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Conformationally restrained β-blocking oxime ethers. 2. Synthesis and β-adrenergic properties of diastereoisomeric *anti* and *syn* 2-(5'-(3'-aryl-substituted)isoxazolidinyl)-*N*-alkylethanolamines*

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Summary — The diastereoisomeric 2-(5'-(3'-aryl)isoxazolidinyl)ethanolamines **1c–h–4c–h** were synthesized as analogs of the corresponding β-blocking isoxazolines unsubstituted on the aromatic ring **1a–4a**, with the aim of checking the effects on the adrenergic properties of the insertion of a methoxy group or a chlorine atom in the *ortho*, *meta* or *para* position of the phenyl ring of **1a–4a**. The relative configurations of **1c–h–4c–h** were assigned on the basis of their ^{1}H-NMR spectral characteristics. The new isoxazolines **1c–h–4c–h** were tested for their affinity towards β_1- and β_2-adrenoceptors by radioligand binding experiments; compounds showing the highest affinity were also assayed for their β-adrenergic activity by functional tests on isolated preparations. The results showed that most of the new compounds (**1c–h–4c–h**) possess a slightly better capacity to interact with the β-receptors, compared with the corresponding analogs unsubstituted on the phenyl ring (**1a–4a**), and that the substitution that leads to compounds with the best properties is the one with the chlorine atom. Quantum mechanical calculations carried out in order to look for possible correlations between the β-adrenergic properties and the conformational and electronic characteristics induced by the presence of the substituents on the phenyl ring of compounds of types **1–4** do not suggest any reasonable explanation for the trend of the affinity data.

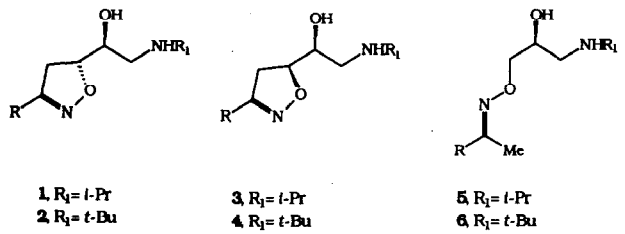
adrenergic drug / β-blocking agent / 2-(5'-(3'-aryl-substituted)isoxazolidinyl)ethanolamine

Introduction

As part of our studies on the stereostructural requirements for the adrenergic β-blocking activity, we synthesized the diastereoisomeric 2-(5'-(3'-phenyl) (**1a–4a**) and 2-(5'-(3'-isopropyl)isoxazolidinyl)ethanolamines (**1b–4b**), which can be viewed as conformationally restricted analogs of the corresponding β-blocking oxime ethers **5a**, **6a** and **5b**, **6b** in which the methyl carbon linked to the imino system is bonded with the carbon adjacent to the ethereal oxygen [1]. Our aim in so doing was to verify whether by constraining the C(1)=NOC(2)C(3) portion of **5a**, **6a** and **5b**, **6b** into the 2 less stable conformations imposed by the cyclic isoxazoline structure, it might be possible to obtain compounds which still possess a β-blocking activity. The new compounds (**1a–4a** and **1b–4b**) were tested *in vitro* both by radioligand binding tests and by functional assays. The phenyl-substituted compounds (**1a–4a**) proved to maintain, in general, appreciable β-adrenergic properties, even if these were somewhat lower than those of the corresponding conformationally free parent compounds **5a** and **6a** [1]; the isopropyl-substituted compounds (**1b–4b**) showed a more marked decrease in the β-adrenergic properties with respect to the corresponding open-chain oxime ethers **5b** and **6b** [1].

One of the possible reasonable explanations for the differences in adrenergic properties found between the 3'-phenyl-(**1a–4a**) and the 3'-isopropyl-substituted (**1b–4b**) isoxazolines might be the presence in **1a–4a** of a suitable distribution of the charge generated by the phenyl system in the area adjacent to the isoxazolinic nucleus, which is capable of playing an indirect role in the interaction with the receptor. If this hypothesis were to prove to be correct, then it would be reasonable to expect modifications of the electronic characteristics of the aromatic system (the phenyl) of **1a–4a** to produce appropriate variations in the adrenergic properties of these types of compounds.

*For preceding paper, see reference [1]. Part of this work was presented at the *X Convegno Naz Div Chim Farm Soc Chim* Italy, in September 1991 [2].



1, R_1 = *t*-Pr
2, R_1 = *t*-Bu

3, R_1 = *t*-Pr
4, R_1 = *t*-Bu

5, R_1 = *t*-Pr
6, R_1 = *t*-Bu

In order to verify this hypothesis and obtain further insights into the activity–structure relationship of the class of isoxazolinic aminoalcohols **1–4**, we planned the synthesis of analogs of **1a–4a** in which the electronic properties of the phenyl group are modulated by means of suitable substitutions in the various positions of the ring.

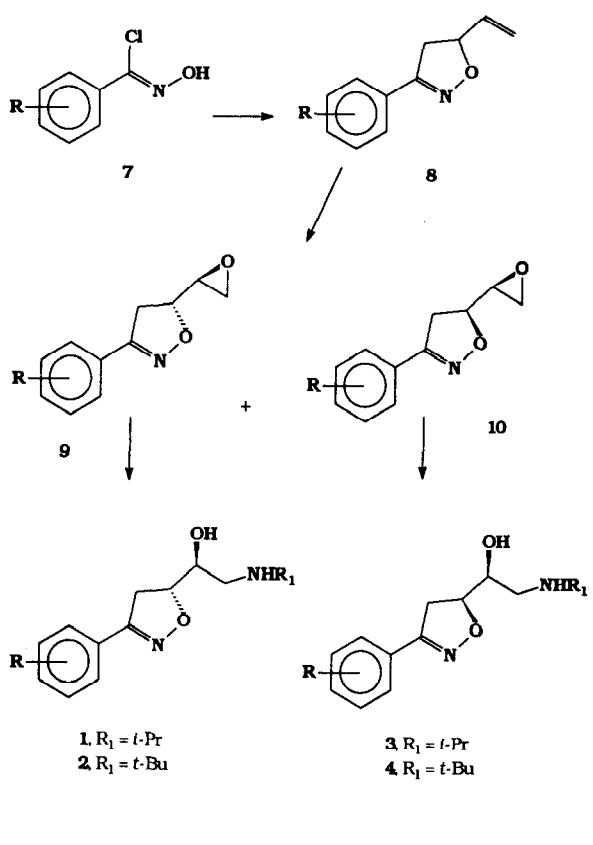
The present paper describes the synthesis and the biopharmacological β-adrenergic properties of a series of isoxazoline derivatives of the type **1–4** (**1c–h–4c–h**)[1] (see table I) in which the phenyl ring is substituted in the *ortho*, *meta*, or *para* position by substituents that can exercise different electronic effects, such as the methoxy group or the chlorine atom.

Chemistry

The *anti* (**1c–h**, **2c–h**) and *syn* (**3c–h**, **4c–h**) 2-(5'-(3'-aryl-substituted)isoxazolidinyl)-*N*-alkylethanolamine derivatives (table I) were prepared using the synthetic procedure previously followed [1] for the synthesis of **1a,b–4a,b** (see scheme 1). Reaction with triethylamine and butadiene of the hydroxamyl chlorides **7c–h**, prepared by treatment of the appropriate arylaldoximes with *N*-chlorosuccinimide, afforded the corresponding 3-aryl-5-vinyl-2-isoxazolines **8c–h**. Oxidation of **8c–h** with *m*-chloroperoxybenzoic acid yielded mixtures of the *anti* (**9c–h**) and *syn* (**10c–h**) epoxides, in a ratio of about 1:1, which were separated by column chromatography. Aminolysis with *i*-$PrNH_2$ or *t*-$BuNH_2$ of **9c–h** and **10c–h** afforded the corresponding *anti* (**1c–h**, **2c–h**) and *syn* (**3c–h**, **4c–h**) aminoalcohols which were purified by crystallization of their oxalate or maleate salts.

The position of the aryl group linked to the isoxazoline system of compounds **8c–h** (and therefore of compounds **9**, **10**, and **1–4**) was assumed on the basis of the knowledge of the regiochemical behavior of the base-catalyzed cycloaddition reactions of hydroxamyl chlorides to unsaturated compounds [3, 4] and, in particular, of the reaction which leads from unsubstituted benzohydroxamyl chloride (**7a**) to 3-phenyl-5-vinyl-2-isoxazoline (**8a**) [1].

The configuration of the *anti* (**1c–h**, **2c–h**) and *syn* (**3c–h**, **4c–h**) isoxazolidine aminoalcohols was assigned on the basis of a comparison of their ^{1}H-NMR spectral characteristics with those of the previously studied phenyl- (**1a–4a**) and isopropyl-substituted (**1b–4b**) compounds [1]. In the *anti* compounds **1c–h** and **2c–h**, both as free bases and as salts, the $J_{5',2}$ values (tables II and III) are higher (5.7–7.6 Hz for the free bases, and 4.8–5.6 Hz for the salts) than those of the *syn* compounds **3c–h** and **4c–h** (3.3–4.1 Hz for the free bases, and 2.6–3.4 Hz for the salts), as has been



1, R_1 = *t*-Pr
2, R_1 = *t*-Bu

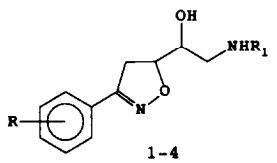
3, R_1 = *t*-Pr
4, R_1 = *t*-Bu

c, R = *o*-MeO ; d, R = *m*-MeO ; e, R = *p*-MeO
f, R = *o*-Cl ; g, R = *m*-Cl ; h, R = *p*-Cl

Scheme 1.

[1]All compounds are racemic mixtures. However, in the schemes and figures the enantiomer in which the relative configuration on C(3) corresponds to that of the natural catecholamines is drawn.

Table I. Chemical data of isoxazoline derivatives **1–4**.

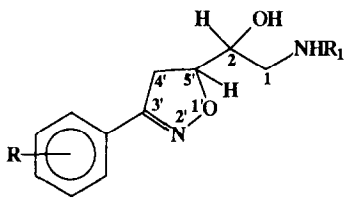


1-4

Compound	*R*	*R1*	*Stereo-isomer*	*Yield (%)*[a]	*Recrystn solvent*[b]	*mp (°C)*	*Formula*[c]
1c·$H_4C_4O_4$	*o*-OMe	*i*-Pr	*anti*	80	A	180-181	$C_{19}H_{26}N_2O_7$
2c·$H_4C_4O_4$	*o*-OMe	*t*-Bu	*anti*	78	A	204-205	$C_{20}H_{28}N_2O_7$
3c·$H_4C_4O_4$	*o*-OMe	*i*-Pr	*syn*	85	A	140-141	$C_{19}H_{26}N_2O_7$
4c·$H_4C_4O_4$	*o*-OMe	*t*-Bu	*syn*	70	A	163-164	$C_{20}H_{28}N_2O_7$
1d·$H_4C_4O_4$	*m*-OMe	*i*-Pr	*anti*	72	A	159-160	$C_{19}H_{26}N_2O_7$
2d·$H_2C_2O_4$	*m*-OMe	*t*-Bu	*anti*	68	B	171-172	$C_{18}H_{26}N_2O_7$
3d·$H_2C_2O_4$	*m*-OMe	*i*-Pr	*syn*	85	B	138-140	$C_{17}H_{24}N_2O_7$
4d·$H_4C_4O_4$	*m*-OMe	*t*-Bu	*syn*	80	A	192-194	$C_{20}H_{28}N_2O_7$
1e·$H_4C_4O_4$	*p*-OMe	*i*-Pr	*anti*	70	A	188-189	$C_{19}H_{26}N_2O_7$
2e·$H_4C_4O_4$	*p*-OMe	*t*-Bu	*anti*	65	A	205-207	$C_{20}H_{28}N_2O_7$
3e·$H_4C_4O_4$	*p*-OMe	*i*-Pr	*syn*	73	A	183-184	$C_{19}H_{26}N_2O_7$
4e·$H_4C_4O_4$	*p*-OMe	*t*-Bu	*syn*	67	A	210-212	$C_{20}H_{28}N_2O_7$
1f·$H_4C_4O_4$	*o*-Cl	*i*-Pr	*anti*	62	B	172-173	$C_{18}H_{23}N_2O_6Cl$
2f·$H_4C_4O_4$	*o*-Cl	*t*-Bu	*anti*	58	B	133-134	$C_{19}H_{25}N_2O_6Cl$
3f·$H_4C_4O_4$	*o*-Cl	*i*-Pr	*syn*	68	B	151-152	$C_{18}H_{23}N_2O_6Cl$
4f·$H_4C_4O_4$	*o*-Cl	*t*-Bu	*syn*	56	B	184-185	$C_{19}H_{25}N_2O_6Cl$
1g·$H_4C_4O_4$	*m*-Cl	*i*-Pr	*anti*	70	B	148-149	$C_{18}H_{23}N_2O_6Cl$
2g·$H_4C_4O_4$	*m*-Cl	*t*-Bu	*anti*	72	B	162-164	$C_{19}H_{25}N_2O_6Cl$
3g·$H_4C_4O_4$	*m*-Cl	*i*-Pr	*syn*	58	B	158-160	$C_{18}H_{23}N_2O_6Cl$
4g·$H_4C_4O_4$	*m*-Cl	*t*-Bu	*syn*	65	B	187-188	$C_{19}H_{25}N_2O_6Cl$
1h·$H_4C_4O_4$	*p*-Cl	*i*-Pr	*anti*	60	B	152-154	$C_{18}H_{23}N_2O_6Cl$
2h·$H_4C_4O_4$	*p*-Cl	*t*-Bu	*anti*	65	B	195-196	$C_{19}H_{25}N_2O_6Cl$
3h·$H_4C_4O_4$	*p*-Cl	*i*-Pr	*syn*	62	B	166-167	$C_{18}H_{23}N_2O_6Cl$
4h·$H_4C_4O_4$	*p*-Cl	*t*-Bu	*syn*	68	B	153-154	$C_{19}H_{25}N_2O_6Cl$

[a]For the epoxide aminolysis; no efforts were made to optimize yields; [b]A, $EtOH/Et_2O$; B, $MeOH/Et_2O$; [c]all compounds were analyzed for C, H, N.

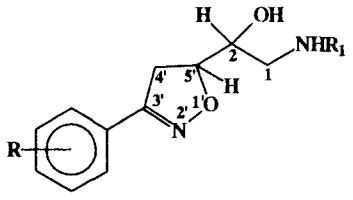
Table II. ^{1}H-NMR data of isoxazoline derivatives **1–4** as free bases.



Compound	*R*	R_1	*Stereoisomer*	*H(1A)*	*H(1B)*	Δ δ [a]	*H(4'A)*	*H(4'B)*	*H(2)*	*H(5')*
1c	*o*-OMe	*i*-Pr	*anti*	2.57 dd (J=12.2, 8.2)	2.84 dd (J=12.2, 3.5)	0.27	3.40 dd (J=16.7, 9.2)	3.40 dd (J=16.7, 9.2)	3.64 ddd (J=8.2, 6.3, 3.5)	4.50 ddd (J=9.2, 9.2, 6.3)
2c	*o*-OMe	*t*-Bu	*anti*	2.53 dd (J=12.1, 7.8)	2.81 dd (J=12.1, 3.9)	0.28	3.41 dd (J=17.1, 9.2)	3.41 dd (J=17.1, 9.1)	3.55 ddd (J=7.8, 6.5, 3.8)	4.49 ddd (J=9.2, 9.1, 6.5)
3c	*o*-OMe	*i*-Pr	*syn*	2.72 d (J=6.8, 0.0)	2.72 d (J=4.9, 0.0)	0.00	3.35 dd (J=17.1, 10.1)	3.43 dd (J=17.1, 9.2)	3.63 ddd (J=6.8, 4.9, 4.1)	4.61 ddd (J=10.1, 9.2, 4.1)
4c	*o*-OMe	*t*-Bu	*syn*	2.69 d (J=6.3, 0.0)	2.69 d (J=6.3, 0.0)	0.00	3.38 dd (J=16.7, 9.7)	3.38 dd (16.7, 9.7)	3.59 ddd (J=6.3, 5.1, 3.8)	4.62 ddd (J=9.7, 9.7, 3.8)
1d	*m*-OMe	*i*-Pr	*anti*	2.54 dd (J=12.2, 8.3)	2.82 dd (J=12.2, 3.5)	0.28	3.24 dd (J=16.7, 10.4)	3.37 dd (J=16.7, 7.9)	3.66 ddd (J=8.3, 6.1, 3.5)	4.56 ddd (J=10.4, 7.9, 6.1)
2d	*m*-OMe	*t*-Bu	*anti*	2.52 dd (J=12.1, 7.9)	2.81 dd (J=12.1, 3.8)	0.29	3.26 dd (J=17.0, 10.3)	3.37 dd (J=17.0, 8.0)	3.57 ddd (J=7.9, 6.5, 3.8)	4.56 ddd (J=10.3, 8.0, 6.5)
3d	*m*-OMe	*i*-Pr	*syn*	2.70 dd (J=11.6, 6.7)	2.76 dd (J=11.6, 6.2)	0.06	3.30 dd (J=16.8, 9.7)	3.33 dd (J=16.8, 9.6)	3.66 ddd (J=6.7, 6.2, 3.8)	4.69 ddd (J=9.7, 9.6, 3.8)
4d	*m*-OMe	*t*-Bu	*syn*	2.71 d (J=5.9, 0.0)	2.71 d (J=5.9, 0.0)	0.00	3.26 dd (J=16.7, 10.6)	3.34 dd (J=16.7, 8.8)	3.61 ddd (J=5.9, 5.9, 3.4)	4.69 ddd (J=10.6, 8.8, 3.4)
1e	*p*-OMe	*i*-Pr	*anti*	2.55 dd (J=12.6, 8.3)	2.83 dd (J=12.2, 3.6)	0.28	3.22 dd (J=16.6, 10.4)	3.34 dd (J=16.6, 7.9)	3.67 ddd (J=8.3, 6.0, 3.6)	4.53 ddd (J=10.4, 7.9, 6.0)
2e	*p*-OMe	*t*-Bu	*anti*	2.53 dd (J=12.1, 7.8)	2.82 dd (J=12.1, 3.8)	0.29	3.27 dd (J=16.7, 10.1)	3.34 dd (J=16.7, 7.9)	3.55 ddd (J=7.8, 7.6, 3.8)	4.52 ddd (J=10.1, 7.9, 7.6)
3e	*p*-OMe	*i*-Pr	*syn*	2.74 d (J=6.5, 0.0)	2.74 d (J=5.1, 0.0)	0.00	3.26 dd (J=17.1, 10.8)	3.26 dd (J=17.1, 9.7)	3.64 ddd (J=6.5, 5.1, 3.9)	4.65 ddd (J=10.8, 9.7, 3.9)
4e	*p*-OMe	*t*-Bu	*syn*	2.72 d (J=6.1, 0.0)	2.72 d (J=5.8, 0.0)	0.00	3.23 dd (J=16.6, 10.2)	3.26 dd (J=16.6, 8.7)	3.61 ddd (J=6.1, 5.8, 3.4)	4.66 ddd (J=10.2, 8.7, 3.4)
1f	*o*-Cl	*i*-Pr	*anti*	2.49 dd (J=12.0, 8.7)	2.76 dd (J=12.0, 3.4)	0.27	3.36 dd (J=17.1, 10.3)	3.47 dd (J=17.1, 8.3)	3.74 ddd (J=8.7, 5.7, 3.4)	4.55 ddd (J=10.3, 6.3, 5.7)
2f	*o*-Cl	*t*-Bu	*anti*	2.54 dd (J=12.0, 7.8)	2.81 dd (J=12.0, 3.8)	0.27	3.44 dd (J=17.2, 9.7)	3.47 dd (J=17.2, 8.3)	3.61 ddd (J=7.8, 6.4, 3.8)	4.57 ddd (J=9.7, 8.3, 6.4)
3f	*o*-Cl	*i*-Pr	*syn*	2.79 d (J=6.0, 0.0)	2.79 d (J=6.0, 0.0)	0.00	3.50 dd (J=16.8, 9.4)	3.50 dd (J=9.6, 9.3)	3.70 ddd (J=6.0, 6.0, 3.8)	4.73 ddd (J=9.4, 9.3, 3.8)
4f	*o*-Cl	*t*-Bu	*syn*	2.79 d (J=5.9, 0.0)	2.79 d (J=5.9, 0.0))	0.00	3.50 dd (J=16.7, 9.2)	3.50 dd (J=9.9, 9.0)	3.64 ddd (J=5.9, 5.9, 3.6)	4.75 ddd (J=9.2, 9.0, 3.6)
1g	*m*-Cl	*i*-Pr	*anti*	2.55 dd (J=12.2, 8.2)	2.83 dd (J=12.2, 3.7)	0.28	3.25 dd (J=16.8, 10.5)	3.34 dd (J=16.8, 8.0)	3.69 ddd (J=8.2, 6.0, 3.7)	4.56 ddd (J=10.5, 8.0, 6.0)
2g	*m*-Cl	*t*-Bu	*anti*	2.74 dd (J=12.1, 9.3)	3.14 dd (J=12.1, 1.9)	0.40	3.26 dd (J=17.1, 10.8)	3.47 dd (J=17.1, 6.7)	3.96 ddd (J=9.3, 6.7, 1.9)	4.52 ddd (J=10.8, 6.7, 6.7)
3g	*m*-Cl	*i*-Pr	*syn*	2.75 d (J=5.2, 0.0)	2.75 d (J=5.2, 0.0)	0.00	3.30 dd (J=17.8, 10.1)	3.30 dd (J=17.8, 9.1)	3.64 ddd (J=5.2, 5.2, 3.6)	4.69 ddd (J=10.1, 9.1, 3.6)
4g	*m*-Cl	*t*-Bu	*syn*	2.72 d (J=5.2, 0.0)	2.72 d (J=5.2, 0.0)	0.00	3.26 dd (J=16.6, 10.6)	3.34 dd (J=16.6, 8.7)	3.60 ddd (J=5.2, 5.2, 3.3)	4.70 ddd (J=10.6, 8.7, 3.3)
1h	*p*-Cl	*i*-Pr	*anti*	2.59 dd (J=12.3, 8.1)	2.86 dd (J=12.3, 3.7)	0.27	3.28 dd (J=16.9, 10.3)	3.36 dd (J=16.9, 7.4)	3.64 ddd (J=8.1, 6.4, 3.7)	4.55 ddd (J=10.3, 7.4, 6.4)
2h	*p*-Cl	*t*-Bu	*anti*	2.52 dd (J=12.0, 8.0)	2.79 dd (J=12.0, 3.6)	0.27	3.24 dd (16.8, 10.2)	3.32 dd (J=16.8, 7.9)	3.57 ddd (J=8.0, 6.8, 3.6)	4.52 ddd (J=10.2, 7.9, 6.8)
3h	*p*-Cl	*i*-Pr	*syn*	2.74 d (J=6.4, 0.0)	2.74 d (J=5.3, 0.0)	0.00	3.26 dd (J=16.7, 10.1)	3.28 dd (J=16.7, 9.3)	3.64 ddd (J=6.4, 5.3, 3.8)	4.66 ddd (J=10.1, 9.3, 3.8)
4h	*p*-Cl	*t*-Bu	*syn*	2.72 d (J=6.2, 0.0)	2.72 d (J=5.8, 0.0)	0.00	3.29 dd (J=16.6, 10.7)	3.36 dd (J=16.6, 8.7)	3.62 ddd (J=6.2, 5.8, 3.3)	4.69 ddd (J=10.7, 8.7, 3.3)

[a] $\Delta\delta = \delta_{H(1B)} - \delta_{H(1A)}$.

Table III. ^{1}H-NMR data of isoxazoline derivatives **1–4** as salts.



Compound	*R*	R_1	*Stereoisomer*	*H(1A)*	*H(1B)*	Δ δ [a]	*H(4'A)*	*H(4'B)*	*H(2)*	*H(5')*
1c	*o*-OMe	*i*-Pr	*anti*	3.17dd (J=13.1,9.8)	3.41 dd (J=13.1,2.9)	0.24	3.57 dd (J=17.8, 7.4)	3.75 dd (J=17.8, 10.5)	4.18 ddd (J=9.8, 5.3, 2.9)	4.88 ddd (J=10.5, 7.4, 5.3)
2c	*o*-OMe	*t*-Bu	*anti*	3.12 dd (J=12.9, 9.9)	3.38 dd (J=12.9, 2.8)	0.26	3.55 dd (J=17.8, 7.5)	3.71 dd (J=17.8, 10.5)	4.14 ddd (J=9.3, 5.2, 2.8)	4.87 ddd (J=10.5, 7.5, 5.2)
3c	*o*-OMe	*i*-Pr	*syn*	3.34 dd (J=12.8, 8.4)	3.39 dd (J=12.8, 2.9)	0.05	3.54 dd (J=18.2, 8.4)	3.78 dd (J=18.2, 10.9)	4.16 ddd (J=8.4, 3.4, 2.9)	4.91 ddd (J=10.9, 8.4, 3.4)
4c	*o*-OMe	*t*-Bu	*syn*	2.94 dd (J=13.0, 9.3)	3.02 dd (J=13.0, 3.6)	0.08	3.17 dd (J=17.7, 7.8)	3.40 dd (J=17.7, 11.0)	3.76 ddd (J=9.3, 3.6, 2.9)	4.53 ddd (J=11.0, 7.8, 2.9)
1d	*m*-OMe	*i*-Pr	*anti*	3.18 dd (J=13.6, 9.9)	3.39 dd (J=13.6, 3.1)	0.21	3.54 dd (J=16.2, 7.4)	3.71 dd (J=16.2, 10.2)	4.21 ddd (J=9.9, 5.6, 3.1)	4.93 ddd (J=10.2, 7.4, 5.6)
2d	*m*-OMe	*t*-Bu	*anti*	3.13 dd (J=12.8, 9.9)	3.40 dd (J=12.8, 2.8)	0.27	3.54 dd (17.8, 7.6)	3.71 dd (J=17.8, 10.6	4.18, ddd (J=9.9, 5.4, 2.8)	4.97 ddd (J=10.6, 7.6, 5.4)
3d	*m*-OMe	*i*-Pr	*syn*	3.34 dd (J=12.6, 8.8)	3.39 dd (J=12.6, 2.9)	0.06	3.57 dd ((J=17.5, 7.9)	3.76 dd (J=17.5, 10.9)	4.18 ddd (J=8.8, 3.3, 2.9)	4.97 ddd (J=10.9, 7.9, 3.3)
4d	*m*-OMe	*t*-Bu	*syn*	3.32 dd (J=13.0, 9.5)	3.39 dd (J=13.0, 3.4)	0.07	3.52 dd (J=17.5, 7.9)	3.78 dd (J=17.5, 11.0)	4.16 ddd (J=9.5, 3.4, 2.9)	4.99 ddd (J=11.0, 7.8, 2.9)
1e	*p*-OMe	*i*-Pr	*anti*	2.03 dd (J=12.8, 10.2)	3.06 dd (J=12.8, 2.8)	0.23	3.17 dd (J=17.4, 7.3)	3.34 dd (J=17.4, 10.7)	3.84 ddd (J=10.2, 4.8, 2.8)	4.59 ddd (J=10.7, 7.3, 4.8)
2e	*p*-OMe	*t*-Bu	*anti*	2.78 dd (J=12.9, 10.4)	3.04 dd (J=12.9, 2.7)	0.24	3.20 dd (J=17.8, 7.7)	3.35 dd (J=17.8, 10.7)	3,82 ddd (J=10.4, 4.9, 2.7)	4.58 ddd (J=10.7, 7.7, 4.9)
3e	*p*-OMe	*i*-Pr	*syn*	3.32 dd (J=12.6, 8.0)	3.38 dd (J=12.6, 2.5)	0.06	3.48 dd ((J=17.6, 8.1)	3.74 dd (J=17.6, 10.7)	4.27 ddd (J=8.0, 3.2, 2.5)	4.95 ddd (J=10.7, 8.1, 3.2)
4e	*p*-OMe	*t*-Bu	*syn*	3.31 dd (J=12.9, 9.3)	3.40 dd (J=12.9, 3.5)	0.09	3.50 dd (J=17.4, 7.8)	3.76 dd (J=17.4, 10.9)	4.16 ddd (J=9.3, 3.5, 3.2)	4.97 ddd (J=10.9, 7.8, 3.2)
1f	*o*-Cl	*i*-Pr	*anti*	2.79dd (J=12.9,10.2)	3.04 dd (J=12.9,2.7)	0.25	3.25 dd (J=17.8, 7.2)	3.43 dd (J=17.8, 10.7)	3.85 ddd (J=10.2, 5.3, 2.7)	4.50 ddd (J=10.7, 7.2, 5.3)
2f	*o*-Cl	*t*-Bu	*anti*	2.74 dd (J=12.7, 10.3)	3.03 dd (J=12.7, 2.5)	0.29	3.26 dd (J=17.8, 7.3)	3.43 dd (J=17.8, 10.6)	3.82 ddd (J=10.3, 4.9, 2.5)	4.59 ddd (J=10.6, 7.3, 4.9)
3f	*o*-Cl	*i*-Pr	*syn*	2.98d (J=12.8, 9.8)	3.08 d (J=12.8, 2.9)	0.10	3.24 dd (J=17.8, 7.6)	3.50 dd (J=17.8, 11.0)	3.79 ddd (J=9.8, 3.4, 2.9)	4.58 ddd (J=11.0, 7.6, 3.4.)
4f	*o*-Cl	*t*-Bu	*syn*	2.94 dd (J=12.8, 9.8)	3.03 dd (J=12.8, 2.7)	0.09	3.23 dd (J=17.8, 7.6)	3.46 dd (J=17.8, 11.1)	3.76 ddd (J=9.8, 3.1, 2.7)	4.60 ddd (J=11.1, 7.6, 3.1)
1g	*m*-Cl	*i*-Pr	*anti*	2.79 dd (J=12.8, 10.3)	3.03 dd (J=12.8, 2.6)	0.24	3.15 dd (J=17.9, 7.7)	3.32 dd (J=17.9, 11.0)	3.82 ddd (J=10.3, 5.2, 2.6)	4.55 ddd (J=11.0, 7.7, 5.2)
2g	*m*-Cl	*t*-Bu	*anti*	2.73 dd (J=12.5, 10.5)	3.03 dd (J=12.5, 2.5)	0.30	3.14 dd (17.5, 7.7)	3.29 dd ()=17.5, 10.5	3.83, ddd (J=10.5, 5.2, 2.5)	4.54 ddd (J=10.5, 7.7, 5.2)
3g	*m*-Cl	*i*-Pr	*syn*	2.95 dd (J=12.6, 8.3)	3.01 dd (J=12.6, 4.2)	0.06	3.13 dd ((J=17.5, 8.9)	3.34 dd (J=17.5, 11.2)	3.79 ddd (J=8.3, 4.2, 2.6)	4.58 ddd (J=11.2, 8.9, 2.6)
4g	*m*-Cl	*t*-Bu	*syn*	3.31 dd (J=12.4, 9.4)	3.36 dd (J=12.4, 3.5)	0.05	3.48 dd (J=17.2, 7.9)	3.74 dd (J=17.2, 11.2)	4.15 ddd (J=9.4, 3.5, 2.6)	4.93 ddd (J=11.2, 7.9, 2.6)
1h	*p*-Cl	*i*-Pr	*anti*	3.19 dd (J=12.9, 9.9)	3.41 dd (J=12.9, 3.2)	0.22	3.52 dd (J=17.5, 7.6)	3.70 dd (J=17.5, 10.7)	4.21 ddd (J=9.9, 5.5, 3.2)	4.94 ddd (J=10.7, 7.6, 5.5)
2h	*p*-Cl	*t*-Bu	*anti*	2.78 dd (J=12.9, 10.4)	3.05 dd (J=12.9, 2.4)	0.27	3.20 dd (J=17.8, 7.7)	3.36 dd (J=17.8, 10.7)	3,79 ddd (J=10.4, 5.2, 2.4)	4.52 ddd (J=10.7, 7.7, 5.2)
3h	*p*-Cl	*i*-Pr	*syn*	3.32 dd (J=13.1, 8.9)	3.40 dd (J=13.1, 3.9)	0.08	3.48 dd ((J=17.6, 7.9)	3.74 dd (J=17.6, 10.9)	4.18 ddd (J=8.9, 3.9, 3.3)	4.98 ddd (J=10.9, 7.9, 3.3)
4h	*p*-Cl	*t*-Bu	*syn*	3.29 dd (J=13.0, 9.4)	3.38 dd (J=13.0, 3.5)	0.09	3.48 dd (J=17.1, 7.9)	3.74 dd (J=17.1, 11.3)	4.15 ddd (J=9.4, 3.5, 3.2)	4.97 ddd (J=11.3, 7.9, 3.2)

[a] $\Delta\delta = \delta_{H(1B)} - \delta_{H(1A)}$.

found for the previously studied *anti* (**1a,b** and **2a,b**) and *syn* (**3a,b** and **4a,b**) derivatives, respectively. In addition, as in the case of the derivatives **1a,b–4a,b**, the chemical shift differences between the H(1) protons [H(1A)] and [H(1B)] in the *anti* compounds **1c–h** and **2c–h** are higher (0.27–0.40 ppm for the free bases, and 0.21–0.30 ppm for the salts) than in the *syn* compounds **3c–h** and **4c–h** (0.00–0.06 ppm for the free bases, and 0.05–0.10 ppm for the salts (tables II and III).

Once the relative configurations of the *anti* (**1c–h**, **2c–h**) and the *syn* (**3c–h**, **4c–h**) aminoalcohols had been established, it was also possible to assign the corresponding starting epoxides **9c–h** and **10c–h** to the *anti* and the *syn* configurations shown. The C(5') and C(2) chiral centers of epoxides **9c–h** and **10c–h** are not involved in their aminolysis reaction to the aminoalcohols , **1c–h**, **2c–h** and **3c–h**, **4c–h**.

The analogies that exist between the spectral parameters of the isoxazoline aminoalcohols **1c–h–4c–h** and those of the corresponding previously studied analogs **1a,b–4a,b** [1] make it possible to extend to the new compounds (**1c–h–4c–h**) the considerations already expressed for compounds **1a,b–4a,b**, as regards their conformational situation around the C(5')–C(2) bond in solution. In particular, the relatively high values of $J_{5',2}$ shown by **1c–h** and **2c–h** (5.7–7.6 Hz for the free bases, and 4.8–5.3 Hz for the salts) indicate that of the 3 classic staggered rotamers α, β, and γ of these compounds (see fig 1), the conformer γ, in which the H(5') and H(2) are in a *trans* relationship, plays an important role in the conformational equilibrium. For the *syn* compounds **3c–h** and **4c–h**, on the other hand, the lower values of their $J_{5',2}$ coupling constants (3.3–4.1 Hz for the free bases, and 3.1–3.4 Hz for the salts) indicate that the γ rotamer should play little or no role in the conformational equilibrium, and, therefore, that rotamers should prevail in which the H(5') and H(2) hydrogens are in a *gauche* relationship, as in the α and β conformers of figure 1. It may be noted that in the γ rotamer of the *anti* compounds **1c–h** and **2c–h**, and in the α and β rotamers of the *syn* compounds **3c–h** and **4c–h**, the formation of a hydrogen bond between the hydrogen linked to the amine nitrogen and the isoxazoline oxygen is possible.

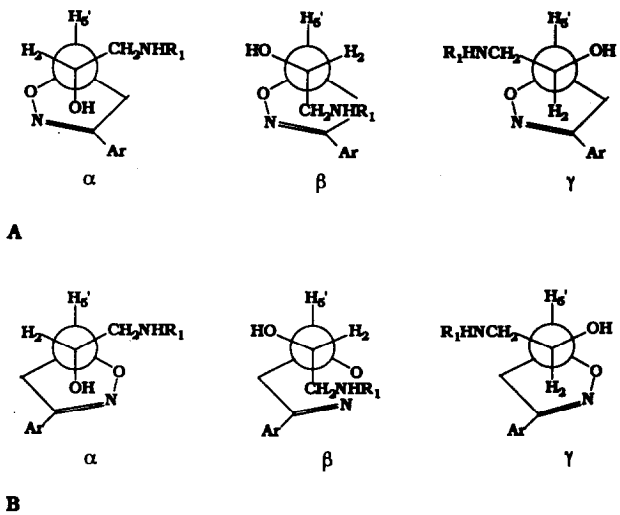


Fig 1. Newman's projections along the C(2)-C(5') bond of the 3 classic staggered rotamers of type *anti* (**A**) and *syn* (**B**) isoxazoline derivatives.

Results

Radioligand binding assays

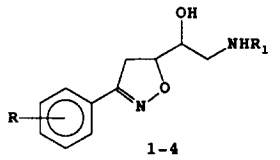
The affinity towards β-adrenoceptors of the 3'-aryl-isoxazolidine derivatives **1c–h–4c–h** and of the reference drug propranolol was checked by binding tests on rat-brain membranes for β_1-adrenoceptors and on bovine lung membranes for β_2-adrenoceptors (table IV). ^{3}H-CGP 26505 [5] was used as a specific tritiated ligand for rat brain β_1-adrenoceptors. ^{3}H-Dihydroalprenolol (^{3}H-DHA) [6] was used to label bovine lung β_2-adrenoceptors in the presence of 50 nM CGP 26505, which displaced ^{3}H-DHA binding from the β_1-adrenoceptor subpopulation, which represents 17% in the bovine lung [7]. Table IV also shows the results previously obtained by us in the same types of tests with the isoxazoline derivatives unsubstituted on the phenyl ring (**1a–4a**) [1].

Rat-brain β_1-adrenoceptors

Among the *N*-isopropyl-substituted *anti* isoxazolines (**1c–h**), only the *p*-MeO- (**1e**) and *p*-Cl-substituted (**1h**) ones showed an affinity higher than that of the corresponding unsubstituted isoxazoline **1a**. As far as the *N*-isopropyl-substituted isoxazolines with the *syn* configuration are concerned (**3c–h**), all the chloro-substituted compounds (**3f–h**) and the only *o*-MeO-substituted one (**3c**) exhibited an affinity higher than that of the phenyl-unsubstituted isoxazoline **3a**.

Among the *anti N-t*-butyl-substituted isoxazolines (**2c–h**), all compounds presented a more marked affinity than the corresponding isoxazoline **2a**, with the only exception of the *m*-MeO-substituted compound **2d**. The *syn N-t*-butyl-substituted isoxazolines (**4c–h**) were found to possess an affinity higher than that of the isoxazoline **4a** which, however, showed a very high K_i value.

Table IV. Radioligand binding affinity of compounds **1–4**.

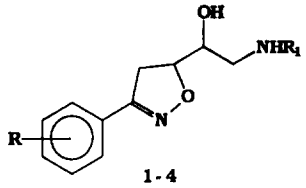


1-4

Compound	*R*	*R1*	*Stereo-isomer*	*β-Adrenergic affinity*[a]	
				Rat brain (β_1) Ki (nM)	*Bovine lung (β_2) Ki (nM)*
1a[b]	H	*i*-Pr	*anti*	3900 (3200-4600)	20000 (17500-22450)
1c	*o*-MeO	*i*-Pr	*anti*	7100 (6000-8200)	7400 (6150-8600)
1d	*m*-MeO	*i*-Pr	*anti*	14400 (13650-15150)	9600 (7700-11400)
1e	*p*-MeO	*i*-Pr	*anti*	2000 (1700-2300)	12000 (10250-13750)
1f	*o*-Cl	*i*-Pr	*anti*	5200 (4300-6050)	16000 (13400-18600)
1g	*m*-Cl	*i*-Pr	*anti*	16500 (12650-18400)	13600 (12500-14650)
1h	*p*-Cl	*i*-Pr	*anti*	560 (515-610)	320 (295-350)
3a[b]	H	*i*-Pr	*syn*	5400 (4500-6300)	34000 (30100-37900)
3c	*o*-MeO	*i*-Pr	*syn*	2000 (1650-2300)	20700 (17450-23900)
3d	*m*-MeO	*i*-Pr	*syn*	12900 (8600-17000)	5300 (4625-5915)
3e	*p*-MeO	*i*-Pr	*syn*	7400 (6300-8490)	10000 (8600-11370)
3f	*o*-Cl	*i*-Pr	*syn*	2000 (1660-2330)	37000 (30600-43360)
3g	*m*-Cl	*i*-Pr	*syn*	1400 (1055-1695)	4300 (3470-5125)
3h	*p*-Cl	*i*-Pr	*syn*	1060 (1017-1110)	370 (348-399)
2a[b]	H	*t*-Bu	*anti*	7100 (6020-8170)	2300 (1880-2700)
2c	*o*-MeO	*t*-Bu	*anti*	1000 (845-1150)	4000 (3390-4600)
2d	*m*-MeO	*t*-Bu	*anti*	8300 (6870-9630)	25000 (17690-32640)
2e	*p*-MeO	*t*-Bu	*anti*	5400 (4660-6140)	26000 (22230-29760)
2f	*o*-Cl	*t*-Bu	*anti*	730 (640-820)	5000 (4190-5810)
2g	*m*-Cl	t-Bu	*anti*	2800 (2470-3040)	18000 (17670-18130)
2h	*p*-Cl	*t*-Bu	*anti*	1090 (960-1220)	1240 (1110-1370)
4a[b]	H	*t*-Bu	*syn*	>100000	13000 (10870-15130)
4c	*o*-MeO	*t*-Bu	*syn*	2500 (2140-2850)	24000 (20600-27300)
4d	*m*-MeO	*t*-Bu	*syn*	9300 (7870-10630)	10200 (8760-11610)
4e	*p*-MeO	*t*-Bu	*syn*	3000 (2545-3450)	10000 (8360-11600)
4f	*o*-Cl	*t*-Bu	*syn*	6100 (5100-7100)	15000 (12700-17270)
4g	*m*-Cl	*t*-Bu	*syn*	4400 (3340-5360)	6600 (6460-6640)
4h	*p*-Cl	*t*-Bu	*syn*	490 (455-530)	330 (205-255)
propranolol				4.9 (3.6-6.1)	1.7 (1.4-1.9)

[a]Geometric means of 5 separate determinations with confidence limits in parentheses. [b]From reference [1].

Table V. β-Blocking activity of compounds **1–4**.



1-4

Compound	*R*	*R_1*	*Stereo-isomer*	*β-Adrenergic activity*[a]	
				Isolated guinea pig atria (β_1) p IC_{50}	*Isolated guinea pig tracheal strip (β_2) p IC_{50}*
1a	H	*i*-Pr	*anti*	4.92±0.06[b]	4.31±0.08[b]
1h	*p*-Cl	*i*-Pr	*anti*	4.23	<3.5
3a	H	*i*-Pr	*syn*	4.50±0.02[b]	—[b]
3g	*m*-Cl	*i*-Pr	*syn*	4.07	5.58
3h	p-Cl	*i*-Pr	*syn*	<3.5	4.46
2a	H	*t*-Bu	*anti*	4.85±0.31[b]	4.89±0.08[b]
2c	*o*-MeO	*t*-Bu	*anti*	—	5.09
2f	*o*-Cl	*t*-Bu	*anti*	4.59	5.41
2h	*p*-Cl	*t*-Bu	*anti*	—	5.17
4a	H	*t*-Bu	*syn*	4.41±0.21[b]	3.75±0.03[b]
4h	*p*-Cl	*t*-Bu	*syn*	3.67	5.23
propranolol				7.39±0.20	7.54±0.18

[a]The values represent the mean of 4–6 experiments for each drug ± standard error. [b]From reference [1].

Bovine lung β_2-adrenoceptors

All the *anti N*-isopropyl-substituted isoxazolines (**1c–h**) presented, on this type of receptor, an affinity higher than that of the corresponding phenyl-unsubstituted analogs **1a** (see table IV). Also the *syn N*-isopropyl isoxazolines (**3c–h**), with the single exception of the *o*-Cl one, showed affinities higher than that of the corresponding isoxazoline **3a**.

The *anti N-t*-butyl isoxazolines, with the only exception of the *p*-Cl derivative **2h**, presented an affinity, for the bovine lung β_2-adrenoceptor, lower than that of the corresponding isoxazoline unsubstituted on the phenyl ring (**2a**). As far as *N-t*-butyl-substituted *syn* isoxazolines (**4c–h**) are concerned, the *m*-(**4d,g**) and *p*-substituted (**4e,h**) ones were found to show a greater affinity for this type of receptor, with respect to the phenyl-unsubstituted analog (**4a**), while the *o*-

substituted isoxazolines **4c**, **f** presented an affinity lower than that of **4a**.

Functional tests

Isoxazoline derivatives that showed in the binding tests on rat brain a β_1-adrenergic receptor affinity index lower than 2000 nM (**1h**, **2c,f,h**, **3g,h** and **4h**) (see table IV) were submitted to functional tests on guinea-pig atria and guinea-pig tracheal strips for their β_1- and β_2-adrenergic activity, respectively. The results obtained are shown in table V, together with those obtained with the reference drug propranolol, and those previously obtained in the same types of tests with the isoxazoline derivatives unsubstituted on the phenyl ring (**1a–4a**) [1].

Guinea-pig atria

Almost all the compounds examined exhibited a β_1-blocking activity, revealed by their ability to antagonize the stimulating effect of isoprenaline. The *p*-Cl-substituted isoxazoline derivative with the *anti* configuration (**1h**) showed an antagonistic activity index (pIC_{50}) which was slightly lower than that of the corresponding isoxazoline derivative unsubstituted on the phenyl ring (**1a**). As regards the *N*-isopropyl-substituted *syn* isoxazolines **3g,h**, the *m*-Cl-substituted one (**3g**) exhibited an antagonistic activity index slightly lower than that of the phenyl-unsubstituted analog (**3a**), while the *p*-Cl substituted one (**3h**) was found to be practically inactive.

As regards the *anti N-t*-butyl isoxazoline derivatives **2c,f,h**, only the *o*-Cl-substituted one (**2f**) showed a pIC_{50} value similar to that of **2a**, while both the *o*-MeO- (**2c**) and *p*-Cl-substituted (**2h**) ones were found to be completely inactive.

The *p*-Cl substituted *syn N-t*-butyl isoxazoline (**4a**) revealed a modest β-blocking activity, with an activity index about one order of magnitude lower than that of the isoxazoline **4a**.

None of the new isoxazolinic compounds shown in table V proved to possess stimulating properties on atrial β_1-adrenoceptors.

Guinea-pig tracheal strips

On β_2-adrenoceptors, the new isoxazolines tested (**1h**, **2c,f,h**, **3g,h**, **4h**), displayed an antagonistic activity towards the isoprenaline-induced response, with activity indices almost always higher than those of the corresponding isoxazolinic compounds unsubstituted on the phenyl ring (**1a–4a**)(table V); the only exception was represented by the *anti p*-Cl-substituted derivative (**1h**), which was found to be practically inactive, while the corresponding phenyl-unsubstituted analog (**1a**) showed an activity, albeit modest.

The *m*-Cl- (**3g**) and the *p*-Cl-substituted (**3h**) *N*-isopropyl isoxazolines with the *syn* configuration exhibited appreciable pIC_{50} values, while the phenyl-unsubstituted analog (**3a**) has previously been found to be devoid of any antagonistic activity [1].

The *anti N-t*-butyl-substituted compounds **2c,f,h** showed activity indices which were not much higher than that previously found for the corresponding phenyl-unsubstituted analog **2a** on the same type of tissue.

The *syn p*-Cl-*N-t*-butyl isoxazoline **4h** exhibited a pIC_{50} value more than one order of magnitude higher than that of the corresponding phenyl-unsubstituted analog **4a**.

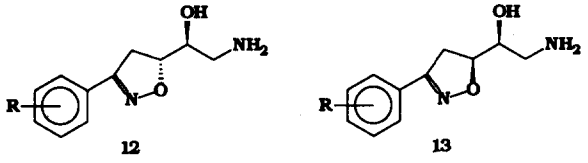
Moreover, on these tracheal β_2-adrenoceptors, none of the new isoxazoline derivatives shown in table V proved to possess any appreciable stimulating activity.

Theoretical calculations

In order to obtain information about some of the molecular characteristics which might play a role in determining the differences in the biopharmacological properties of compounds **1c–h**–**4c–h**, theoretical calculations were carried out on the conformational profile and the molecular electrostatic potential (MEP) of their *N*-unsubstituted analogs (**12c–h** and **13c–h**). 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 [1, 8, 9].

Conformational analysis

The conformational analysis of **12c–h** and **13c–h** was performed by using a full geometry optimization carried out using the semiempirical program MOPAC [10] at AM1 level; the energies of the optimized conformations were then recalculated at the *ab initio* SCF–MOLCAO level, using a minimal STO3G basis set.



a, R = H; **c**, R = *o*-MeO; **d**, R = *m*-MeO; **e**, R = *p*-MeO
f, R = *o*-Cl; **g**, R = *m*-Cl; **h**, R = *p*-Cl

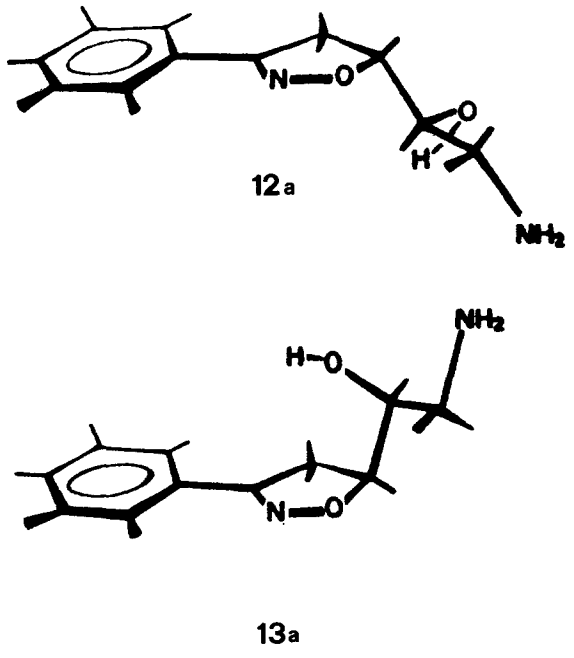


Fig 2. Perspective views of the model compounds **12a** and **13a** in their low-energy conformations.

For the optimization, the starting conformations of the *anti* (**12c–h**) and *syn* (**13c–h**) compounds were built by using the preferred conformations previously found for the corresponding phenyl-unsubstituted model compounds **12a** and **13a** (fig 2), in which the phenyl ring is slightly rotated with respect to the isoxazoline system (7°) and the conformational situation around the C(5')-C(2) bond corresponds to that of the classic staggered rotamer γ of figure 1A and α of figure 1B for **12a** and **13a**, respectively [1]. In the case of the *ortho*- and *meta*-substituted compounds, the substituent was placed on the opposite side with respect to the isoxazoline nitrogen.

The results indicate that the optimization procedure does not substantially modify the starting conformations; only a lack of coplanarity was found between the aryl ring and the isoxazoline ring in a few compounds: in both *anti* (**12f**) and *syn* (**13f**) *o*-Cl-substituted compounds, the angle between these rings is about 70° and in the *syn* *o*-MeO-substituted compound (**13c**), it is about 30°.

Molecular electrostatic potential

The molecular electrostatic potential (MEP) of **12a,c–h** and **13a,c–h** was calculated on the solvent-accessible molecular surface [11] by using the STO3G wavefunctions; the calculations were made for the optimized conformations.

The minima generated by the nitrogen and the oxygen of the isoxazoline system together with the mean value on the aromatic ring are listed in table VI; the MEP values on the ethanolaminic portion were practically identical for all compounds considered and are not therefore reported.

These data show that the kind of substitution influences the MEP values to a certain extent: the chlorine makes them less negative, whereas the methoxyl group, on the contrary, makes them more negative.

The position of the substituent also influences the MEP values, even if it seems to be less important. Generally, the *ortho*-substituted compounds show the most negative, and the *meta*-substituted ones the least negative values. However, the differences are fairly small, and the trend is less regular for the MEP values on the aromatic ring.

A comparison between equally substituted compounds indicates that generally the most negative values are shown by derivatives with the *syn* geometry.

Discussion and conclusions

Binding data for β_1-receptors indicate that most isoxazoline compounds substituted on the phenyl ring (**1c–h–4c–h**) present an affinity for this receptor that is slightly higher than that of the corresponding phenyl-unsubstituted compounds (**1a–4a**). In particular, the introduction either of a methoxyl group or a chlorine atom in the *ortho* or *para* positions of the phenyl of **1a–4a** leads to compounds with a higher

Table VI. Molecular electrostatic potential values (V, kcal/mol) of compounds **12a,c–h** and **13a,c–h** on the molecular surface, calculated for the minimized conformations.

Compd	*R*	*V(N)*[a]	*V(O)*[b]	*V(ϕ)*[c]
12a	H	-43.5	-33.7	+0.8
12c	*o*-OCH_3	-46.3	-34.6	-0.8
12d	*m*-OCH_3	-44.1	-31.2	-1.7
12e	*p*-OCH_3	-45.0	-32.7	-1.3
12f	*o*-Cl	-46.8	-34.8	+0.9
12g	*m*-Cl	-40.5	-28.7	+1.2
12h	*p*-Cl	-42.0	-29.2	-1.5
13a	H	-44.4	-31.4	-1.2
13c	*o*-OCH_3	-46.5	-30.7	-2.3
13d	*m*-OCH_3	-45.5	-33.8	-2.1
13e	*p*-OCH_3	-45.9	-34.8	-1.2
13f	*o*-Cl	-49.2	-35.5	-0.9
13g	*m*-Cl	-42.6	-32.5	+1.3
13h	*p*-Cl	-43.2	-32.6	-0.1

affinity, with the exceptions of the *anti* *o*-MeO- (**1c**) and *o*-Cl-substituted (**1f**) isoxazolines, and the *p*-MeO-substituted one (**3e**).

As regards the effects of the substitutions in the *meta* position of the phenyl of **1a–4a**, the introduction of the chlorine atom leads to an increase in the affinity for β_1-receptors, with the exception of the *anti* isoxazoline **1f**, whereas the methoxyl group only has a positive effect in the case of **4d**, and negative effects in the cases of **1d–3d**.

A comparison of the K_i values of the *anti* compounds with those of the corresponding isomers with the *syn* configuration does not appear to reveal any substantial differences in their ability to interact with β_1-receptors.

For the isoxazolines that were submitted to functional tests (**1f**, **2c,f,h**, **3g,h** and **4h**), the activity indices for β_1-receptors were not completely in agreement with the K_i values obtained in the binding tests. All these compounds, while presenting K_i values lower than those of the corresponding phenyl-unsubstituted compounds (**1a–4a**), were found to possess a lower β_1-blocking activity in the cases of **1h**, **2f**, **3g,h** and **4a**, or even no activity at all, in the cases of **2c,h**. The differences between the trend of the binding test results and those of functional tests might be partly attributable to differences between animal species and kinds of tissues used in the 2 types of tests [12].

Moreover, for β_2-receptors, the results obtained in the binding tests indicate that most of the isoxazolinic compounds substituted on the aromatic ring possess a slightly higher affinity than those of the corresponding compounds unsubstituted on the phenyl ring (**1a–4a**). The introduction of either the methoxyl group or the chlorine atom into the *meta* or *para* position of the phenyl ring of **1a–4a** generally leads to compounds which display an improvement in the affinity, except for the *p*-MeO- (**2e**) and *m*-MeO- (**2d**) and *m*-Cl-substituted (**2g**) *anti* *N*-*t*-butyl isoxazolines.

As regards the effects of substitution in the *ortho* position of the phenyl ring of **1a–4a**, the insertion of the methoxyl group leads to an improvement in the affinity for β_2-receptors, as in the cases of **1c** and **3c**, but has a negative effect in the cases of **2c** and **4c**. On the other hand, the insertion of a chlorine atom has a positive effect only when it leads to the *anti* isoxazoline **1f**, seeing that it gives compounds with a lower affinity for β_2-receptors in the cases of **2f**, **3f**, and **4f**.

A comparison between the affinity index values of isoxazoline derivatives with the *anti* configuration with those of the corresponding isomers with the *syn* configuration does not reveal any substantial differences between the capacities of these 2 types of compounds to interact with β_2 receptors.

The results obtained for the isoxazolines **1h**, **2c,f,h**, **3g,h** and **4h** in the functional tests carried out on guinea-pig tracheal receptors are in quite good agreement with those obtained for the same compounds in the binding tests carried out on bovine lung β_2-receptors, apart from the *anti* (**1h**) and *syn* (**3h**) *p*-Cl derivatives, which exhibit a low β_2-blocking activity, even if they are among the compounds possessing a higher affinity for β_2-receptors.

Compounds **1c–h–4c–h** were prepared and tested with the aim of checking the effects on the β-adrenergic properties of the substitution on the phenyl ring of **1a–4a**. Actually, most of the new compounds substituted on the phenyl proved to possess a slightly better capacity to interact with β-receptors than the corresponding unsubstituted compounds **1a–4a**. The substitution which led to compounds with the best properties was found to be with the chlorine atom. In general, the variations in the β-adrenergic properties with respect to **1a–4a** appear to be influenced not only by the type of the substituent and its position on the aromatic ring, but also by the configuration and the type of the substituent on the aminic nitrogen. However, it would appear to be rather difficult to demonstrate any relationship between these findings. Only in the case of the *p*-chloro substitution was it found that all the compounds both with the *anti* and the *syn* configuration, and *N*-isopropyl- and *N*-*tert*-butyl-substituted, proved to possess β-adrenergic properties which, at least as regards the affinity, were in all cases markedly better than the corresponding phenyl-unsubstituted compounds.

A comparison of the conformational features of the model compounds of the isoxazoline derivatives **1c–h–4c–h** and of the corresponding phenyl-unsubstituted analogs **1a–4a** does not suggest any reasonable explanation for the trend of the binding data: while compounds like **2c** and **2h**, whose corresponding model compounds exhibit different conformational profiles, show quite similar β-adrenergic affinity indices, compounds like **1d** and **1h**, corresponding to model compounds with practically the same conformational characteristics, show markedly different affinity indices.

Likewise, the analysis of the MEP trends of the model compounds **12a,c–h** and **13a,c–h** on their common molecular portions is of no help in the formulation of any reasonable hypothesis about the influence of the electronic characteristics on the binding properties of **1c–h–4c–h** and **1a–4a** to β-receptors. Isoxazoline derivatives like **2c** and **2e** corresponding to model compounds with substantially similar MEP trends (**12c** and **12e**) proved to possess β-adrenergic properties that differ more than those of compounds like **1f** and **1h**, which correspond to model compounds with different MEP trends (**12f** and **12h**).

An analysis of the effects on β-adrenergic properties of substitutions on the aromatic ring of 3'-aryl-

substituted isoxazoline compounds of types **1–4** did not reveal any possible correlation between their structure and their activity. The data obtained could not be rationalized even by means of a theoretical study of their geometries and of the molecular reactivity of the model compounds **12** and **13**.

The above appears to indicate that the aromatic ring of 3'-aryl-substituted isoxazoline compounds of types **1–4** should not play any direct role in the interaction of these compounds with β-adrenergic receptors.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken on paraffin oil mulls on a Perkin-Elmer Model 1310 instrument. ^{1}H-NMRspectra of all compounds were routinely detected with a Varian EM 360 A instrument in a *ca* 5% solution of $CDCl_3$ (for the neutral compounds or the free bases) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The ^{1}H-NMR spectral study of **1c–h–4c–h** was performed with a Bruker AC-200 instrument, and the spectral parameters were refined by a Mole (Laocoon) program, using an Atari PC 3 computer. The parameters obtained were correct to within ± 0.2 Hz. Preparative MPLCs were carried out through glass columns containing 230–400 mesh silica gel, using a chromatographic apparatus consisting of a Buchi 681 pump, a Knauer differential refractometer detector, and a Philips PM 8220 pen recorder. *E*-Benzaldoximes substituted on the phenyl ring were prepared by the method described in reference [13]. Boiling points refer to the air bath temperature of bulb-to-bulb distillation carried out by using a Buchi GKR 51 apparatus. Evaporations were made *in vacuo* (rotating evaporator). $MgSO_4$ was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with the theoretical values to within ± 0.4%.

*General procedure for the synthesis of 3-aryl-5-vinyl-2-isoxazolines **8c–h***

A stirred solution of the appropriate benzaldoxime substituted on the phenyl ring (86 mmol) in DMF (500 ml) was treated in portions with *N*-chlorosuccinimide (13.0 g, 100 mmol). The resulting mixture was stirred at 45°C for 3 h and then diluted with H_2O (200 ml) and extracted with Et_2O. The organic layers were washed with brine, dried, and evaporated to yield a crude residue consisting almost exclusively of the aroylhydroxamyl chloride **7c–h** [14, 15] which, without further purification, was dissolved in anhydrous $CHCl_3$ (30 ml) and then added dropwise to a stirred and cooled (0°C) solution of 1,3-butadiene (14.2 g, 0.26 mol) and Et_3N (12.1 g, 0.12 mol) in anhydrous $CHCl_3$ (50 ml). After 3 h at room temperature, the reaction mixture was washed with brine, dried, and evaporated to yield a solid (in the case of **8c,e**) or an oily residue (in the case of **8d,f–h**) which was purified by crystallization or distillation, respectively. **8c** (10.5 g, 57 %): mp 122–123°C (*i*-PrOH); ^{1}H-NMR δ 3.10–3.69 (m, 2H), 3.88 (s, 3H), 4.87–6.04 (m, 4H), 6.83–7.85 (m, 4H). Anal for $C_{12}H_{13}NO_2$ (C, H, N). **8d** (12.3 g, 66%): bp 60°C (0.1 mmHg); ^{1}H-NMR δ 3.08–3.67 (m, 2H), 3.86 (s, 3H), 4.90–6.04 (m, 4H), 6.86–7.83 (m, 4H). Anal for $C_{12}H_{13}NO_2$ (C, H, N). **8e** (9.6 g, 52%): mp 126–128°C (*i*-PrOH); ^{1}H-NMR δ 2.66–3.53 (m, 2H), 3.86 (s, 3H), 4.73–5.83 (m, 4H), 6.60–7.43 (m, 4H). Anal for $C_{12}H_{13}NO_2$ (C, H, N). **8f** (8.3 g, 46%): bp 78°C (0.1 mmHg); ^{1}H-NMR δ 3.04–3.40 (m, 2H), 4.90–5.50 (m, 4H), 6.87–7.88 (m, 4H). Anal for $C_{11}H_{10}ClNO$ (C, H, N). **8g** (8.6 g, 48%): bp 82°C (0.2 mmHg); ^{1}H-NMR δ 3.08–3.39 (m, 2H), 4.85–6.00 (m, 4H), 6.90–7.70 (m, 4H). Anal for $C_{11}H_{10}ClNO$ (C, H, N). **8h** (8.7 g, 49%): bp 87–88°C (0.1 mmHg); ^{1}H NMR δ 3.08–3.39 (m, 2H), 4.85–6.00 (m, 4H), 6.90–7.70 (m, 4H). Anal for $C_{11}H_{10}ClNO$ (C, H, N).

*Procedure for the preparation of anti-**9c–h** and syn-2-((3'-aryl)-5'-isoxazolidinyl)oxiranes **10c–h***

A stirred solution of **8c–h** (0.07 mol) in anhydrous CH_2Cl_2 (70 ml) was treated as previously described for analogous compounds [1]. The crude residue, consisting almost exclusively of a 1:1 mixture of the diastereoisomeric *anti* (**9c–h**) and *syn* epoxides (**10c–h**) was submitted to MPLC on silica gel, eluting with a 4:2:1 hexane/$CHCl_3$/AcOEt mixture, for the methoxy-substituted compounds, or with a 2:1:1 hexane/$CHCl_3$/AcOEt mixture, for the chloro-substituted ones, and collecting 25 ml fractions. For all crude reaction mixtures obtained from **8c–h**, the first fractions afforded pure *anti* epoxides **9c–h**, whereas the subsequent fractions yielded the *syn* epoxides **10c–h**. **9c** (6.29 g, 41%): mp 115–118°C (AcOEt/hexane); ^{1}H-NMR δ 2.62 (dd, 1H, J = 2.3 and 4.2 Hz), 2.75–3.3 (m, 4H), 4.44 (ddd, 1H, J = 4.2, 7.6 and 9.0 Hz). Anal for $C_{12}H_{13}NO_3$ (C, H, N). **9d** (6.14 g, 40%): mp 109–111°C (AcOEt/hexane); ^{1}H-NMR δ 2.68 (dd, 1H, J = 3.0 and 4.5 Hz), 2.73–3.40 (m, 4H), 4.55 (ddd, 1H, J = 4.8, 7.8 and 10.2 Hz). Anal for $C_{12}H_{13}NO_3$ (C, H, N). **9e** (6.14 g, 40%): mp 117–118°C (AcOEt/hexane); ^{1}H-NMR δ 2.65 (dd, 1H, J = 2.8 and 4.0 Hz), 2.72–3.32 (m, 4H), 4.48 (ddd, 1H, J = 4.6, 8.1 and 10.0 Hz). Anal for $C_{12}H_{13}NO_3$ (C, H, N). **9f** (6.10 g, 39%): mp 118–121°C ($CHCl_3$/hexane); ^{1}H-NMR δ 2.74 (dd, 1H, J = 2.7 and 4.1 Hz), 2.80–3.40 (m, 4H), 4.51 (ddd, 1H, J = 4.7, 8.8, 10.2 Hz). Anal for $C_{11}H_{10}ClNO_2$ (C, H, N). **9g** (4.58 g, 29%): mp 120–123°C (AcOEt/hexane); ^{1}H-NMR δ 2.90 (dd, 1H, J = 3.9 and 4.2 Hz), 2.82–3.43 (m, 4H), 4.49 (ddd, 1H, J = 5.0, 7.9 and 10.2 Hz). Anal for $C_{11}H_{10}ClNO_2$ (C, H, N). **9h** (5.25 g, 33%): mp 122–123°C ($CHCl_3$/hexane); ^{1}H-NMR δ 2.87 (dd, 1H, J = 3.3 and 4.0 Hz), 2.80–3.45 (m, 4H), 4.48 (ddd, 1H, J = 5.5, 9.0 and 10.3 Hz). Anal for $C_{11}H_{10}ClNO_2$ (C, H, N). **10c** (5.67 g, 37%): mp 121–123°C (AcOEt/hexane); ^{1}H-NMR δ 2.65 (d, 2H, J = 3.8 Hz), 2.93–3.38 (m, 3H), 4.44 (ddd, 1H, J = 4.2, 7.6 and 9.0 Hz). Anal for $C_{12}H_{13}NO_3$ (C, H, N). **10d** (5.52 g, 36%): mp 112–115°C (AcOEt/hexane); ^{1}H-NMR δ 2.71 (d, 2H, J = 4.0 Hz), 2.80–3.20 (m, 3H), 4.60 (ddd, 1H, J = 4.4, 8.0 and 10.0 Hz). Anal for $C_{12}H_{13}NO_3$ (C, H, N). **10e** (5.83 g, 38%): mp 118–119°C (AcOEt/hexane); ^{1}H-NMR δ 2.70 (d, 2H, J = 3.9 Hz), 2.89–3.28 (m, 3H), 4.52 (ddd, 1H, J = 4.2, 7.9 and 9.8 Hz). Anal for $C_{12}H_{13}NO_3$ (C, H, N). **10f** (5.80 g, 37%): mp 125–127°C ($CHCl_3$/hexane); ^{1}H-NMR δ 2.93 (d, 2H, J = 4.0 Hz), 3.12–3.48 (m, 3H), 4.76 (ddd, 1H, J = 4.5, 8.2 and 9.9 Hz). Anal for $C_{11}H_{10}ClNO_2$ (C, H, N). **10g** (4.23 g, 27%): mp 125–128°C ($CHCl_3$/hexane); ^{1}H-NMR δ 2.87 (d, 2H, J = 3.8 Hz), 3.03–3.37 (m, 3H), 4.68 (ddd, 1H, J = 4.3, 7.6 and 9.7 Hz). Anal for $C_{11}H_{10}ClNO_2$ (C, H, N). **10h** (4.2 g, 27%): mp 129–131°C ($CHCl_3$/hexane); ^{1}H-NMR δ 2.91 (d, 2H, J = 3.8 Hz), 3.08–3.39 (m, 3H), 4.73 (ddd, 1H, J = 4.5, 8.4 and 10.1 Hz). Anal for $C_{11}H_{10}ClNO$ (C, H, N).

*General procedure for the preparation of **1c–h–4c–h***

The appropriate epoxide (**9c–h**, **10c–h**) (1.0 mmol) was treated as previously reported for the preparation of **1a–4a** [1]. The residue was dissolved in Et_2O and treated, in the case of

aminoalcohols **1c–h**, **2c,e–h**, **3c,e–h**, and **4c–h**, with a small excess of malic acid in a 4:1 anhydrous Et_2O/EtOH mixture, or, in the case of compounds **2d** and **3d**, with oxalic acid in a 7:3 Et_2O/MeOH mixture. The crude salts were then filtered and crystallized from the appropriate solvent (see table I). For analytical and spectral data, see tables I and III.

The salts of **1c–h–4c–h** were converted into the free bases by treating an aqueous solution of the salt with 10% aqueous K_2CO_3 and then extracting the free base with Et_2O. The organic layers were filtered and evaporated to give practically pure **1c–h–4c–h** (for ^{1}H-NMR spectral data, see table II). Anal for $C_{15}H_{22}N_2O_3$ (**1c–e**, **3c–e**), $C_{16}H_{24}N_2O_3$ (**2c–e**, **4c–d**), $C_{14}H_{19}ClN_2O_2$ (**1f–h**, **3f–h**), and $C_{15}H_{21}ClN_2O_2$ (**2f–h**, **4f–h**) (C, H, N).

Radioligand binding methods

Rat-brain β_1-adrenoceptors

β_1-Adrenoceptors were assayed in rat cortical membranes following the method previously described [1] and using ^{3}H-CGP 26505 [5] (1-(2-(3-carbamoyl-4-hydroxy)phenoxy)ethylamino)-3-(4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy-2-propanol) as the specific ligand (Du Pont de Nemours, New England Nuclear Division, specific activity 28.4 Ci/mmol).

Bovine lung β_2-adrenoceptors

β_2-Adrenoceptor binding was studied in bovine lung using ^{3}H-dihydroalprenolol (DHA) [6] as the ligand (Du Pont de Nemours, New England Nuclear Division, specific activity 48.1 Ci/mmol).

Membranes obtained as previously described [1] were suspended in phosphate buffer (4 mg/ml proteins) and incubated with 1 nM ^{3}H-DHA in the presence of 50 nM CGP 26505. After incubation at 25°C for 30 min, the samples were filtered on Whatman GF/BC fibre-glass filters and washed with 3 x 5 ml of phosphate buffer, dried and added to 8 ml Ready Protein Beckman scintillation cocktail. No specific binding was measured in the presence of 35 μM *l*-isoprenaline.

The affinity of drugs for the specific binding sites was expressed as the molar concentration inhibiting specific binding by 50% (IC_{50}). These values were calculated by log probit analysis of the displacement curves obtained for each compound by using 5 concentrations ranging from 10^{-7} M to 10^{-3} M. The dissociation constant (K_i) was derived from the equation of Cheng and Prusoff [16]. The ligand affinities (K_d) of ^{3}H-CGP 26505 and ^{3}H-DHA were 0.7 and 1.0 nM, respectively.

Pharmacological methods

Guinea-pig atria

The ability of the tested compounds to interact with β_1-adrenoceptors was investigated, as previously described [1], by assaying their effects on the contractile force of isolated guinea-pig atria.

Guinea-pig tracheal strips

The efficacy of the tested compounds on β_2-adrenoceptors was experimented on preparations of tracheal smooth musculature following the method previously described [1].

For both preparations, the antagonistic activity of the tested compounds towards β_1- and β_2-adrenoceptors was expressed as pIC_{50}, *ie* the negative log of the molar concentration that reduced the response to isoprenaline by 50% [17]. All compounds were tested at concentrations ranging from 10^{-9} to 10^{-3} M. Each antagonistic activity index was obtained by at least 5 active concentrations.

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