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Chiral 2-(3'-(5'-*p*-chlorophenyl)isoxazolidinyl)ethanolamines as conformationally restrained analogs of methyloxyiminomethyl (MOIM) β-adrenergic antagonists: synthesis, configuration and β-adrenergic properties*

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Summary — The chiral *N*-isopropyl- and *N*-*t*-butyl-substituted 2-(3'-(5'-*p*-chlorophenyl)isoxazolidinyl)ethanolamines **2**, **3**, which can be viewed as conformationally restrained analogs of the corresponding methyloxyiminomethyl (MOIM) β-adrenergic antagonists **1**, were synthesized from optically active precursors with a known absolute configuration. The structure and configuration of the intermediate and final products **2**, **3** were assigned on the basis of a comparison of the ^{1}H-NMR spectral data of all compounds, crystallographic analysis of one of the intermediates [(2*R*,5'*S*)-**7**] and knowledge of the configuration of the chiral starting compounds **4**. The new isoxazoline derivatives **2**, **3** were tested for their affinity towards β_1- and β_2-adrenoceptors by radioligand binding experiments; compounds showing affinity indices lower than 10 μM on β_1-adrenoceptors were also assayed for their β-adrenergic activity by functional tests on isolated preparations. The results showed that the cyclic derivatives **2**, **3** possess a capacity to interact with β-receptors which is clearly lower than that of the corresponding MOIM analogs **1**.

adrenergic drug / β-blocking agent / chiral 2-(3'-(5'-*p*-chlorophenyl)isoxazolidinyl)ethanolamine

Introduction

In previous studies on the stereoelectronic requirements for the interaction of adrenergic drugs with β-receptors, we found that the methyleneaminoxymethyl moiety (C=NOCH$_2$, MAOMM) of type **B** compounds can act as a bioisoster of the aryl group (Ar) of type **A** β-blocking agents [3, 4]. Further research in the same area showed that completely aliphatic compounds of type **C**, which differ from the β-blockers of type **B** due to the presence of a methyloxyiminomethyl moiety (CH$_2$ON=C, MOIMM) with the *E* configuration in the place of the MAOMM, also possess adrenergic properties similar to those of type **B** β-blocking drugs [5]. Subsequent studies showed that type **C** compounds, in which an Ar linked to the CH$_2$ carbon of the MOIMM is present, often possess slightly better β-adrenergic properties with respect to completely aliphatic compounds, at least as regards their affinity [6]. Among the MOIM derivatives studied (**C**), the *p*-chlorophenyl-substituted one in which R' is a *t*-butyl group (**1b**) appeared to possess the most interesting β-adrenergic properties [6]; consequently, the enantiomers of this compound [(*S*)-**1b** and (*R*)-**1b**] and of its *N*-isopropyl analog **1a** [(*S*)-**1a** and (*R*)-**1a**] were synthesized, in order to evaluate their enantiomeric specificity [7]. The chiral compounds (*S*)-**1a**,**b**, in which the geometry of the carbon linked to the hydroxyl group resembles that of the same carbon in the natural catecholamines with the *R* configuration, proved to interact better with β-receptors, even if the stereochemical selectivity between the enantiomeric pairs is not so marked as in catecholamines and in type **A** adrenergic drugs [7]. The similar biopharmacological properties of MOIM (**C**) and MAOM (**B**) derivatives were rationalized on the basis of analogies between their conformational and electronic characteristics; both MAOM and MOIM moieties preferentially exist in fully extended planar conformations,

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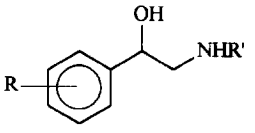
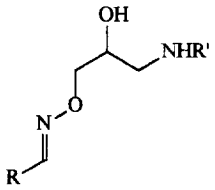
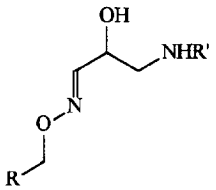
where they do not present any substantial differences in their chemical reactivity [5]. Furthermore, the results obtained with type **C** derivatives do not exclude the possibility that compounds of this type are able to interact with β-receptors even if the MOIMM is arranged in conformations different from the preferential one.



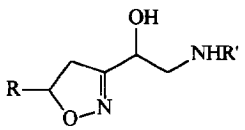
A



B



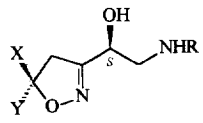
C



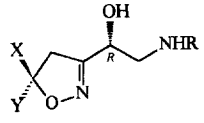
D

Type **D** compounds can be considered as formally obtained from the corresponding type **C** compounds by means of a rotation of 180° around the O–N bond of the MOIMM and the insertion of a methylenic bridge between the two carbon atoms of the moiety. The study of the β-adrenergic properties of compounds of type **D** appeared to offer the chance of verifying whether by constraining the C–O–N=C portion of type **C** compounds into the less stable conformation imposed by the cyclic isoxazolinic structure, it might still be possible to obtain compounds able to interact with β-receptors.

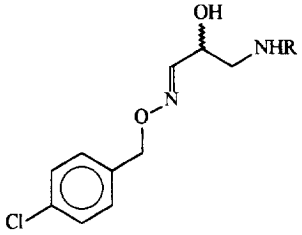
On the basis of these considerations, we synthesized the chiral *N*-alkyl-substituted 2-(3'-(5'-*p*-chlorophenyl)isoxazolidinyl)ethanolamines (2*S*,5'*R*)-**2a,b**, (2*S*,5'*S*)-**3a,b** and their enantiomers (2*R*,5'*S*)-**2a,b**, (2*R*,5'*R*)-**3a,b**, in order to compare their β-adrenergic properties with those of the corresponding optically-active open-chain compounds (*S*)-**1a,b** and (*R*)-**1a,b**.



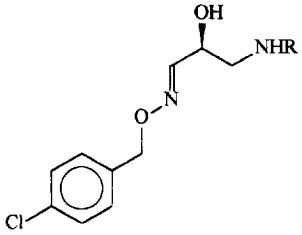
(2*S*,5'*R*)-**2**; X = *p*-Cl-Ph, Y = H
(2*S*,5'*S*)-**3**; X = H, Y = *p*-Cl-Ph



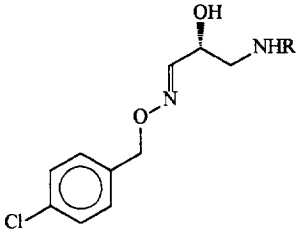
(2*R*,5'*S*)-**2**; X = H, Y = *p*-Cl-Ph
(2*R*,5'*R*)-**3**; X = *p*-Cl-Ph, Y = H



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



(*S*)-**1a,b**



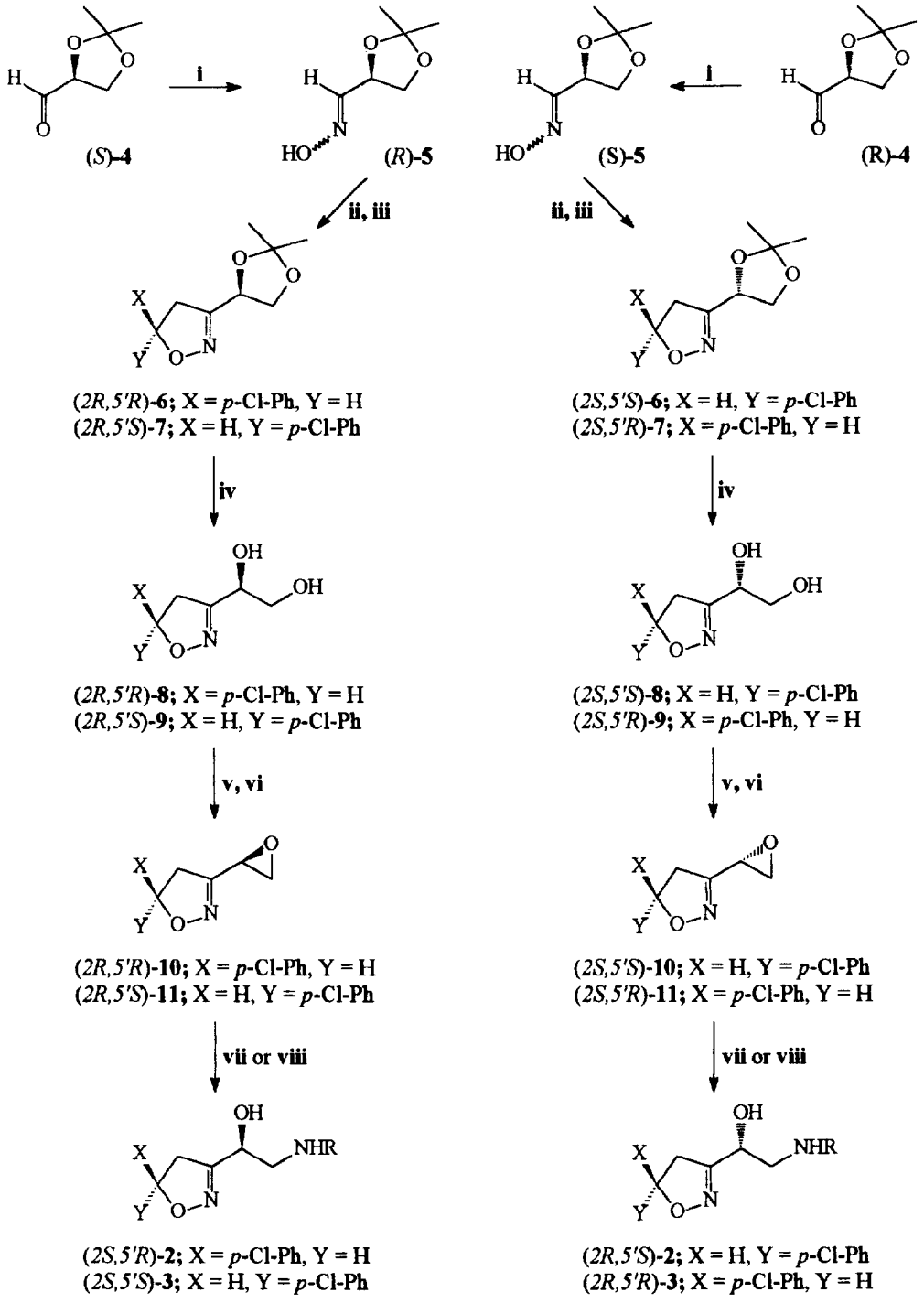
(*R*)-**1a,b**

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

Chemistry

The optically active compounds **2** and **3** were synthesized by the procedure shown in scheme 1.

Enantiomerically pure (*S*)-[(*S*)-**4**] [8] and (*R*)-isopropylideneglyceraldehyde [(*R*)-**4**] [9], obtained by the methods reported in the literature [8, 9], were transformed into the corresponding oximes (*R*)-**5** and (*S*)-**5** [9] respectively, by reaction with H_2NOH·HCl. Treatment of (*R*)-**5** and (*S*)-**5** with *N*-chlorosuccinimide yielded the corresponding chloroximates which, without purification, were transformed into the 1:1 diastereoisomeric mixtures of the corresponding isoxazoline derivatives (2*R*,5'*R*)-**6**, (2*R*,5'*S*)-**7** and (2*S*,5'*S*)-**6**, (2*S*,5'*R*)-**7**, respectively, by base-catalyzed (Et_3N) dipolar cycloaddition to *p*-chlorostyrene. Compounds **6** and **7** were separated by column chromatography and then hydrolyzed to the corresponding isoxazolinic diols (2*R*,5'*R*)-**8**, (2*R*,5'*S*)-**9** and (2*S*,5'*S*)-**8**, (2*S*,5'*R*)-**9** by treatment with HCl in acetone. Reaction of **8** and **9** with TsCl, followed by treatment of the crude product with MeONa, afforded the corresponding epoxides (2*R*,5'*R*)-**10**, (2*R*,5'*S*)-**11** and (2*S*,5'*S*)-**10**, (2*S*,5'*R*)-**11**. Aminolysis of **10** and **11** with



Scheme 1. Reaction conditions: (i) $H_2NOH{\cdot}HCl$, Et_3N, absolute MeOH, 4 h at 10 °C; (ii) NCS, pyridine, anhydrous $CHCl_3$; (iii) *p*-chlorostyrene, Et_3N, anhydrous $CHCl_3$, 16 h at rt; (iv) 1N HCl, acetone, 30 min at 100 °C; (v) TsCl, pyridine, 16 h at 4 °C; (vi) Na, absolute MeOH, anhydrous Et_2O, 1 h at 0 °C; (vii) *i*-$PrNH_2$, EtOH/benzene 2:1, 72 h at rt; (viii) *t*-$BuNH_2$, EtOH/benzene 2:1, 72 h at rt.

i-$PrNH_2$ or *t*-$BuNH_2$ yielded the corresponding optically pure aminoalcohols (2*S*,5'*R*)-**2a,b**, (2*S*,5'*S*)-**3a,b** and (2*R*,5'*S*)-**2a,b**, (2*R*,5'*R*)-**3a,b** respectively.

The structure and configuration of the intermediate compounds (**5–11**) and the final products (**2, 3**) were assigned on the basis of a comparison of the ^{1}H-NMR spectral data of all compounds, crystallographic analysis of one of the isoxazolidinyl derivatives [(2*R*,5'*S*)-**7**] and knowledge of the configuration of the starting optically pure isopropylideneglyceraldehydes (*S*)-**4** and (*R*)-**4**.

The X-ray structure of (2*R*,5'*S*)-**7** was unequivocally established bearing in mind that this product was obtained starting from the (*S*)-glyceraldehyde derivative (*S*)-**4**, and that in the synthetic sequence which leads from (*S*)-**4** to (*R*)-**5** and then to (2*R*,5'*S*)-**7**, the dioxolanic chiral center of (*S*)-**4** and of (*R*)-**5** is not involved. An ORTEP [10] plot of the X-ray structure of (2*R*,5'*S*)-**7**, showing the crystallographic atom-numbering scheme, is given in figure 1; atomic coordinates are shown in table I. Compound (2*R*,5'*S*)-**7** presents the aromatic ring linked to the chiral C(3) carbon (ie, the carbon in the 5' position of the isoxazolinic system). This carbon possesses the *S* configuration, while the other chiral carbon C(4) shows the *R* configuration. The atoms of the isoxazolinic system are practically coplanar and the aromatic ring is strongly rotated (~80°) with respect to the isoxazolinic plane.

Once the stereochemistry of (2*R*,5'*S*)-**7** is established, it is possible to assign the proper stereochemistry also to its diastereoisomer (2*R*,5'*R*)-**6**. This compound, which derives from the same intermediate that yields (2*R*,5'*S*)-**7**, should possess the same configuration as (2*R*,5'*S*)-**7** on the dioxolanic chiral carbon and should therefore differ from (2*R*,5'*S*)-**7** in the configuration (*R*) of the isoxazolinic chiral carbon. The enantiomeric relationship between the dioxolanic compounds (2*R*,5'*R*)-**6** and (2*S*,5'*S*)-**6** and between (2*R*,5'*S*)-**7** and (2*S*,5'*R*)-**7** is confirmed by a comparison of their ^{1}H-NMR spectral characteristics, which appear to be practically identical for (2*R*,5'*R*)-**6** and (2*S*,5'*S*)-**6** and for (2*R*,5'*S*)-**7** and (2*S*,5'*R*)-**7**.

The determination of the complete stereochemistry of the four isoxazolinic intermediates **6, 7** makes it possible to attribute the configurations of the two chiral centers of all the intermediates **8–11** deriving from **6, 7** and also of the final aminoalcohols **2a,b**, **3a,b**; in the synthetic sequences leading from **6, 7** to the corresponding aminoalcohols **2, 3**, through the diols **8, 9** and then the epoxides **10, 11**, neither of the chiral centers of **6–11** is involved. The enantiomeric relationships between the pairs of diols (2*R*,5'*R*)-**8** and (2*S*,5'*S*)-**8** and (2*R*,5'*S*)-**9** and (2*S*,5'*R*)-**9**, epoxides (2*R*,5'*R*)-**10** and (2*S*,5'*S*)-**10** and (2*R*,5'*S*)-**11** and (2*S*,5'*R*)-**11** and aminoalcohols (2*S*,5'*R*)-**2** and (2*R*,5'*S*)-**2** and (2*S*,5'*S*)-**3** and (2*R*,5'*R*)-**3**, both *N*-isopropyl- and *N*-*t*-butyl-substituted, are confirmed by the identical ^{1}H-NMR spectral data of the couples of enantiomers. Further support for the attribution of the relative configurations to compounds **6–11** and **2, 3** comes from an examination of their specific rotation values which, among the couples of enantiomeric forms, are very similar in their absolute values, but with opposite signs (see *Experimental protocols*).

The optical purity of the aminoalcohols (2*S*,5'*R*)-**2a,b** and (2*S*,5'*S*)-**3a,b**, and their corresponding enantiomers (2*S*,5'*S*)-**2a,b** and (2*R*,5'*R*)-**3a,b**, was checked by chiral HPLC analysis. None of the isoxazolinic compounds **2, 3** revealed the presence of any detectable enantiomeric or diastereoisomeric impurity.

Results

Radioligand binding assays

The β-adrenergic affinities of isoxazolines **2a,b** and **3a,b**, and the reference drug dichloroisoproterenol (table II) were determined by binding tests carried out on rat brain and bovine lung membrane preparations for β_1- and β_2-adrenoceptors respectively. ^{3}H-CGP 26505 [11] was used as a specific tritiated ligand for β_1-adrenoceptors, whereas ^{3}H-DHA [12] was utilized to label β_2-adrenoceptors, in the presence of 50 nM CGP 26505, which displaced ^{3}H-DHA from the β_1-adrenoceptor subpopulation (which in the bovine lung represents 17% of the total population [13]). Table II also shows the results previously obtained by us in the same types of tests with the chiral open-chain oxime ethers (*S*)-**1a,b** and (*R*)-**1a,b** [7].

Rat brain β_1-adrenoceptors

All the isoxazoline derivatives **2, 3** showed an affinity for this type of receptor lower than that of the corresponding conformationally free analogs **1**. The affinity of the isoxazolinic compounds with the *S* configuration on C(2) was found to be 30 to 75 times lower than that of the corresponding oxime ethers (*S*)-**1a,b**; the drop in affinity of the isoxazolinic compounds with the *R* configuration on C(2) was, on the contrary, less marked: from twice as low, in the case of (2*R*, 5'*R*)-**3b**, to ten times as low, in the case of (2*R*,5'*S*)-**2b**. Among the compounds with the *R* configuration on C(2), those that displayed a less marked drop in affinity compared with the corresponding analogs **1** proved to be those with the *R* configuration on C(5'); among the compounds with the S configuration on C(2), the drop in affinity was less pronounced for the compound with the *S* or *R* configuration on the C(5') carbon, depending on whether the substituent on the amino nitrogen was the *i*-Pr or the *t*-Bu group respectively.

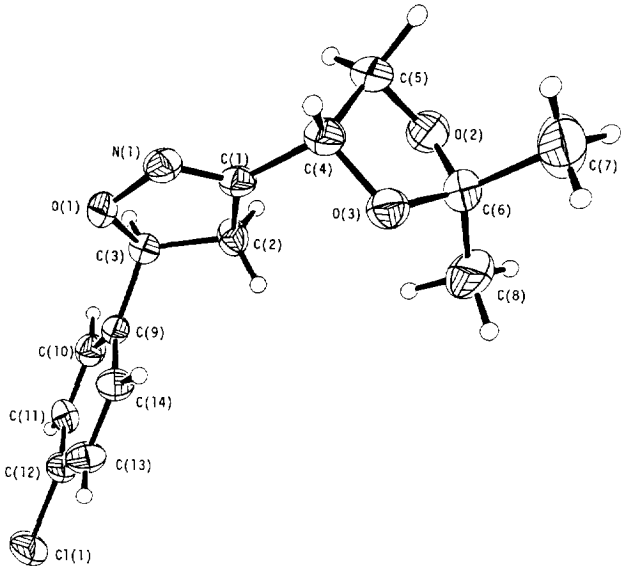


Fig 1. ORTEP 2 plot (50% probability level for thermal ellipsoids) of the X-ray structure of (2*R*,5'*S*)-**7** showing the crystallographic atom numbering scheme.

Bovine lung β_2-adrenoceptors

On this type of β-adrenoceptor, the isoxazolinic compounds **2**, **3** exhibited an affinity markedly lower than that of the corresponding oxime ethers **1**. The affinity of the isoxazolinic compounds with the *S* configuration on the C(2) carbon was more than two orders of magnitude lower than that of the corresponding conformationally-free compounds (*S*)-**1a,b**; the isoxazolinic compounds with the *R* configuration on C(2) showed a less marked decrease in affinity, which varied from about 20 times lower, in the case of (2*R*,5'*S*)-**3a**, to about 90 times lower, in the case of (2*R*,5'*S*)-**2b**. Among the compounds with the *R* configuration on C(2), those with the *R* configuration on C(5') displayed a more limited loss of affinity compared with the corresponding oxime ethers (*R*)-**1a,b**; for the compounds with the *S* configuration on C(2), on the other hand, the decrease in affinity was less marked for the *N*-isopropyl-substituted isoxazoline with the *S* configuration on C(5') and for the *N-tert*-butyl-substituted one with the *R* configuration on the C(5') carbon.

Functional tests

The isoxazoline derivatives that showed an affinity index lower than 10 μM in the binding tests on rat brain β_1-adrenoceptors [(2*S*,5'*R*)-**2b**, (2*S*,5'*S*)-**3b** and (2*R*,5'*R*)-**3b**] together with the reference drug dichloroisoproterenol, were submitted to functional tests on guinea-pig atria and guinea-pig tracheal strips for their β_1- and β_2-adrenergic activities respectively. The results obtained are shown in table II, together with those previously obtained in the same types of tests with the optically active oxime ethers (*S*)-**1a,b** and (*R*)-**1a,b** [7].

Guinea-pig atria β_1-adrenoceptors

On this type of receptor, only compound (2*S*,5'*S*)-**3b** exhibited an appreciable activity index, albeit about one order of magnitude lower than that of the corresponding oxime ether (*S*)-**1b**. Compounds (2*S*,5'*R*)-**2b** and (2*R*,5'*R*)-**3b** were practically inactive. No stimulating properties were detected for the isoxazolinic compounds tested.

Guinea-pig tracheal strip β_2-adrenoceptors

On β_2-adrenoceptors, the isoxazolinic compounds tested displayed a modest antagonistic activity towards isoprenaline-induced responses, with activity indices about three orders of magnitude lower than those of the corresponding oxime ethers, (*S*)-**1b** and (*R*)-**1b**. The isoxazolinic compounds tested proved to be devoid of any stimulating activity on β_2-adrenoceptors.

Theoretical calculations

The conformational and electronic characteristics of compounds **2**, **3** were studied by means of theoretical calculations performed on model compounds **13**, **14**, which are the *N*-unsubstituted analogs of **2**, **3** respectively. In previous papers, it had been found that this simplification did not significantly alter either the conformational or the electronic properties of the rest of the molecule [3, 5, 14]. Only compounds in which the geometry of the carbon linked to the hydroxyl group resembles that of the natural catecholamines were taken into consideration. Conformational analysis of model compound **12**, corresponding to oxime ethers **1**, had indicated that the C(α)–C–O–N=C portion is planar and the phenyl ring is perpendicular to this plane [6]. The conformational energy of model compounds **13** and **14** was determined as a function of the torsion around the bond between the isoxazolinic portion and the ethanolaminic side chain, using the molecular mechanics program Discover [15]. Two minima were found, with energies which differ by less than 0.4 kcal/mol. In one of these conformations the ethanolaminic side chain and the C=N–O portion of the isoxazoline ring of both **13** and **14**, are practically identical to the corresponding molecular portion of **12** (see fig 2).

The molecular electrostatic potential (MEP) of **12**–**14** was calculated at the ab initio STO-3G level,

Table I. Atomic coordinates x 10^4.

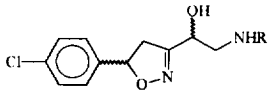
Atom	*X/A*	*Y/B*	*Z/C*
Cl(1)	7590 (1)	1265 (0)	4186 (1)
O(1)	5797 (1)	4499 (4)	8752 (1)
O(2)	1158 (1)	1803 (4)	9032 (1)
O(3)	1589 (1)	5012 (3)	7990 (1)
N(1)	4718 (2)	5708 (4)	9019 (1)
C(1)	3748 (2)	4350 (4)	8739 (1)
C(2)	3999 (2)	1968 (4)	8203 (2)
C(3)	5437 (2)	2038 (4)	8290 (1)
C(4)	2488 (2)	5169 (4)	8962 (1)
C(5)	1944 (2)	3425 (5)	9734 (2)
C(6)	632 (2)	3235 (5)	8127 (2)
C(7)	–556 (3)	4566 (9)	8314 (3)
C(8)	439 (3)	1523 (8)	7154 (3)
C(9)	5944 (1)	1816 (4)	7251 (1)
C(10)	6689 (2)	–208 (4)	7077 (2)
C(11)	7192 (2)	–386 (5)	6127 (2)
C(12)	6943 (2)	1458 (5)	5363 (2)
C(13)	6187 (2)	3451 (5)	5505 (2)
C(14)	5684 (2)	3624 (4)	6455 (1)

considering compounds **12–14** in their low energy conformations shown in figure 2. Figure 3 shows the solid contour corresponding to a MEP value of –10 kcal/mol of **12–14**. The analysis of the MEP trend of **12–14** reveals that the positions of the minima generated by the oximethereal oxygen lone pairs are different in the two types of compounds; no other significant difference was found in the reactivity of the ON=C group, whether introduced into the isoxazoline ring or not, or in the rest of the molecules.

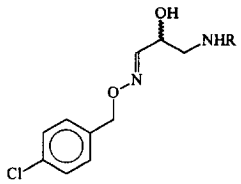
Discussion and conclusions

A comparison of the K_i values of the isoxazolinic compounds **2, 3** with those of the corresponding open-chain oxime ethers with the same chirality on C(2), shows that in all cases the β-adrenergic affinity of the cyclic derivatives **2, 3** is markedly lower than that of the corresponding conformationally-free compounds. The reduction in affinity for both types of β-receptors is more marked for the isoxazolinic compounds with the *S* configuration on C(2) than for the isoxazolinic

Table II. Radioligand binding affinity for β-adrenoceptors and β-adrenergic activity of chiral compounds **2, 3** and **1**.



2, 3



1

Compound[a]	*R*	*β-Adrenergic binding affinity*[b] K_i *(μM)*		*β-Adrenoceptor 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)*
(2*S*,5'*R*)-**2a**	*i*-Pr	52.4 (47.2–57.6)	53.9 (48.7–59.1)	NT	NT
(2*S*,5'*S*)-**3a**	*i*-Pr	32.1 (28.2–36.0)	38.3 (34.4–42.2)	NT	NT
(*S*)-**1a**	*i*-Pr	1.1 (0.95–1.25)[e]	0.263 (0.249–0277)[e]	4.81 ± 0.16[e]	6.10 ± 0.22[e]
(2*R*,5'*S*)-**2a**	*i*-Pr	32.2 (28.3–36.1)	44.3 (39.4–49.3)	NT	NT
(2*R*,5'*R*)-**3a**	*i*-Pr	20.3 (18.4–22.3)	34.9 (31.1–38.7)	NT	NT
(*R*)-**1a**	*i*-Pr	3.66 (3.41–3.91)[e]	1.84 (1.64–2.04)[e]	4.23 ± 0.08[e]	5.97 ± 0.24[e]
(2*S*,5'*R*)-**2b**	t-Bu	4.0 (3.6–4.4)	42.0 (37.4–46.5)	< 3.5	4.37
(2*S*,5'*S*)-**3b**	*t*-Bu	9.0 (8.1–9.9)	47.0 (41.8–52.2)	4.61	4.04
(*S*)-**1b**	*t*-Bu	0.119 (0.109–0.129)[e]	0.075 (0.070–0.080)[e]	5.42 ± 0.01[e]	7.27 ± 0.09[e]
(2*R*,5'*S*)-**2b**	*t*-Bu	13.7 (12.4–15.0)	31.0 (27.7–34.2)	NT	NT
(2*R*,5'*R*)-**3b**	*t*-Bu	5.2 (4.5–5.8)	17.0 (15.4–18.6)	< 3.5	3.86
(*R*)-**1b**	*t*-Bu	2.62 (2.42–2.82)[e]	0.35 (0.32–0.38)[e]	4.54 ± 0.07[e]	6.30 ± 0.15[e]
Dichloroisoproterenol		0.058 (0.050–0.066)	0.144 (0.124–0.168)	6.80 ± 0.21	6.15 ± 0.37

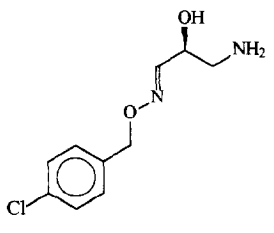
[a] Chiral isoxazoline derivatives **2, 3** were tested as maleates whereas dichloroisoproterenol was used as hydrochloride; [b] geometric means of five separate determinations with confidence limits in parentheses; [c] the values represent the mean of three to five experiments for each drug ± standard error; [d] pIC_{50} is the negative logarithm of the molar concentration that reduces the response to isoproterenol by 50%; NT = not tested; [e] from reference [6].

isomers with the *R* configuration on the same carbon. As a consequence, while among the oxime ethers **1** those with the *S* configuration (*S*)-**1** appear to interact better with the receptors than their enantiomeric form (*R*)-**1** [7], in the case of the isoxazolinic compounds **2**, **3**, the affinity appears to be independent of the configuration of the C(2) carbon. However, the chirality on the C(5') carbon of the cyclic compounds **2**, **3** would appear not to have any decisive effect on their affinity, even if, with the exception of compound (2*S*,5'*R*)-**2a**, among the diastereomeric pairs with the same configuration on C(2), the compounds with the *R* configuration on C(5') appear to be able to interact slightly better with β-receptors than the corresponding diastereoisomer with the *S* configuration on the same carbon atom.

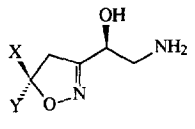
As regards the activity, the pIC_{50} values shown by the isoxazolinic compounds submitted to functional tests (ie, both the enantiomers (2*S*,5'*S*)-**3b** and (2*R*,5'*R*)-**3b**, and (2*S*,5'*R*)-**2b**) indicate that these compounds are very poor antagonists on β_2-adrenoceptors; on β_1-adrenoceptors, only (2*S*,5'*S*)-**3b** possesses a certain β-blocking activity.

The results of the present study demonstrate that by constraining the MOIMM of type **C** β-blocking drugs such as (*S*)-**1** and (*R*)-**1** into a conformation different from the preferred one, like the one imposed by the cyclic isoxazolinic structure, compounds are obtained which, in comparison with the corresponding open-chain compounds **1**, present a decrease in β-adrenergic affinity. The extent of this decrease appears to be more marked for the cyclic compounds for which the geometry of the C(2) chiral carbon corresponds to that of the more active MOIM derivatives with the *S* configuration (*S*)-**1a**,**b** and to that of the natural catecholamines.

A comparison of the conformational and electronic properties of compounds **2**, **3** with those of the corresponding oximethereal derivatives **1**, determined by theoretical calculations, may suggest some hypotheses for the decrease in the β-adrenergic properties observed on passing from the open-chain compounds **1** to the conformationally-restrained isoxazoline derivatives **2**, **3**. Figure 2, showing the overlap of the molecular structure of the model compounds **12–14**,



(*S*)-**12**



(2*S*,5'*R*)-**13**; X = *p*-Cl-Ph, Y = H
(2*S*,5'*S*)-**14**; X = H, Y = *p*-Cl-Ph

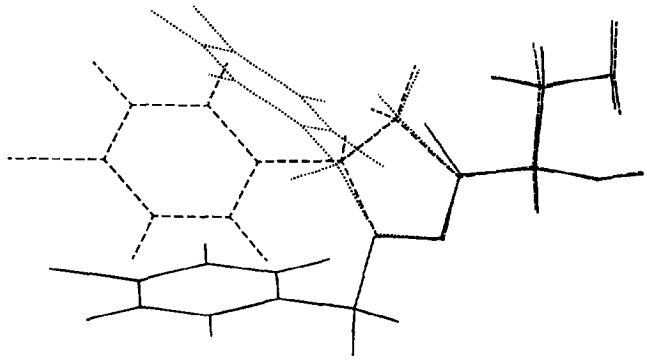


Fig 2. Overlap of molecular structures of the oxime ether **12** (solid line) and isoxazoline analogs **13** (dashed line) and **14** (dotted line), in their low energy conformations which permit the superimposition of the ethanolaminic side-chain and of the C=N–O portion.

indicates a good correspondence between the side-chain, as well as the O–N=C portion of the MOIMM of isoxazolinic and open-chain compounds. The aromatic portions, on the contrary, occupy spatial regions which are quite different with respect to the remaining superimposed molecular portions, and are oriented in a markedly different way with respect to the plane including the isoxazoline ring of **13**, **14** and the MOIMM of **12**. The MEP analysis also indicates a difference in the reactivity pattern of the two kinds of molecules due to the different spatial position of the negative minimum generated by the oxygen of the MOIMM.

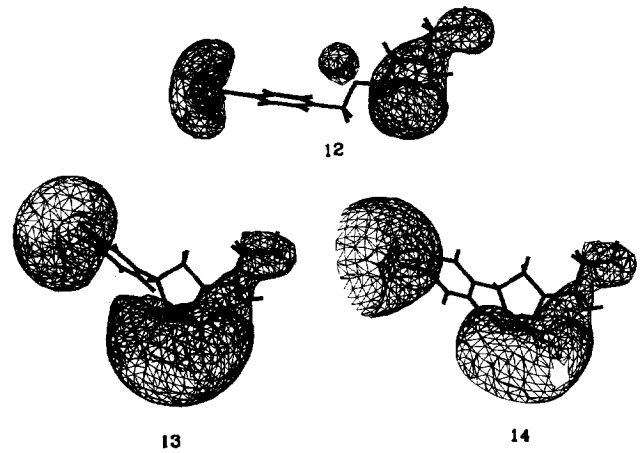


Fig 3. Compounds **12–14** and their isopotential surfaces corresponding to an MEP value of –10 kcal/mol.

On the basis of the above considerations, it may be hypothesized that the differences in the capacities of the type **C** compounds (*S*)-**1** and (*R*)-**1** and type **D** compounds **2**, **3** to interact with β-adrenoceptors may be due to the differences in their MEP trends. An alternative hypothesis might involve differences in the spatial position of the aryl group, which may exert a different steric effect depending on whether it is inserted into a relatively flexible structure, like that of the type **C** compounds (*S*)-**1** and (*R*)-**1**, or a relatively rigid one, like that of the cyclic analogs of type **D** **2**, **3**. The hypothesis of a negative steric effect played by the aryl of **2**, **3** in the interaction with β-adrenoceptors might also explain the absence of a dependence of the β-adrenergic properties of the optically active compounds **2**, **3** on the chirality of the C(2) carbon. For **2**, **3**, the aryl group might at least partly hinder the approach of the molecule to the β-adrenoceptor, thus making it of negligible importance, for the purposes of the biopharmacological properties, whether the geometry of the C(2) carbon of **2**, **3** resembles or not that of the corresponding atom of the oxime ethers **1** with the *S* configuration and the natural catecholamines.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Boiling points refer to the air bath temperature of bulb-to-bulb distillation, carried out using a Büchi GKR-51 apparatus. 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 all compounds were routinely recorded with a Varian CFT-20 instrument operating at 80 MHz in a ca 2% solution of $CDCl_3$ (for the neutral compounds and free bases) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ respectively as the internal standard. ^{1}H NMR spectra of the final products and of compounds **6**, **7** were also measured with a Bruker AC-200 instrument. Preparative MPLCs were carried out through glass columns containing 230-400 mesh silica gel, using a chromatographic apparatus consisting of a Büchi 681 pump, a Knauer differential refractometer detector, and a Philips PM 8220 pen recorder. High-performance liquid chromatography analysis was performed using a chromatographic system consisting of a Water Associates liquid chromatograph, equipped with a U6 K injector, a 6000 A solvent delivery system and a UV detector Model 480 set at 265 nm. Analyses were carried out using a Chiracel OD-H column, and eluting with a 90:10 hexane/*i*-PrOH mixture at a flow rate of 1.12 mL/min for the *N*-isopropyl-substituted compounds **2a**, **3a**, and with a 70:30 hexane/*i*-PrOH mixture at a flow of 1.4 mL/min for the *N*-*t*-butyl-substituted compounds **2b**, **3b**. 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%.

General procedure for the preparation of (R)- and (S)-2,3-O-isopropylideneglyceraldehydeoxime, (R)-5 and (S)-5

A solution of hydroxylamine hydrochloride (6.6 g, 95 mmol) and Et_3N (13.3 mL, 95 mmol) in absolute MeOH (64 mL) was added, dropwise, under nitrogen, to a stirred and cooled (10 °C) solution of crude (*S*)- or (*R*)-isopropylideneglyceraldehyde [(*S*)-**4** [8] or (*R*)-**4**] [9]] in AcOEt (300 mL) for (*S*)-**4**, or in THF (110 mL) for (*R*)-**4**; these solutions were obtained, as reported in the literature, starting from 5,6-*O*-isopropylidene-L-ascorbic acid (8.24 g, 38.1 mmol) [8] or 1,2,5,6-di-*O*-isopropylidene-D-mannitole (10.0 g, 38.1 mmol) [9] respectively. The resulting solution was stirred for 4 h at 10 °C, filtered and evaporated to dryness. The residue was dissolved in $CHCl_3$ and washed with saturated aqueous $NaHCO_3$ (three times) and H_2O (twice). Evaporation of the organic layer afforded an oily residue consisting almost exclusively of (*R*)-**5** or (*S*)-**5** [9] as 70:30 mixtures (GLC) of *E*/*Z* isomers. (*R*)-**5** (2.86 g): bp 45 °C (0.04 mmHg); ^{1}H-NMR δ 1.44 (s, 6H), 3.68–5.28 (m, 3H), 6.90 (d, 1H, *J* = 4.0 Hz, CH=N), 7.43 (d, 1H, *J* = 6.8 Hz, CH=N), 8.73–9.33 (br, 1H). (*S*)-**5** (9.0 g): bp 70 °C (0.07 mmHg) [9]; ^{1}H-NMR δ 1.45 (s, 6H), 3.66–5.33 (m, 3H), 6.95 (d, 1H, *J* = 4.0 Hz, CH=N), 7.45 (d, 1H, *J* = 6.8 Hz, CH=N), 8.43–9.23 (br, 1H).

General procedure for the preparation of the dioxolanes (2R,5'R)-6, (2R,5'S)-7, (2S,5'S)-6 and (2S,5'R)-7

The appropriate oxime (*R*)-**5** or (*S*)-**5** (5.0 g, 34.4 mmol) was added, at room temperature in a single portion, to a stirred mixture of *N*-chlorosuccinimide (4.6 g, 34.4 mmol) in anhydrous $CHCl_3$ (31 mL) and pyridine (0.17 mL). After 15 min, the resulting solution was cooled (0 °C) and then treated with *p*-chlorostyrene (5.4 mL, 44.7 mmol) and, dropwise, with a solution of Et_3N (7.2 mL, 51.6 mmol) in anhydrous $CHCl_3$ (7.5 mL). After 16 h at room temperature, the reaction mixture was washed with brine, dried and then evaporated to dryness. The crude residue, consisting almost exclusively of a 1:1 mixture (GLC) of (2*R*,5'*R*)-**6** and (2*R*,5'*S*)-**7** or (2*S*,5'*S*)-**6** and (2*S*,5'*R*)-**7**, respectively, was submitted to MPLC on silica gel, eluting with a 4:2:1 hexane/$CHCl_3$/AcOEt mixture and collecting 25 mL fractions. The first fractions yielded the (2*R*,5'*R*)-**6** and (2*S*,5'*S*)-**6** compounds respectively, whereas the subsequent fractions yielded the (2*R*,5'*S*)-**7** and (2*S*,5'*R*)-**7** compounds respectively. (2*R*,5'*R*)-**6** (1.96 g, 20%): $[\alpha]_D$ = −177° (c = 0.75, $CHCl_3$); ^{1}H-NMR δ 1.39 (s, 3H), 1.41 (s, 3H), 2.94 (dd, 1H, *J* = 17.8 and 7.9 Hz), 3.52 (dd, 1H, *J* = 17.8 and 11.1 Hz), 3.95 (dd, 1H, *J* = 8.7 and 5.8 Hz), 4.23 (dd, 1H, *J* = 8.7 and 6.8 Hz), 4.98 (dd, 1H, *J* = 6.8 and 5.8 Hz), 5.61 (dd, 1H, *J* = 11.1 and 7.9 Hz), 7.24 (d, 2H, *J* = 8.7 Hz), 7.34 (d, 2H, *J* = 8.7 Hz). Anal for $C_{14}H_{16}NO_3Cl$ (C, H, N). (2*R*,5'*S*)-**7** (1.16 g, 12%): mp 76–78 °C; $[\alpha]_D$ = +220° (c = 0.80, $CHCl_3$); ^{1}H-NMR δ 1.40 (s, 3H), 1.43 (s, 3H), 3.02 (dd, 1H, *J* = 17.4 and 8.4 Hz), 3.45 (dd, 1H, *J* = 17.4 and 10.9 Hz), 4.07 (dd, 1H, *J* = 8.7 and 5.9 Hz), 4.26 (dd, 1H, *J* = 8.7 and 6.8 Hz), 4.97 (dd, 1H, *J* = 6.8 and 5.9 Hz), 5.58 (dd, 1H, *J* = 10.9 and 8.4 Hz), 7.27 (d, 2H, *J* = 8.7 Hz), 7.35 (d, 2H, *J* = 8.7 Hz). Anal for $C_{14}H_{16}NO_3Cl$ (C, H, N). (2*S*,5'*S*)-**6** (2.02 g, 21%): $[\alpha]_D$ = +175° (c = 0.85, $CHCl_3$); ^{1}H-NMR δ 1.37 (s, 3H), 1.43 (s, 3H), 2.95 (dd, 1H, *J* = 17.8 and 7.9 Hz), 3.50 (dd, 1H, *J* = 17.8 and 11.1 Hz), 3.93 (dd, 1H, *J* = 8.7 and 5.8 Hz), 4.21 (dd, 1H, *J* = 8.7 and 6.8 Hz), 5.00 (dd, 1H, *J* = 6.8 and 5.8 Hz), 5.62 (dd, 1H, *J* = 11.1 and 7.9 Hz), 7.22 (d, 2H, *J* = 8.7 Hz), 7.33 (d, 2H, *J* = 8.7 Hz). Anal for $C_{14}H_{16}NO_3Cl$ (C, H, N). (2*S*,5'*R*)-**7** (1.54 g, 16%): mp 78–79 °C; $[\alpha]_D$ = −218° (c = 0.80, $CHCl_3$); ^{1}H-NMR δ 1.41 (s, 3H), 1.45 (s, 3H), 3.03 (dd, 1H, *J* = 17.4 and 8.4 Hz), 3.46 (dd, 1H, *J* = 17.4 and 10.9 Hz), 4.04 (dd, 1H,

J = 8.7 and 5.9 Hz), 4.28 (dd, 1H, J = 8.7 and 6.8 Hz), 4.96 (dd, 1H, J = 6.8 and 5.9 Hz), 5.57 (dd, 1H, J = 10.9 and 8.4 Hz), 7.25 (d, 2H, J = 8.7 Hz), 7.34 (d, 2H, J = 8.7 Hz). Anal for $C_{14}H_{16}NO_3Cl$ (C, H, N).

*General procedure for the preparation of the diols (2R,5'R)-**8**, (2R,5'S)-**9**, (2S,5'S)-**8** and (2S,5'R)-**9***

A stirred solution of the appropriate dioxolane [(2*R*,5'*R*)-**6**, (2*R*,5'*S*)-**7**, (2*S*,5'*S*)-**6** or (2*S*,5'*R*)-**7**] (1.0 g, 3.55 mmol) in acetone (1 mL) and 1N HCl (2.1 mL), was heated at 100 °C for 30 min. The organic solvent was evaporated and the resulting aqueous mixture was extracted with CH_2Cl_2. Evaporation of the organic layers yielded a solid residue which was crystallized from AcOEt/hexane. (2*R*,5'*R*)-**8** (0.63 g, 73%): mp 110–112 °C; $[\alpha]_D$ = –234° (c = 0.96, MeOH); ^{1}H-NMR δ 1.48–2.71 (br, 2H), 2.77–3.92 (m, 4H), 4.53 (dd, 1H, J = 4.8 and 4.0 Hz), 5.51 (dd, 1H, J = 10.4 and 8.8 Hz), 7.22 (brs, 4H). Anal for $C_{11}H_{12}NO_3Cl$ (C, H, N). (2*R*,5'*S*)-**9** (0.76 g, 88.4%): mp 92–94 °C; $[\alpha]_D$ = +236° (c = 0.99, MeOH); ^{1}H-NMR δ 1.42–2.68 (br, 2H), 2.74–3.94 (m, 4H), 4.52 (dd, 1H, J = 4.8 and 4.0 Hz), 5.53 (dd, 1H, J = 10.4 and 8.8 Hz), 7.26 (brs, 4H). Anal for $C_{11}H_{12}NO_3Cl$ (C, H, N). (2*S*,5'*S*)-**8** (0.53 g, 62%): mp 108–110 °C; $[\alpha]_D$ = +230° (c = 1.05, MeOH); ^{1}H-NMR δ 1.43–2.70 (br, 2H), 2.75–4.00 (m, 4H), 4.53 (dd, 1H, J = 4.8 and 4.0 Hz), 5.51 (dd, 1H, J = 10.4 and 8.8 Hz), 7.22 (brs, 4H). Anal for $C_{11}H_{12}NO_3Cl$ (C, H, N). (2*S*,5'*R*)-**9** (0.54 g, 63%): mp 92–94 °C; $[\alpha]_D$ = –235° (c = 1.08, MeOH); ^{1}H-NMR δ 1.44–2.70 (br, 2H), 2.73–3.95 (m, 4H), 4.51 (dd, 1H, J = 4.8 and 4.0 Hz), 5.54 (dd, 1H, J = 10.4 and 8.8 Hz), 7.26 (brs, 4H). Anal for $C_{11}H_{12}NO_3Cl$ (C, H, N).

*General procedure for the preparation of the epoxides (2R,5'R)-**10**, (2R,5'S)-**11**, (2S,5'S)-**10** and (2S,5'R)-**11***

A stirred and cooled (0 °C) solution of the appropriate diol [(2*R*,5'*R*)-**8**, (2*R*,5'*S*)-**9**, (2*S*,5'*S*)-**8** or (2*S*,5'*R*)-**9**] (0.9 g, 3.72 mmol) in pyridine (2 mL) was treated, portionwise, with *p*-toluenesulphonylchloride (0.71 g, 3.72 mmol). After 1 h of stirring at 0 °C, the mixture was kept at 4 °C for 16 h and then dissolved in Et_2O and washed with 1N aqueous HCl (three times), saturated aqueous $NaHCO_3$ and H_2O (twice). Evaporation of the organic layer afforded an oily residue which was directly dissolved in absolute MeOH (1 mL) and anhydrous Et_2O (0.5 mL). The resulting solution, stirred and cooled at 0 °C, was treated, portionwise over about 1 h, with Na (0.077 g, 3.35 mmol). The mixture was stirred at 0 °C for 1 h and then diluted with Et_2O, filtered and evaporated to yield a semisolid residue, consisting almost exclusively of the appropriate epoxide, which was directly used for the following transformations. (2*R*,5'*R*)-**10** (0.71 g): ^{1}H-NMR δ 2.42–3.56 (m, 4H), 3.87 (dd, 1H, J = 4.5 and 3.2 Hz), 5.55 (dd, 1H, J = 10.4 and 8.8 Hz), 7.26 (brs, 4H). (2*R*,5'*S*)-**11** (0.67 g): ^{1}H-NMR δ 2.62–3.41 (m, 4H), 3.87 (dd, 1H, J = 4.0 and 2.4 Hz), 5.56 (dd, 1H, J = 10.4 and 8.8 Hz), 7.26 (brs, 4H). (2*S*,5'*S*)-**10** (0.69 g): ^{1}H-NMR δ 2.41–3.55 (m, 4H), 3.86 (dd, 1H, J = 4.5 and 3.2 Hz), 5.56 (dd, 1H, J = 10.4 and 8.8 Hz), 7.26 (brs, 4H). (2*S*,5'*R*)-**11** (0.68 g): ^{1}H-NMR δ 2.61–3.41 (m, 4H), 3.86 (dd, 1H, J = 4.0 and 2.4 Hz), 5.56 (dd, 1H, J = 10.4 and 8.8 Hz), 7.26 (brs, 4H).

*General procedure for the preparation of the isoxazoline ethanolamine maleates (2S,5'R)-**2a,b**•$C_4H_4O_4$, (2S,5'S)-**3a,b**•$C_4H_4O_4$, (2R,5'S)-**2a,b**•$C_4H_4O_4$ and (2R,5'R)-**3a,b**•$C_4H_4O_4$*

A solution of the appropriate epoxide [(2*R*,5'*R*)-**10**, (2*R*,5'*S*)-**11**, (2*S*,5'*S*)-**10** or (2*S*,5'*R*)-**11**] (0.3 g, 1.34 mmol) in an anhydrous 2:1 EtOH/benzene mixture (3.6 mL) was treated with the appropriate amine (i-$PrNH_2$ or t-$BuNH_2$, 8.5 mmol) and stirred for 72 h at room temperature. Evaporation of the organic layer yielded a solid residue which was dissolved in anhydrous Et_2O and added, dropwise, under stirring at 0 °C, to a solution of maleic acid (0.155 g, 1.34 mmol) in acetone (0.5 mL). Addition of anhydrous Et_2O gave a solid precipitate which was crystallized from MeOH/Et_2O to yield the appropriate pure maleate. (2*S*,5'*R*)-**2a**•$C_4H_4O_4$ (0.35 g, 65%): mp 114–116 °C; ^{1}H-NMR δ 1.09 and 1.10 (2d, 6H, J = 6.4 Hz), 2.85–3.55 (m, 5H), 5.47 (dd, 1H, J = 10.6 and 8.7 Hz), 6.03 (s, 2H), 7.08 and 7.18 (2d, 4H, J = 8.5 Hz). Anal for $C_{18}H_{23}N_2O_6Cl$ (C, H, N). (2*S*,5'*S*)-**3a**•$C_4H_4O_4$ (0.32 g, 60%): mp 108–110 °C; ^{1}H-NMR δ 1.09 and 1.10 (2d, 6H, J = 6.6 Hz), 2.81–3.52 (m, 5H), 5.45 (dd, 1H, J = 10.9 and 8.2 Hz), 6.25 (s, 2H), 7.07 and 7.18 (2d, 4H, J = 8.6 Hz). Anal for $C_{18}H_{23}N_2O_6Cl$ (C, H, N). (2*R*,5'*S*)-**2a**•$C_4H_4O_4$ (0.35 g, 65%): mp 112–114 °C; ^{1}H-NMR δ 1.08 and 1.10 (2d, 6H, J = 6.4 Hz), 2.86–3.57 (m, 5H), 5.45 (dd, 1H, J = 10.6 and 8.7 Hz), 6.04 (s, 2H), 7.08 and 7.16 (2d, 4H, J = 8.5 Hz). Anal for $C_{18}H_{23}N_2O_6Cl$ (C, H, N). (2*R*,5'*R*)-**3a**•$C_4H_4O_4$ (0.33 g, 62%): mp 108–110 °C; ^{1}H-NMR δ 1.09 and 1.11 (2d, 6H, J = 6.6 Hz), 2.83–3.53 (m, 5H), 5.44 (dd, 1H, J = 10.9 and 8.2 Hz), 6.26 (s, 2H), 7.09 and 7.19 (2d, 4H, J = 8.6 Hz). Anal for $C_{18}H_{23}N_2O_6Cl$ (C, H, N). (2*S*,5'*R*)-**2b**•$C_4H_4O_4$ (0.48 g, 87%): mp 135–137 °C; ^{1}H-NMR δ 1.20 (s, 9H), 2.92–3.49 (m, 4H), 5.52 (dd, 1H, J = 10.9 and 8.2 Hz), 6.08 (s, 2H), 7.14 and 7.24 (2d, 4H, J = 8.7 Hz). Anal for $C_{19}H_{25}N_2O_6Cl$ (C, H, N). (2*S*,5'*S*)-**3b**•$C_4H_4O_4$ (0.47 g, 85%): mp 159–161 °C; ^{1}H-NMR δ 1.20 (s, 9H), 2.89–3.53 (m, 4H), 5.53 (dd, 1H, J = 11.0 and 8.2 Hz), 6.08 (s, 2H), 7.15 and 7.25 (2d, 4H, J = 8.7 Hz). Anal for $C_{19}H_{25}N_2O_6Cl$ (C, H, N). (2*R*,5'*S*)-**2b**•$C_4H_4O_4$ (0.48 g, 87%): mp 134–136 °C; ^{1}H-NMR δ 1.20 (s, 9H), 2.93–3.50 (m, 4H), 5.51 (dd, 1H, J = 10.9 and 8.2 Hz), 6.08 (s, 2H), 7.15 and 7.25 (2d, 4H, J = 8.7 Hz). Anal for $C_{19}H_{25}N_2O_6Cl$ (C, H, N). (2*R*,5'*R*)-**3b**•$C_4H_4O_4$ (0.48 g, 87%): mp 160–162 °C; ^{1}H-NMR δ 1.20 (s, 9H), 2.89–3.53 (m, 4H), 5.54 (dd, 1H, J = 11.0 and 8.2 Hz), 6.08 (s, 2H), 7.16 and 7.25 (2d, 4H, J = 8.7 Hz). Anal for $C_{19}H_{25}N_2O_6Cl$ (C, H, N).

The maleate salts were converted into the free bases by adding them to a stirred and cooled (0 °C) mixture of 1N aqueous NaOH and $CHCl_3$. The organic layers were filtered and evaporated to give the pure bases. (2*S*,5'*R*)-**2a**: mp 106–108 °C; $[\alpha]_D$ = –179° (c = 0.96, $CHCl_3$); ^{1}H-NMR δ 1.00 (d, 6H, J = 6.3 Hz), 2.28–2.57 (br, 2H), 2.65–2.86 (m, 3H), 2.95 (dd, 1H, J = 17.6 and 8.1 Hz), 3.43 (dd, 1H, J = 17.6 and 10.8 Hz), 4.35–4.45 (m, 1H), 5.49 (dd, 1H, J = 10.8 and 8.1 Hz), 7.19 and 7.27 (2d, 4H, J = 8.6 Hz); HPLC: t_R = 12.30 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*S*,5'*S*)-**3a**: mp 92–94 °C; $[\alpha]_D$ = +200° (c = 0.98, $CHCl_3$); ^{1}H-NMR δ 1.07 (d, 6H, J = 6.4 Hz), 2.20–2.53 (br, 2H), 2.71–3.10 (m, 4H), 3.53 (dd, 1H, J = 17.4 and 11.0 Hz), 4.37–4.50 (m, 1H), 5.55 (dd, 1H, J = 11.0 and 8.1 Hz), 7.27 and 7.34 (2d, 4H, J = 8.6 Hz); HPLC: t_R = 12.00 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*R*,5'*S*)-**2a**: mp 105–108 °C; $[\alpha]_D$ = +181° (c = 1.00, $CHCl_3$); ^{1}H-NMR δ 1.01 (d, 6H, J = 6.3 Hz), 2.01–2.42 (br, 2H), 2.63–2.85 (m, 3H), 2.96 (dd, 1H, J = 17.6 and 8.1 Hz), 3.45 (dd, 1H, J = 17.6 and 10.8 Hz), 4.33–4.43 (m, 1H), 5.50 (dd, 1H, J = 10.8 and 8.1 Hz), 7.20 and 7.28 (2d, 4H, J = 8.6 Hz); HPLC: t_R = 12.92 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*R*,5'*R*)-**3a**: mp 90–93 °C; $[\alpha]_D$ = –203° (c = 0.96, $CHCl_3$); ^{1}H-NMR δ 1.08 (d, 6H, J = 6.4 Hz), 2.12–2.46 (br, 2H), 2.73–3.12 (m, 4H), 3.52 (dd, 1H, J = 17.4 and 11.0 Hz), 4.39–4.52 (m, 1H), 5.56 (dd, 1H, J = 11.0 and 8.1 Hz), 7.26 and 7.34 (2d, 4H, J = 8.6 Hz); HPLC: t_R = 12.47 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*S*,5'*R*)-**2b**: mp 120–123 °C; $[\alpha]_D$ = –162° (c = 1.02, $CHCl_3$); ^{1}H-NMR δ 1.10 (s, 9H), 1.65–2.40 (br, 2H), 2.86–2.98 (m, 2H), 3.02 (dd, 1H, J = 17.6 and

8.3 Hz), 3.49 (dd, 1H, J = 17.6 and 10.7 Hz), 4.40 (dd, 1H, J = 5.8 and 5.0 Hz), 5.56 (dd, 1H, J = 10.7 and 8.3 Hz), 7.27 and 7.35 (2d, 4H, J = 8.5 Hz); HPLC: t_R = 6.25 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*S*,5'*S*)-**3b**: mp 114–116 °C; $[\alpha]_D$ = +146° (c = 1.04, $CHCl_3$); ^{1}H-NMR δ 1.11 (s, 9H), 2.18–2.85 (br, 2H), 2.90–3.22 (m, 3H), 3.54 (dd, 1H, J = 17.5 and 10.9 Hz), 4.35–4.52 (m, 1H), 5.55 (dd, 1H, J = 10.9 and 8.1 Hz), 7.27 and 7.34 (2d, 4H, J = 8.6 Hz); HPLC: t_R = 9.43 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*R*,5'*S*)-**2b**: mp 120–122 °C; $[\alpha]_D$ = +162° (c = 1.07, $CHCl_3$); ^{1}H-NMR δ 1.10 (s, 9H), 1.52–2.30 (br, 2H), 2.85–2.97 (m, 2H), 3.04 (dd, 1H, J = 17.6 and 8.3 Hz), 3.50 (dd, 1H, J = 17.6 and 10.7 Hz), 4.42 (dd, 1H, J = 5.8 and 5.0 Hz), 5.55 (dd, 1H, J = 10.7 and 8.3 Hz), 7.28 and 7.35 (2d, 4H, J = 8.5 Hz); HPLC: t_R = 7.59 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N). (2*R*,5'*R*)-**3b**: mp 115–117 °C; $[\alpha]_D$ = −144° (c = 0.98, $CHCl_3$); ^{1}H-NMR δ 1.12 (s, 9H), 2.13–2.74 (br, 2H), 2.90–3.24 (m, 3H), 3.55 (dd, 1H, J = 17.5 and 10.9 Hz), 4.33–4.53 (m, 1H), 5.57 (dd, 1H, J = 10.9 and 8.1 Hz), 7.26 and 7.33 (2d, 4H, J = 8.6 Hz); HPLC: t_R = 7.94 min. Anal for $C_{14}H_{19}N_2O_2Cl$ (C, H, N).

Crystallography

Crystals of (2*R*,5'*S*)-**7**, mp 75–77 °C, were obtained by slow evaporation of a hexane/*i*-Pr_2O solution: $C_{14}H_{16}ClNO_3$, M = 281.74, monoclinic, space group P_{21}, a = 10.749 (5) Å, b = 5.385 (1) Å, c = 12.610 (5) Å, β = 99.43 (4), V = 720.05 (47) Å^3, Z = 2, D = 1.2995 g·cm^3, λ (Mo-Kα) = 0.71069 Å, $F(000)$ = 296.0.

Cell parameters and the orientation matrix were obtained by least-squares refinement using 24 reflections in the range 12° < θ < 22°. The data collection, at room temperature, was performed on a Philips PW1100 diffractometer using Mo-Kα radiation, θ/2θ scan mode, scan speed 3°–10°·min^{-1}, scan width (1.20 + 0.14 tan θ)° and θ in the range 3°–30°. One standard reflection was measured every 100. The reflections [total 3809; observed 3099 ($I_{obs} > 3\sigma(I)$), after merging 2720] were corrected for Lorentz and polarization; no absorption correction was applied. The final R value was 0.039 (R_w = 0.040).

All the calculations were performed on the Gould computer system of the Centro di Strutturistica Diffrattometrica del CNR, Parma, Italy. The structure was solved using the SIR92 system program [16] on a RISC 6000 system; the refinement (anisotropic for heavy atoms and isotropic for the hydrogens) was performed by the full-matrix least-squares method using the program SHELX76 [17] with the form factors included there. Geometric parameters were calculated with PARST [18]; the drawing was made using the ORTEP 2.0 program [10]. Final atomic parameters and structure factors have been deposited at the Crystallographic Data Centre, Cambridge.

Radioligand binding methods

Rat brain β_1-receptors

β_1-Receptors were assayed in rat cortical membranes as previously described [5], using ^{3}H-CGP 26505 [11] (1-[[2-(3-carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]phenoxy]-2-propanol) as the specific ligand (DuPont de Nemours, New England Nuclear Division; specific activity 28.4 Ci/mmol).

Bovine lung β_2-receptors

β_2-Receptor binding was studied in bovine lung as previously described [5], using ^{3}H-dihydroalprenolol (^{3}H-DHA) [12] 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 (2*S*,5'*R*)-**2b**, (2*S*,5'*S*)-**3b** and (2*R*,5'*R*)-**3b** on β_2-adrenoceptors was evaluated on isolated preparations obtained from adult male Dunkin-Hartley guinea pigs, weighing 300–350 g. The efficacy of the tested compounds on β_1- and β_2-adrenoceptors was tested on preparations of isolated guinea-pig atria and of tracheal smooth musculature respectively, following the methods previously described [5].

For both β_1 and β_2 preparations, the antagonistic activities of the tested compounds towards β_1- and β_2-adrenoceptors were expressed as pIC_{50}, ie, the negative log of the molecular concentration that reduced the response to isoprenaline by 50% [19]. All compounds were tested at concentrations ranging from 10^{-9} M to 10^{-3} M. Each antagonistic activity index was obtained from at least five active concentrations.

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