.21	6.15 ± 0.37
Propranolol		5.0 (3.7–6.0)	1.9 (1.6–2.1)	7.35 ± 0.18	7.51 ± 0.18

[a]MOIM derivatives (*S*)-**1a,b**, (*R*)-**1a,b** and (*R,S*)-**1a,b** were tested as oxalates whereas dichloroisoproterenol and propranolol were used as hydrochlorides. [b]Geometric means of five separate determinations with confidence limits in parentheses. [c]The values represent the mean of three to five experiments for each drug ± standard error. [d]pIC_{50} is the negative logarithm of the molar concentration that reduces the response to isoprenaline by 50%. [e]From reference [20].

guinea-pig tracheal strips for their β_1- and β_2-adrenergic activities, respectively. The results obtained are shown in table I, together with those previously obtained in the same types of tests with the racemic derivative (*R,S*)-**1b** [20].

Guinea-pig atria β_1-adrenoceptors

The MOIM derivatives with the *S* configuration, (*S*)-**1a** and (*S*)-**1b**, showed antagonistic activities 3.7 and 7.6 times higher than those of the corresponding enantiomers with the *R* configuration, (*R*)-**1a** and (*R*)-**1b**, respectively. The β_1-blocking activities of (*S*)-**1a** and (*S*)-**1b** were also found to be 1.5- and 2-fold higher than those of the corresponding racemic compounds (*R,S*)-**1a** and (*R,S*)-**1b**, while those of (*R*)-**1a** and (*R*)-**1b** were 2.5 and 4 times lower than those of (*R,S*)-**1a** and (*R,S*)-**1b**, respectively. No stimulating properties were detected for the optically active compounds (*S*)-**1a,b** and (*R*)-**1a,b**.

Guinea-pig tracheal strip β_2-adrenoceptors

On this type of β-receptor, (*S*)-**1b** was 9.3 times as potent as the corresponding enantiomer (*R*)-**1b**, while (*S*)-**1a** was slightly more potent than (*R*)-**1a**. For the *N*-isopropyl-substituted compounds (**1a**), the activity indices of the two enantiomers (*S*)-**1a** and (*R*)-**1a** were practically equal to that of the racemic mixture (*R,S*)-**1a**. For the *N-tert*-butyl-substituted compounds (**1b**), the activities of the *S* and *R* enantiomers ((*S*)-**1b** and (*R*)-**1b**) were 3.2 times higher and 3-fold lower, respectively, than that of the racemate (*R,S*)-**1b**. The MOIM compounds (*S*)-**1a,b** and (*R*)-**1a,b** proved to be devoid of any stimulating activity on β_2-adrenoceptors.

Discussion and conclusions

The results of the binding and functional tests on β_1- and β_2-adrenoceptors shown in table I indicate that the MOIM compounds **1a,b** possess an activity trend that is substantially in agreement with that of the affinity. In both types of experiments, the β-adrenergic properties of the enantiomers with the *S* configuration ((*S*)-**1a,b**) are better, even if not always markedly so, than those of the corresponding enantiomers with the *R* configuration ((*R*)-**1a,b**). Moreover, the racemic compounds (*R,S*)-**1a,b** show affinity and activity indices whose values are intermediate between those of the corresponding enantiomeric forms, (*S*)-**1a,b** and (*R*)-**1a,b**.

As regards β_2/β_1-selectivity, it appears that all the optically active MOIM compounds present a certain degree of β_2-selectivity, at least with respect to the activity, in analogy with observations for the racemic mixtures. In contrast, when the affinity is considered, only compounds (*S*)-**1a** and (*R*)-**1b** appear to display any appreciable β_2-selectivity, whereas (*R*)-**1a** and (*S*)-**1b** appear to possess a very slight degree of selectivity for β_2-adrenoceptors.

The synthesis and study of the β-adrenergic properties of the optically active (*S*)- and (*R*)-MOIM derivatives **1a,b** aimed at verifying the existence of a possible enantiomeric specificity at the level of β_1-

and β_2-adrenoceptors, on the part of the new type **D** β-adrenergic antagonists. In fact, compounds (*S*)-**1a,b**, in which the geometry of the chiral carbon adjacent to the hydroxyl group resembles that of the natural catecholamines with the *R* configuration, proved to interact better with β-receptors, even if the stereochemical selectivity among the enantiomeric pairs is not so marked as in type **A** or **B** β-adrenergic blocking drugs [7, 17, 29].

These results seem to indicate, however, that for MOIM-type β-blocking agents (**D**), the chirality influences the biopharmacological β-adrenergic characteristics, as is the case for type **A** and **B** drugs, but in contrast to findings for MAOM-type drugs (**C**). An explanation for the different behavior of type **D** compounds compared with type **C** compounds may be offered by an examination of the molecular structures of compounds (*S*)-**1a,b** and (*R*)-**1a,b**. For the enantiomeric forms of type **D** derivatives, the fact that their N=CHCH(OH) portion is more rigid than the corresponding $OCH_2CH(OH)$ moiety of compounds **C**, makes it impossible to find conformations which allow a quasi-superimposition of the enantiomers, of the kind used to explain the absence of stereochemical selectivity in type **C** compounds [17, 18].

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films on a Mattson 1000 Series FTIR spectrometer. ^{1}H NMR spectra of the mixtures of *E* and *Z* compounds and of the pure intermediates and final products were obtained with a Bruker AC-200 instrument in a ca 2% solution of $CDCl_3$ (for the neutral compounds) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The relative percentages of *E* and *Z* isomers were evaluated on the basis of the integrals of the CH=N protons in the ^{1}H NMR spectra of the crude mixtures. TLC was carried out on 0.25 or 0.50 mm layer silica-gel plates (Merck F_{254}) containing a fluorescent indicator; spots were detected under UV light (254 nm). HPLC analysis was performed using a chromatographic system consisting of a Waters Associates liquid chromatograph, equipped with a U6 K injector, a 6000 A solvent delivery system and a UV detector Model 480 set at 254 nm. Analyses were carried out using a Chiracel OD-H column, eluting with a 90:3:0.1 hexane/*i*-PrOH/Et_2NH mixture, at a flow rate of 1.3 mL/min. Evaporations were made in vacuo (rotating evaporator); Na_2SO_4 was always used as the drying agent. Elemental analyses were performed in our analytical laboratory and agreed with theoretical values to within ±0.4%.

(E,R)- and (Z,R)-4-[(p-Chlorophenylmethyl)oxyiminomethyl]-2,2-dimethyl-1,3-dioxolane (E,R)-3 and (Z,R)-4
A solution of *O*-*p*-chlorophenylmethylhydroxylamine hydrochloride (2.35 g, 12.1 mmol) [23] and Et_3N (1.68 mL, 12.1 mmol) in absolute MeOH (8 mL) was added, dropwise, to a stirred and cooled (0–10 °C) solution of crude (*S*)-isopropylideneglyceraldehyde ((*S*)-**2**) in AcOEt (100 mL) obtained, as reported in literature [21], starting from 5,6-isopropylidene-L-ascorbic acid (2.0 g, 9.3 mmol). The resulting solution was stirred for 4 h at 10 °C, filtered and evaporated to yield an oily residue, which was subjected to flash chromatography on silica gel (230-400 mesh), eluting with a 9:1 hexane/AcOEt mixture. Evaporation of the fractions containing the more chromophoric substances gave an oil (2.05 g) consisting almost exclusively of a mixture of (*E,R*)-**3** and (*Z,R*)-**4** in a ratio of about 2:1 (GLC), which was directly used for the subsequent transformation. (*E,R*)-**3**: ^{1}H NMR δ 1.37 and 1.41 (2s, 6H), 3.85 (dd, 1H, *J* = 8.5 and 6.3 Hz), 4.13 (dd, 1H, *J* = 8.5 and 6.6 Hz), 4.61 (ddd, 1H, *J* = 7.1, 6.6 and 6.3 Hz), 5.02 (s, 2H), 7.28 (m, 4H), 7.39 (d, 1H, *J* = 7.1 Hz, CH=N). (*Z,R*)-**4**: ^{1}H NMR δ 6.93 (d, 1H, *J* = 4.0 Hz, CH=N).

(E,S)- and (Z,S)-4-[(p-Chlorophenylmethyl)oxyiminomethyl]-2,2-dimethyl-1,3-dioxolane (E,S)-3 and (Z,S)-4
A solution of *O*-*p*-chlorophenylmethylhydroxylamine hydrochloride (6.0 g, 30.9 mmol) [23] and Et_3N (4.29 mL, 30.9 mmol) in absolute MeOH (200 mL) was added, dropwise, to a stirred and cooled (0–10 °C) solution of crude (*R*)-isopropylideneglyceraldehyde ((*R*)-**2**) obtained by treating a solution of 1,2,5,6-di-*O*-isopropylidene-D-mannitol (3.22 g, 12.3 mmol) in anhydrous THF (32 mL) with $(AcO)_4Pb$ (5.45 g, 12.3 mmol) [22]. The resulting mixture was stirred for 4 h at 10 °C, filtered and evaporated to yield an oily residue which was submitted to flash chromatography on silica gel (230–400 mesh), eluting with a 9:1 hexane/AcOEt mixture. Evaporation of the fractions containing the more chromophoric substances afforded a 2:1 mixture (GLC) of practically pure (*E,S*)-**3** and (*Z,S*)-**4** (5.48 g), which was directly used for the subsequent reaction. For the ^{1}H NMR data of (*E,S*)-**3** and (*Z,S*)-**4**, see (*E,R*)-**3** and (*Z,R*)-**4**, respectively.

(E,R)-N-(2,3-Dihydroxypropylidene)(p-chlorophenylmethyloxy)amine (E,R)-5
A stirred and cooled (0 °C) solution of the 2:1 mixture of (*E,R*)-**3** and (*Z,R*)-**4** (1.8 g, 6.7 mmol) in 4:1 THF/H_2O (30 mL) was treated with CF_3COOH (1.6 mL, 20.9 mmol) and the resulting stirred mixture was left to warm to room temperature and then evaporated to dryness. The residue was dissolved in $CHCl_3$ and the solution was evaporated again. This operation was repeated twice and the crude residue was again dissolved in $CHCl_3$, washed (H_2O), filtered and evaporated. The resulting semisolid residue, consisting of a 3:1 mixture of (*E,R*)-**5** and (*Z,R*)-*N*-(2,3-dihydroxypropylidene)(*p*-chlorophenylmethyloxy)amine (*Z,R*)-**6** (^{1}H NMR δ 6.77 (d, 1H, *J* = 4.1 Hz, CH=N)), was crystallized from *i*-Pr_2O/hexane to give pure (*E,R*)-**5** (0.97 g, 95% calculated on (*E,R*)-**3** in the *E/Z* starting mixture). (*E,R*)-**5**: mp 84–85 °C; $[\alpha]_D$ = –10.0° (c = 0.98, $CHCl_3$); ^{1}H NMR δ 3.74 (m, 2H), 4.35 (br, 1H), 5.04 (s, 2H), 7.26 (d, 2H, *J* = 8.6 Hz), 7.33 (d, 2H, *J* = 8.6 Hz), 7.50 (d, 1H, *J* = 4.8 Hz, CH=N). Anal for $C_{10}H_{12}NO_3Cl$ (C, H, N).

(E,S)-N-(2,3-Dihydroxypropylidene)(p-chlorophenylmethyloxy)amine (E,S)-5
A solution of the 2:1 mixture of (*E,S*)-**3** and (*Z,S*)-**4** (2.0 g, 7.4 mmol) in 4:1 THF/H_2O (35 mL) was treated, as described for the preparation of (*E,R*)-**5**, with CF_3COOH (1.4 mL, 18.3 mmol). The crude residue consisting of a 3:1 mixture of (*E,S*)-**5** and (*Z,S*)-*N*-(2,3-dihydroxypropylidene)(*p*-chlorophenylmethyloxy)amine (*Z,S*)-**6** (^{1}H NMR δ 6.77 (d, 1H, *J* = 4.1 Hz, CH=N)), was crystallized from *i*-Pr_2O/hexane to yield pure (*E,S*)-**5** (1.11 g, 98% calculated on (*E,S*)-**3** in the *E/Z*

starting mixture). (*E,S*)-**5**: mp 83–85 °C; $[\alpha]_D$ = +10.2° (c = 0.98, $CHCl_3$); ^1H NMR: see spectral data of (*E,R*)-**5**. Anal for $C_{10}H_{12}NO_3Cl$ (C, H, N).

(E,R)-N-(2,3-Epoxypropylidene)(p-chlorophenylmethyloxy)-amine (E,R)-7
A stirred and cooled (0 °C) solution of (*E,R*)-**5** (1.8 g, 7.8 mmol) in pyridine (4.2 mL) was treated portionwise with *p*-toluensulfonylchloride (1.5 g, 7.8 mmol). After 1 h of stirring at 0 °C, the mixture was kept at 4 °C for 12 h, and then dissolved in Et_2O and washed with 1 N acqueous HCl (three times), saturated aqueous $NaHCO_3$, and H_2O (twice). Evaporation of the organic layer afforded an oily residue (2.5 g) which was directly dissolved in *t*-BuOH (22 mL). The resulting solution was cooled to 10 °C and treated portionwise, under stirring, with *t*-BuOK (0.94 g, 8.4 mmol). The mixture was stirred at room temperature for 6 h and then diluted with petroleum ether (bp 60–80 °C), filtered and evaporated to yield a semisolid residue consisting almost exclusively of (*E,R*)-**7** (1.37 g, 82%) which was directly used for the following transformations. An analytical sample of (*E,R*)-**7** was obtained by preparative TLC using a 9:1 hexane/AcOEt mixture as the eluent. Pure (*E,R*)-**7** was obtained as a vitreous solid: ^1H NMR δ 2.80 (dd, 1H, *J* = 5.0 and 2.6 Hz), 3.04 (dd, 1H, *J* = 5.0 and 4.1 Hz), 3.52 (ddd, 1H, *J* = 8.0, 4.1 and 2.6 Hz), 5.07 (s, 2H), 6.99 (d, 1H, *J* = 8.0 Hz, CH=N), 7.28 (d, 2H, *J* = 8.8 Hz), 7.34 (d, 2H, *J* = 8.8 Hz). Anal for $C_{10}H_{10}NO_2Cl$ (C, H, N). Treatment of a $CDCl_3$ solution of pure (*E,R*)-**7** with 1 molar equivalent of CF_3COOD for 45 min afforded a 7:3 mixture of (*E,R*)-**7** and (*Z,R*)-*N*-(2,3-epoxypropylidene)(*p*-chlorophenylmethyloxy)amine (*Z,R*)-**8** (^1H NMR δ 6.38 (d, 1H, *J* = 7.0 Hz, CH=N)).

(E,S)-N-(2,3-Epoxypropylidene)(p-chlorophenylmethyloxy)-amine (E,S)-7
A solution of (*E,S*)-**5** (1.4 g, 6.1 mmol) in pyridine (3.3 mL) was treated, as described for the preparation of (*E,R*)-**7**, with *p*-toluensulfonylchloride (1.16 g, 6.1 mmol). Subsequent treatment of the crude product with *t*-BuOK (0.74 g, 6.6 mmol) in *t*-BuOH (17 mL), as described above for the synthesis of (*E,R*)-**7**, yielded a semisolid residue consisting almost exclusively of (*E,S*)-**7** (0.92 g, 71%) which was directly used for the following transformations. An analytical sample of (*E,S*)-**7** was obtained by preparative TLC eluting with a 9:1 hexane/AcOEt mixture. Pure (*E,S*)-**7** was a vitreous solid: ^1H NMR: see spectral data of (*E,R*)-**7**. Anal for $C_{10}H_{10}NO_2Cl$ (C, H, N). Treatment of a $CDCl_3$ solution of pure (*E,S*)-**7** with 1 molar equiv of CF_3COOD gave an 8:2 mixture of (*E,S*)-**7** and (*Z,S*)-*N*-(2,3-epoxypropylidene)(*p*-chlorophenylmethyloxy)amine (*Z,S*)-**8** (^1H-NMR δ 6.38 (d, 1H, *J* = 7.0 Hz, CH=N)).

*(E,S)-N-[3-(Isopropylamino)-2-hydroxypropylidene](p-chlorophenylmethyloxy)amine oxalate (E,S)-**1a**·$H_2C_2O_4$*
A solution of (*E,R*)-**7** (0.55 g, 2.6 mmol) in an anhydrous 2:1 EtOH/benzene mixture (7 mL) and *i*-$PrNH_2$ (1 mL, 11.7 mmol) was stirred for 96 h at room temperature. Evaporation of the organic layer yielded a solid residue which was dissolved in anhydrous Et_2O and then treated dropwise, under stirring at 0 °C, with a solution of $H_2C_2O_4$·$2H_2O$ (0.29 g, 2.3 mmol) in MeOH (2 mL). Addition of anhydrous Et_2O gave a solid precipitate which was crystallized from MeOH/Et_2O to yield pure (*E,S*)-**1a**·$H_2C_2O_4$ (0.56 g, 60%): mp 125–127 °C; ^1H NMR δ 1.27 (2d, 6H, *J* = 6.5 Hz), 3.14 (dd, 1H, *J* = 13.1 and 8.5 Hz), 3.26 (dd, 1H, *J* = 13.1 and 4.2 Hz), 3.41 (sept, 1H, *J* = 6.5 Hz), 4.55 (ddd, 1H, *J* = 8.5, 4.9 and 4.2 Hz), 5.07 (s, 2H), 7.24 and 7.41 (2d, 4H, *J* = 8.8 Hz), 7.53 (d, 1H, *J* = 4.9 Hz, CH=N). Anal for $C_{15}H_{21}N_2O_6Cl$ (C, H, N).

The oxalate salt of (**E,S**)-**1a** was converted into the free base by adding it to a stirred and cooled (0 °C) mixture of 1 N aqueous NaOH and CH_2Cl_2. The organic layer was filtered and evaporated to give pure (*E,S*)-**1a**: mp 56–58 °C; $[\alpha]_D$ = –24.8° (c = 1.00, $CHCl_3$); ^1H NMR δ 1.08 (d, 6H, *J* = 6.3 Hz), 2.75 (dd, 1H, *J* = 12.1 and 7.3 Hz), 2.80 (sept, 1H, *J* = 6.3 Hz), 2.86 (dd, 1H, *J* = 12.1 and 4.3 Hz), 4.26 (ddd, 1H, *J* = 12.1, 7.3 and 4.3 Hz), 5.06 (s, 2H), 7.30 and 7.36 (2d, 4H, *J* = 8.7 Hz), 7.46 (d, 1H, *J* = 5.0 Hz, CH=N); HPLC: t_R= 12.09 min. Anal for $C_{13}H_{19}N_2O_2Cl$ (C, H, N).

When the oxalate salt of (*E,S*)-**1a** was kept for 45 min in D_2O, a 5:1 mixture of (*E,S*)-**1a**·$H_2C_2O_4$ and (*Z,S*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene](*p*-chlorophenylmethyloxy)amine oxalate (*Z,S*)-**9a**·$H_2C_2O_4$ (^1H NMR δ 6.90 (d, 1H, *J* = 4.8 Hz, CH=N)) was obtained (^1H NMR); this ratio was found to be practically unchanged after 4 h in the same conditions (^1H NMR).

*(E,S)-N-[3-(tert-Butylamino)-2-hydroxypropylidene](p-chlorophenylmethyloxy)amine hemioxalate (E,S)-**1b**·1/2$H_2C_2O_4$*
A stirred solution of (*E,R*)-**7** (0.65 g, 3.0 mmol) and *t*-$BuNH_2$ (1.4 mL, 13.5 mmol) in an anhydrous 2:1 EtOH/benzene mixture (8 mL) was kept at room temperature for 144 h and then evaporated and treated, as in the preparation of (*E,S*)-**1a**·$H_2C_2O_4$, with $H_2C_2O_4$·$2H_2O$ (0.34 g, 2.7 mmol). Crystallization from MeOH/Et_2O of the solid product yielded pure (*E,S*)-**1b**·1/2$H_2C_2O_4$ (1.1 g, 55%): mp 184–185 °C; ^1H NMR δ 1.33 (s, 9H), 3.13 (dd, 1H, *J* = 12.8 and 8.8 Hz), 3.27 (dd, 1H, *J* = 12.8 and 3.8 Hz), 4.53 (ddd, 1H, *J* = 8.8, 4.9 and 3.8 Hz), 5.09 (s, 2H), 7.36 and 7.43 (2d, 4H, *J* = 8.8 Hz), 7.55 (d, 1H, *J* = 4.9 Hz, CH=N). Anal for $C_{30}H_{44}N_4O_8Cl_2$ (C, H, N).

The hemioxalate salt of (*E,S*)-**1b** was converted into the free base by following the procedure described to obtain (*E,S*)-**1a**. (*E,S*)-**1b**: mp 73–74 °C; $[\alpha]_D$ = –36.3° (c = 0.98, $CHCl_3$); ^1H NMR δ 1.12 (s, 9H), 2.72 (dd, 1H, *J* = 12.0 and 7.0 Hz), 2.84 (dd, 1H, *J* = 12.0 and 4.4 Hz), 4.21 (ddd, 1H, *J* = 7.0, 4.9 and 4.4 Hz), 5.08 (s, 2H), 7.31 and 7.37 (2d, 4H, *J* = 8.7 Hz), 7.47 (d, 1H, *J* = 4.9 Hz, CH=N); HPLC: t_R= 11.76 min. Anal for $C_{14}H_{21}N_2O_2Cl$ (C, H, N).

When pure (*E,S*)-**1b**·1/2$H_2C_2O_4$ was kept for 45 min in D_2O, an approximately 5:1 mixture of (*E,S*)-**1b**·1/2$H_2C_2O_4$ and (*Z,S*)-*N*-[3-(*tert*-butylamino)-2-hydroxy-propylidene](*p*-chlorophenylmethyloxy)amine hemioxalate (*Z,S*)-**9b**·1/2$H_2C_2O_4$ (^1H NMR δ 6.90 (d, 1H, *J* = 4.8 Hz, CH=N)) was obtained (^1H NMR). This ratio appeared to be practically unchanged after 4 h in the same D_2O solution (^1H NMR).

*(E,R)-N-[3-(Isopropylamino)-2-hydroxypropylidene](p-chlorophenylmethyloxy)amine oxalate (E,R)-**1a**·$H_2C_2O_4$*
A stirred solution of (*E,S*)-**7** (0.30 g, 1.42 mmol) in an anhydrous 2:1 EtOH/benzene mixture (4 mL) and *i*-$PrNH_2$ (0.55 mL, 6.4 mmol) was kept at room temperature for 96 h and then evaporated to dryness. The crude residue was dissolved in Et_2O and treated, as described in the preparation of (*E,S*)-**1a**·$H_2C_2O_4$, with $H_2C_2O_4$·$2H_2O$ (0.16 g, 1.28 mmol). Crystallization of the solid product from MeOH/Et_2O afforded pure (*E,R*)-**1a**·$H_2C_2O_4$ (0.32 g, 63%): mp 125–127 °C; ^1H NMR: see spectral data of (*E,S*)-**1a**·$H_2C_2O_4$. Anal for $C_{15}H_{21}N_2O_6Cl$ (C, H, N).

The oxalate salt of (*E,R*)-**1a** was converted into the free base by following the procedure described for the preparation of (*E,S*)-**1a**. (*E,R*)-**1a**: mp 56–58 °C; $[\alpha]_D$ = +24.3° (c = 1.01, $CHCl_3$); ^1H NMR: see spectral data of (*E,S*)-**1a**; HPLC: t_R = 9.93 min. Anal for $C_{13}H_{19}N_2O_2Cl$ (C, H, N).

When pure (*E,R*)-**1a**·$H_2C_2O_4$ was kept for 45 min in D_2O, an approximately 5:1 mixture of (*E,R*)-**1a**·$H_2C_2O_4$ and (*Z,R*)-*N*-[3-

(isopropylamino)-2-hydroxypropylidene](*p*-chlorophenylmethyloxy)amine oxalate (*Z,R*)-**9a**•$H_2C_2O_4$ (^{1}H NMR δ 6.91 (d, 1H, J = 4.8 Hz, CH=N)) was obtained (^{1}H NMR). This ratio was found to be practically unchanged after 4 h in the same conditions (^{1}H NMR).

*(E,R)-N-[3-(tert-Butylamino)-2-hydroxypropylidene](p-chlorophenylmethyloxy)amine oxalate (E,R)-**1b**•$H_2C_2O_4$*

A stirred solution of (*E,S*)-**7** (0.45 g, 2.13 mmol) and *t*-$BuNH_2$ (1 mL, 9.6 mmol) in an anhydrous 2:1 EtOH/benzene mixture (6 mL) was kept at room temperature for 144 h and then evaporated. The crude residue was dissolved in Et_2O and treated, as described for the preparation of (*E,S*)-**1a**•$H_2C_2O_4$, with $H_2C_2O_4$•$2H_2O$ (0.24 g, 1.92 mmol). Crystallization from MeOH/Et_2O of the solid precipitate afforded pure (*E,R*)-**1b**•$H_2C_2O_4$ (0.46 g, 58%): mp 108–110 °C; ^{1}H NMR: see spectral data of (*E,S*)-**1b**•$1/2H_2C_2O_4$. Anal for $C_{16}H_{23}N_2O_6Cl$ (C, H, N).

Compound (*E,R*)-**1b**•$H_2C_2O_4$ was converted into the free base by following the procedure described for the preparation of (*E,S*)-**1a**. (*E,R*)-**1b**: mp 73–74 °C; $[\alpha]_D$ = +38.3° (c = 0.96, $CHCl_3$); ^{1}H NMR: see spectral data of (*E,S*)-**1b**; HPLC: t_R = 8.96 min. Anal for $C_{14}H_{21}N_2O_2Cl$ (C, H, N).

When pure (*E,R*)-**1b**•$H_2C_2O_4$ was kept for 45 min in D_2O, an approximately 5:1 mixture of (*E,R*)-**1b**•$H_2C_2O_4$ and (*Z,R*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene] (*p*-chlorophenylmethyloxy)amine oxalate (*Z,R*)-**9b**•$H_2C_2O_4$ (^{1}H NMR δ 6.90 (d, 1H, J = 4.8 Hz, CH=N) was obtained (^{1}H NMR). This ratio was found to be practically unchanged after 4 h in the same conditions (^{1}H NMR).

Radioligand binding methods

Rat brain β_1-adrenoceptors

β_1-Adrenoceptors were assayed in rat cortical membranes, as previously described [19], using 1-[[2-(3-carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]phenoxy]2-propanol (^{3}H-CGP 26505) [26] as the specific ligand (DuPont de Nemours, New England Nuclear Division; specific activity 2.5 Ci/mmol).

Bovine lung β_2-adrenoceptors

β_2-Adrenoceptor binding was studied in bovine lung, as previously described [19], using ^{3}H-dihydroalprenolol (^{3}H-DHA) [27] as the ligand (DuPont de Nemours, New England Nuclear Division; specific activity 48.1 Ci/mmol), in the presence of CGP 26505.

Pharmacological methods

Guinea-pig atria and guinea-pig tracheal strips

The activity of compounds **1a** and **1b** on atrial β_1- and tracheal β_2-adrenoceptors was evaluated on isolated preparations obtained from adult male Dunkin–Hartley guinea pigs, weighing 300–350 g. The antagonistic and agonistic action of the compounds under examination on β_1- and β_2-adrenoceptors, was experimented on preparations of isolated guinea-pig atria and tracheal smooth musculature, respectively, following the methods previously described [19].

For both β_1 and β_2 preparations, the antagonistic activity of the compounds tested towards β_1- and β_2-adrenoceptors was expressed as pIC_{50}, ie, the negative logarithm of the molar concentration that reduced the response to isoprenaline by 50% [23]. All compounds were tested at concentrations ranging from 10^{-9} M to 10^{-3} M. Each antagonistic activity index was obtained by at least five active concentrations.

Acknowledgment

This work was partly supported by a grant from the Consiglio Nazionale delle Ricerche and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

References

1 Ammon HL, Balsamo A, Macchia B, Macchia F, Howe DB, Keefe WE (1975) *Experientia* 31, 644–645
2 Ammon HL, Howe DB, Erhardt WD et al (1977) *Acta Cryst* B33, 21–29
3 Petrongolo C, Macchia B, Macchia F, Martinelli A (1977) *J Med Chem* 20, 1645–1653
4 Macchia B, Macchia F, Martinelli A (1980) *Eur J Med Chem* 15, 515–520
5 Macchia B, Macchia F, Martinelli A (1983) *Eur J Med Chem* 18, 85–90
6 Main BG (1990) *Comprehensive Medicinal Chemistry*. Pergamon Press, Oxford, 3, 187–227
7 Ruffolo RR Jr (1991) *Tetrahedron* 47, 9953–9980
8 Leclerc G, Amlaiky N, Rouot B (1982) *Eur J Med Chem* 17, 69–74
9 Easson LH, Stedman E (1933) *Biochem J* 27, 1257–1266
10 Leclerc G, Bieth N, Schwartz J (1980) *J Med Chem* 23, 620–624
11 Bouzoubaa M, Leclerc G, Decker N, Schwartz J, Andermann G (1984) *J Med Chem* 27, 1291–1294
12 Macchia B, Balsamo A, Lapucci A et al (1985) *J Med Chem* 28, 153–160
13 Balsamo A, Gentili D, Lapucci A, Macchia M, Martinelli A, Orlandini E (1994) *Il Farmaco* 49, 759–766
14 Imbs JL, Miesch F, Schwartz J et al (1977) *Br J Pharmacol* 60, 357–362
15 Leclerc G, Mann A, Wermuth CG, Bieth N, Schwartz J (1977) *J Med Chem* 20, 1657–1662
16 Gentili D, Lapucci A, Macchia B et al (1995) *Il Farmaco* 50, 519–526
17 Baldwin JJ, McClure DE, Gross DM, Williams M (1982) *J Med Chem* 25, 931–936
18 Bouzoubaa M, Leclerc G, Rakhit S, Andermann G (1985) *J Med Chem* 28, 896–900
19 Macchia B, Balsamo A, Breschi MC et al (1994) *J Med Chem* 37, 1518–1525
20 Balsamo A, Breschi MC, Chiellini G et al (1995) *Eur J Med Chem* 30, 743–755
21 Jung ME, Shaw TJ (1980) *J Am Chem Soc* 102, 6304–6311
22 Danklmaier J, Hönig H (1988) *Liebigs Ann Chem* 1149–1153
23 Balsamo A, Belfiore MS, Macchia M et al (1994) *Eur J Med Chem* 29, 787–794
24 Rosenberg S, Silver SM, Sayer JM, Jencks WP (1974) *J Am Chem Soc* 7986–7998
25 Karabatsos GJ, Hsi N (1967) *Tetrahedron* 23, 1079–1095
26 Dooley DJ, Bittiger H, Reymann NC (1986) *Eur J Pharmacol* 130, 137–139
27 Nahorski SR, Richardson A (1979) *Br J Pharmacol* 66, 469–470
28 Minneman K, Hegstrand LR, Molinoff PB (1979) *Mol Pharmacol* 16, 34–46
29 Howe R, Shanks RG (1966) *Nature (Lond)* 210, 1336–1338
30 Hernauder M, Prieto D, Simonsen V, Rivera L, Barabona MV, Garcia S (1992) *Br J Pharmacol* 107, 924–931