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Laboratory note

Conformationally restrained β-blocking oxime ethers. 3. Synthesis and β-adrenergic antagonistic activity of diastereomeric *anti* and *syn* 2-(5'-(3'-methyl)isoxazolidinyl)-*N*-alkylethanolamines*

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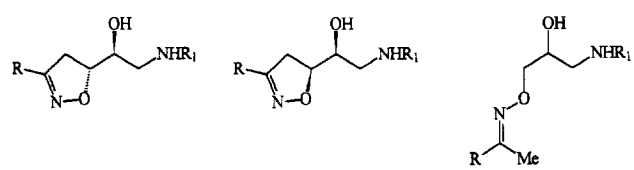
Summary — The diastereomeric *anti* (**1d**, **2d**) and *syn* (**3d**, **4d**) 2-(5'-(3'-methyl)isoxazolidinyl)-*N*-alkylethanolamines were synthesized and assayed for their β_1- and β_2-adrenergic antagonistic activity by functional tests on isolated preparations. The pharmacological results, which were compared with those previously obtained for the corresponding isoxazoline analogs (**1b–4b**) substituted in the 3'-position with an isopropyl group instead of the methyl group in **1d–4d**, indicated that the β-adrenergic antagonistic activity of the 3'-alkyl-substituted compounds **1–4** is not substantially influenced by the size of the alkyl substituent.

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

Introduction

In a previous report [2] from this laboratory, we described the synthesis and the β-blocking adrenergic properties of the diastereomeric 2-(5'-(3'-phenyl)-(**1a–4a**) and 2-(5'-(3'-isopropyl)isoxazolidinyl)ethanolamines (**1b–4b**) designed as semirigid analogs of the corresponding β-blocking oxime ethers **5a**, **6a** and **5b**, **6b**, in which the conformational freedom around the N-O bond is restricted by linking the methyl carbon adjacent to the imino system with the carbon adjacent to the ethereal oxygen. The 3'-phenyl-substituted compounds **1a–4a**, tested in vitro by both radioligand binding tests and functional assays, proved to maintain, in general, appreciable β-adrenergic properties, even if these were somewhat lower than those of the corresponding conformationally free oxime ethers **5a** and **6a** [2]. In contrast, the 3'-isopropyl-substituted compounds **1b–4b** showed, in the same types of tests, a more marked decrease in the β-adrenergic properties with respect to the corresponding open-chain oxime ethers **5b** and **6b** [2].



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

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

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

a, R = Ph; **b**, R = *i*-Pr; **c**, R = Ar; **d**, R = Me

Reasonable explanations for the differences in β-adrenergic properties found between the 3'-phenyl-(**1a–4a**) and the 3'-isopropyl-substituted (**1b–4b**) isoxazolines might be offered by either a possible role of the phenyl system in the interaction of compounds **1a–4a** with β-receptors, or a negative steric effect of the isopropyl group of **1b–4b**, which, unlike the planar phenyl group of **1a–4a**, might hinder the access of **1b–4b** to β-adrenoceptors.

On the basis of the first hypothesis, modifications in the electronic characteristics of the aromatic system of **1a–4a** might have been expected to produce variations in the adrenergic properties of these types of compounds. We therefore carried out the synthesis and

*For preceding paper, see reference [1].

evaluation of the biopharmacological β-adrenergic properties of a series of isoxazoline analogs of **1a–4a** (**1c–4c**) [1], in which the electronic properties of the phenyl ring of **1a–4a** were modulated by means of substituents that exert a variety of electronic effects, linked in the three possible positions of the aromatic ring. The appreciable β-adrenergic properties of several of the **1c–4c** compounds, which in some cases were better than those of the corresponding analogs not substituted on the phenyl ring of **1a–4a**, confirmed the hypothesis of the importance of the presence of the aromatic system in **1a,c–4a,c** for interaction with the receptor [1, 2]. However, as it proved to be impossible to detect any relationship between the substitution on the aromatic moiety and the adrenergic properties, the direct interaction of this system appeared to be excluded [1].

The results of this study did not contradict the alternative hypothesis formulated previously, namely that the low activity of **1b–4b** compared with that of **1a–4a**, might be due to steric factors linked with the hindrance of the isopropyl group of **1b–4b** [2].

One way of verifying the possible importance of steric reasons for the modest β-adrenergic properties of **1b–4b** appeared to be the synthesis and study of the β-adrenergic antagonistic activity of isoxazolinic compounds of types **1–4** containing, in the 3' position, an aliphatic substituent presenting less steric hindrance than the isopropyl group. This paper describes the synthesis and β-adrenergic antagonistic activity of the diastereomeric isoxazoline derivatives **1d–4d**[1] which present such a small-sized group as methyl in the 3'-position of the isoxazolinic nucleus.

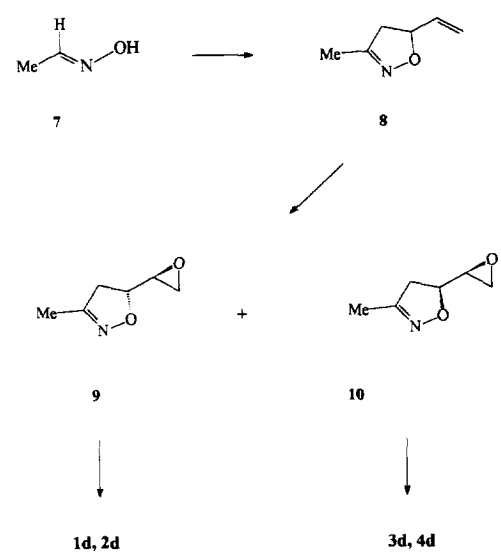
Chemistry

The *anti* (**1d**, **2d**) and *syn* (**3d**, **4d**) 2-(5'-(3'-methyl)-isoxazolidinyl)-*N*-alkyl-ethanolamine derivatives were prepared following the synthetic route previously used for the preparation of **1a,b–4a,b** [2] and **1c–4c** [1] (scheme 1). The acetaldoxime (**7**) was chlorinated by *N*-chlorosuccinimide to the corresponding acetylhydroxamyl chloride which, without being isolated, was treated with butadiene in the presence of triethylamine to afford 3-methyl-5-vinyl-2-isoxazoline **8** [3]. Oxidation of **8** with *m*-chloroperoxybenzoic acid yielded an approximately 1:1 mixture of the *anti* (**9**) and *syn* (**10**) epoxides, which were separated by preparative medium pressure liquid chromatography (MPLC). Aminolysis of epoxides **9** and **10** with *i*-$PrNH_2$ or *t*-$BuNH_2$ gave the corresponding *anti* (**1d**, **2d**) and *syn* (**3d**, **4d**) aminoalcohols, which were isolated as maleate salts.

The structure of compound **8**, and in particular the position of the olefinic chain on the isoxazolinic nucleus, was attributed by analogy with that of the analogous intermediates obtained in the synthetic procedure leading to **1a,b–4a,b** [2] and **1c–4c** [1].

The configuration of the *anti* (**1d**, **2d**) and *syn* (**3d**, **4d**) isoxazoline aminoalcohols was assigned on the basis of the analogies existing between their ^1H NMR spectral data (see *Experimental protocols*) and those of the previously studied isoxazolidine analogs (**1a,b–4a,b** and **1c–4c**), whose structure had been unequivocally determined by studies of protonic nuclear magnetic resonance and by an X-ray analysis of the isoxazolinic compound **2a** [1, 2]. The *anti* compounds **1d** and **2d**, as free bases, exhibited $J_{5',2}$ values higher than those of the *syn* isomers **3d** and **4d**, as previously found for *anti* (**1a–c** and **2a–c**) and *syn* (**3a–c** and **4a–c**) analogs. In addition, in analogy with findings for **1a–c**, **2a–c** and **3a–c**, **4a–c**, compounds **1d** and **2d**, as free bases, showed differences in the chemical shifts of the two H(1) protons, which were higher than those found for the same protons in the *syn* isomers **3d** and **4d** [1, 2].

The relative configuration of epoxides **9** and **10**, *anti* and *syn*, respectively, was then assigned on the basis of those of the *anti* (**1d**, **2d**) and *syn* (**3d**, **4d**) aminoalcohols, bearing in mind that in the aminolysis reactions of epoxides **9** and **10** leading to aminoalcohols **1d**, **2d** and **3d**, **4d**, respectively, the two chiral centers of **9** and **10** are not involved.



Scheme 1.

[1]Compounds **1d–4d** were synthesized and tested as racemates. However, in the scheme and formulas, only the enantiomer in which the relative configuration on C(3) corresponds to that of the natural cathecholamines is shown.

As regards the conformational situation in solution, the analogies between the coupling constant values of the new (**1d–4d**) and the corresponding previously studied analogs (**1a–c–4a–c**) [1, 2] made it possible to also assign conformational profiles to **1d–4d** similar to those previously determined for **1a–c–4a–c**.

Results and conclusion

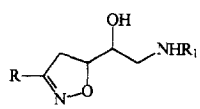
The isoxazoline derivatives **1d–4d**, their corresponding oxime open-chain analogs **5d**, **6d** [4], and the reference drug dichloroisoproterenol were tested on isolated guinea-pig atria and on guinea-pig tracheal strips for their antagonistic activity on β_1- and β_2-adrenoceptors, respectively (see table I). Table I also shows the values previously obtained in the same types of tests with the 3'-isopropyl-substituted isoxazolines **1b–4b** [2].

On atrial β_1-adrenoceptors, both the *anti* (**1d**, **2d**) and *syn* (**3d**, **4d**) compounds proved to be devoid of any β_1-adrenergic activity. The oxime ether derivatives **5d** and **6d**, in contrast, showed appreciable activity indices. On the same adrenoceptors, only the 3'-isopropyl-substituted isoxazolines **1b** and **3b** had previously shown modest activity indices, while **2b** and **4b** had proved to be devoid of any activity [2].

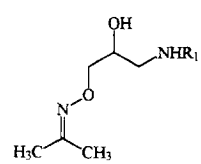
As regards tracheal β_2-adrenoceptors, among the isoxazoline derivatives **1d–4d**, only the *anti-N*-isopropyl-substituted compound **1d** exhibited a significant pIC_{50} value. The oxime ethers **5d** and **6d**, in contrast, showed β_2-blocking activities slightly lower or higher, respectively, than that of the reference drug dichloroisoproterenol. On β_2-adrenoceptors, all compounds **1b–4b** had previously been found to possess a weak antagonistic activity [2].

The differences observed between the results obtained by us for compounds **5d** and **6d** on both types of β-adrenoceptors and those previously reported by other authors for the same isolated tissues [4], may be tentatively attributed to the different strains of guinea pig used in the old and new tests. As regards β_1-adrenoceptors, the differences may be attributed to the fact that previous values were obtained by measuring the

Table I. β-Adrenoreceptor antagonistic activity of compounds **1d–6d**.



1d-4d



5d, 6d

Compound	*R*	R_1	*Stereoisomer*	*β-Adrenergic activity*[a] pIC_{50}	
				Isolated guinea-pig atria (β_1)	*Isolated guinea-pig tracheal strips* (β_2)
1b·$H_4C_4O_4$	*i*-Pr	*i*-Pr	*anti*	3.59 ± 0.31[b]	3.49 ± 0.22[b]
2b·$H_4C_4O_4$	*i*-Pr	*t*-Bu	*anti*	–[b]	3.91 ± 0.12[b]
3b·$H_4C_4O_4$	*i*-Pr	*i*-Pr	*syn*	3.60 ± 0.40[b]	3.54 ± 0.59[b]
4b·$H_4C_4O_4$	*i*-Pr	*t*-Bu	*syn*	–[b]	4.39 ± 0.06[b]
1d·$H_4C_4O_4$	Me	*i*-Pr	*anti*	–	4.17 ± 0.14[b]
2d·$H_4C_4O_4$	Me	*t*-Bu	*anti*	–	3.5[c]
3d·$H_4C_4O_4$	Me	*i*-Pr	*syn*	–	–
4d·$H_4C_4O_4$	Me	*t*-Bu	*syn*	3.5[c]	–
5d·$H_4C_4O_4$	–	*i*-Pr		4.51 ± 0.06[d]	5.29 ± 0.10[e]
6d·HCl	–	*t*-Bu		4.72 ± 0.06[f]	7.08 ± 0.09[g]
Dichloroisoproterenol				6.94 ± 0.23	6.01 ± 0.55

[a]The values represent the mean of three to five experiments for each drug ± standard error. [b]From reference [2]. [c]Approximate value. [d]Reference [4]: pA_2 6.24 ± 0.19. [e]Reference [4]: pA_2 6.71 ± 0.16. [f]Reference [4]: pA_2 6.51 ± 0.05. [g]Reference [4]: pA_2 7.65 ± 0.32.

chronotropic effects of the drugs on preparations made up only of right atria, while we evaluated the inotropic response to the same drugs on the right atria of preparations made up of both atria.

Compounds **1d–4d** were synthesized with the purpose of testing the effects on the β-adrenergic activity of the substitution of the 3'-isopropyl group of **1b–4b** with a substituent presenting less steric hindrance, such as the methyl group. A comparison of the activity indices of **1d–4d** with those previously obtained for the corresponding compounds **1b–4b** does not reveal any substantial variation in the β-adrenergic activity of **1d–4d** compared with that of **1b–4b**. This indicates that for compounds of types **1–4** substituted in the 3' position with an aliphatic group, the size of the substituent does not appear to have any appreciable effect on the ability of these compounds to interact with β-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 with an FTIR Mattson 1000 Unicam spectrometer, as liquid films for the oils, or as paraffin oil mulls for the solids. ^{1}H NMR spectra of all compounds were routinely detected with a Varian CFT-20 instrument operating at 80 MHz in a ca 2% 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 standards, respectively. The ^{1}H NMR spectral study of **1d–4d** was performed with a Bruker AC-200 instrument, and spectral parameters were refined by a MOLE (LAOCOON) program, using an Atari PC 3 computer. The parameters obtained should be correct to within ± 0.2 Hz. The electron impact mass spectra were recorded on a Hewlett Packard 5988A spectrometer by direct introduction at a nominal electron energy of 70 eV and a source temperature of 300 °C. GLCs were performed on a C Erba model 4200 apparatus with a flame ionization detector and a 1.5 m × 2.50 mm neopentylglycolsuccinate 10% on chromosorb W 80/100 mesh column. 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. 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%.

3-Methyl-5-vinyl-2-isoxazoline 8

Acetaldoxime **7** (15.0 g, 0.25 mol) was added at room temperature in a single portion to a stirred mixture of *N*-chlorosuccinimide (33.9 g, 0.25 mol) in anhydrous $CHCl_3$ (230 mL) and pyridine (1.2 mL). After 15 min, the resulting solution was cooled (0 °C) and then treated successively with an excess of 1,3-butadiene (41.0 g, 0.75 mol) and, dropwise, with a solution of Et_3N (38.3 g, 0.38 mol) in anhydrous $CHCl_3$ (56 mL). After 3 h at room temperature, the reaction mixture was washed with brine, dried, and evaporated to yield a crude oily residue which was purified by chromatography on a silica gel column (70–230 mesh) eluting with a 7:3 petroleum ether/AcOEt mixture to yield pure **8** (12.0 g, 40%): bp 65 °C/5 mmHg (reference [3]: bp 69 °C/9 mm Hg).

anti- and syn-2-((3'-Methyl)-5'-isoxazolidinyl)oxirane 9 and 10

A stirred mixture of **8** (2.0 g, 18.0 mmol) and $NaHCO_3$ (3.02 g, 36.0 mmol) in anhydrous CH_2Cl_2 (16 mL) was cooled to 0 °C and then treated dropwise with a solution of 70% *m*-chloroperoxybenzoic acid (8.83 g, 36.0 mmol) in anhydrous CH_2Cl_2 (55 mL). The resulting mixture was stirred under nitrogen at room temperature for 72 h, and then filtered, washed (3% aqueous K_2CO_3, 1 N aqueous $Na_2S_2O_3$ and brine), dried, and evaporated to dryness. The crude residue, consisting almost exclusively of a 1:1 mixture of the diastereomeric *anti*-epoxide **9** and *syn*-epoxide **10** (GLC), 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 afforded pure *anti*-epoxide **9**, whereas the subsequent fractions yielded the *syn* epoxide **10**. **9** (0.35 g, 15%): oil; ^{1}H NMR δ 1.94 (m, 3H), 2.55 (dd, 1H, J = 4.8 and 2.5 Hz), 2.78 (dd, 1H, J = 4.8 and 4.0 Hz), 2.76 (dd, 1H, J = 17.1 and 7.3 Hz), 2.95 (dd, 1H, J = 17.1 and 10.4 Hz), 3.03 (ddd, 1H, J = 4.5, 4.0 and 2.5 Hz), 4.46 (ddd, 1H, J = 10.4, 7.3 and 4.5 Hz). Anal $C_6H_9NO_2$ (C, H, N). **10** (0.30 g, 13%): oil, ^{1}H NMR δ 1.93 (m, 3H), 2.74 (d, 1H, J = 0.0 and 3.4 Hz), 2.74 (d, 1H, J = 0.0 and 3.4 Hz), 2.81 (dd, 1H, J = 17.1 and 7.7 Hz), 3.03 (dd, 1H, J = 17.1 and 10.8 Hz), 3.04 (ddd, 1H, J = 4.5, 3.4 and 3.4 Hz), 4.49 (ddd, 1H, J = 10.8, 7.7 and 4.5 Hz). Anal $C_6H_9NO_2$ (C, H, N).

General procedure for the preparation of 1d–4d

A stirred solution of the appropriate epoxide **9** or **10** (0.20 g, 1.57 mmol) and *i*-$PrNH_2$ or *t*-$BuNH_2$ (7.2 mmol) in a 1:2 anhydrous benzene/EtOH mixture (4.2 mL) was stirred at room temperature for 72 h, and then evaporated to dryness. The crude oily residue was taken up in 10% aqueous HCl and brine, and the resulting mixture was washed with Et_2O, alkalinized with solid K_2CO_3, and then extracted with $CHCl_3$. The washed (H_2O) and filtered $CHCl_3$ layer was evaporated, and the residue was dissolved in Et_2O and treated with a small excess of maleic acid in a 4:1 a hydrous Et_2O/EtOH mixture. The crude product was filtered and crystallized from EtOH/Et_2O to yield the pure maleate salt of **1d–4d**. **1d**·$H_4C_4O_4$ (0.30 g, 63%): mp 164–166 °C; ^{1}H NMR δ 1.08 (2d, 6H, J = 6.6 Hz), 1.74 (s, 3H), 2.65–3.05 (m, 4H), 3.21, (hept, 1H, J = 6.6 Hz), 3.72 (m, 1H), 4.34 (m, 1H). Anal $C_{13}H_{22}N_2O_6$ (C, H, N). MS *m/z* 187 (MH^+). **2d**·$H_4C_4O_4$ (0.40 g, 80%): mp 171–174 °C; ^{1}H NMR δ 1.12 (s, 9H), 1.73 (s, 3H), 2.60–3.05 (m, 1H), 4.34 (m, 1H). Anal $C_{14}H_{24}N_2O_6$ (C, H, N). MS *m/z* 201 (MH^+). **3d**·$H_4C_4O_4$ (0.25 g, 53%): mp 120–122 °C; ^{1}H NMR δ 1.05 (2d, 6H, J = 6.6 Hz), 1.75 (s, 3H), 2.74 (dd, 1H, J = 18.0 and 7.6 Hz), 2.91 (dd, 1H, J = 13.0 and 9.3 Hz), 3.00 (dd, 1H, J = 13.0 and 3.4 Hz), 3.25 (hept, 1H, J = 6.6 Hz), 3.71 (ddd, 1H, J = 9.3, 3.4 and 3.2 Hz), 4.40 (ddd, 1H, J = 10.9, 7.6 and 3.2 Hz). Anal $C_{13}H_{22}N_2O_6$ (C, H, N). MS *m/z* 187 (MH^+). **4d**·$H_4C_4O_4$ (0.33 g, 66%): mp 160–163 °C; ^{1}H NMR δ 1.14 (s, 9H), 1.74 (s, 3H), 2.74 (dd, 1H, J = 17.8 and 7.5 Hz), 2.86 (dd, 1H, J = 13.2 and 9.8 Hz), 2.98 (dd, 1H, J = 13.2 and 3.1 Hz), 3.02 (dd, 1H, J = 17.8 and 10.8 Hz), 3.67 (ddd, 1H, J = 9.8, 3.1 and 3.1 Hz), 4.00 (ddd, 1H, J = 10.8, 7.5 and 3.1 Hz). Anal $C_{14}H_{24}N_2O_6$ (C, H, N). MS *m/z* 201 (MH^+).

The salts of **1d–4d** were converted into the free bases by treating an aqueous solution of the salt with 10% aqueous K_2CO_3 and extracting the free base with Et_2O. The organic layers were filtered and evaporated to give practically pure **1d–4d**. **1d**: oil; ^{1}H NMR δ 0.99 (2d, 6H, J = 6.2 Hz), 1.92 (s,

3H), 2.47 (dd, 1H, J = 12.2 and 8.5 Hz), 2.72 (hept, 1H, J = 6.2 Hz), 2.75 (dd, 1H, J = 12.2 and 3.5 Hz), 2.89 (dd, 1H, J = 17.4 and 8.0 Hz), 2.95 (dd, 1H, J = 17.4 and 10.1 Hz), 3.60 (ddd, 1H, J = 8.5, 5.9 and 3.5 Hz), 4.35 (ddd, 1H, J = 10.1, 8.0 and 5.9 Hz). Anal $C_9H_{18}N_2O_2$ (C, H, N). **2d**: oil; ^{1}H NMR δ 1.03 (s, 9H), 1.92 (s, 3H), 2.45 (dd, 1H, J = 12.0 and 7.9 Hz), 2.73 (dd, 1H, J = 12.0 and 3.8 Hz), 2.87 (dd, 1H, J = 17.2 and 8.1 Hz), 2.95 (dd, 1H, J = 17.2 and 9.9 Hz), 3.50 (ddd, 1H, J = 7.9, 6.4 and 3.8 Hz), 4.34 (ddd, 1H, J = 9.9, 8.1 and 6.4 Hz). Anal $C_{10}H_{20}N_2O_2$ (C, H, N). **3d**: oil; ^{1}H NMR δ 1.04 (2d, 6H, J = 6.3 Hz), 1.96 (s, 3H), 2.68 (dd, 1H, J = 11.6 and 6.5 Hz), 2.70 (dd, 1H, J = 11.6 and 4.1 Hz), 2.76 (hept, 1H, J = 6.3 Hz), 2.90 (dd, 1H, J = 16.8 and 9.2 Hz), 2.95 (dd, 1H, J = 16.8 and 9.4 Hz), 3.57 (ddd, 1H, J = 6.5, 4.1 and 3.8 Hz), 4.50 (ddd, 1H, J = 9.4, 9.2 and 3.8 Hz). Anal $C_9H_{18}N_2O_2$ (C, H, N). **4d**: oil; ^{1}H NMR δ 1.03 (s, 9H), 1.92 (s, 3H), 2.63 (dd, 1H, J = 11.6 and 8.5 Hz), 2.65 (dd, 1H, J = 11.6 and 3.7 Hz), 2.90 (dd, 1H, J = 16.8 and 9.3 Hz), 2.91 (dd, 1H, J = 16.8 and 9.4 Hz), 3.49 (ddd, 1H, J = 7.6, 3.7 and 3.6 Hz), 4.47 (ddd, 1H, J = 9.4, 9.3 and 3.6 Hz). Anal $C_{10}H_{20}N_2O_2$ (C, H, N).

*3-[(Isopropylideneamino)oxy]-1-(alkylamino)-2-propanolol derivatives **5d**·$H_4C_4O_4$ and **6d**·HCl*

Compounds **5d**·$H_4C_4O_4$ and **6d**·HCl were obtained following the synthetic route described previously [4]. **5d**·$H_4C_4O_4$: mp 118–120 °C (reference [4]: 119 °C). **6d**·HCl: mp 129–131 °C (reference [4]: 131 °C)

Pharmacological methods

Guinea-pig atria

The ability of the compounds to interact with β_1-adrenoceptors was investigated by assaying their effects on the contractile force of isolated guinea-pig atria. Atria were taken from albino male guinea pigs, average weight 300 g, sacrificed by cervical dislocation after light ether anesthesia. The specimens, consisting of both atria, were rapidly dissected from the ventriculi and cleaned to obtain strips that were suspended in organ baths containing saline solution at 32 °C gassed with pure O_2. The composition of the saline solution in mM was as follows: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4 \cdot 7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; glucose 5.5. The organs were connected to an isometric transducer (mod Basile 7003) and left to stabilize for 45 min before starting the experiments. They were then submitted to increasing doses of isoprenaline to obtain dose–response curves with the method of single doses. Spontaneous activity and responses to drugs were recorded with a microdynamometer (mod Basile 7050).

The antagonistic action of the drugs was evaluated as their ability to reduce, in a dose-dependent manner and after 15 min incubation time, the inotropic response to a stated concentration of isoprenaline (1×10^{-8} M) corresponding to the dose that promoted about 75% of the maximal response to this reference drug.

Guinea-pig tracheal strips

The efficacy of the compounds tested on β_2-receptors was experimented on preparations of tracheal smooth musculature. These organs were taken from the same animals employed for atria assays. The organs were carefully dissected out, transferred to a dish with saline solution and cut helically to obtain strips of 30 × 4 mm. The composition of the saline solution in mM was: NaCl 118; KCl 4.75; $CaCl_2$ 2.50; $MgSO_4 \cdot 7H_2O$ 1.19; KH_2PO_4 1.19; $NaHCO_3$ 25; glucose 11.5. Each strip was mounted in a 10 mL organ bath containing the physiological solution kept at 37 °C and gassed with carbogen (5% CO_2/95% O_2). The tracheal preparations were submitted to a tension of 0.5 g and connected to an isotonic force displacement transducer (mod Basile 7006), which recorded the responses of the organs to drug administration on a microdynamometer (mod Basile 7050). The tissues were allowed to stabilize for 1 h before starting the experiments. A dose–effect curve to isoprenaline was obtained in each organ by the method of cumulative doses, and then the antagonistic activities of the compounds under test were assessed.

The antagonistic effects of the drugs on these receptors were evaluated on the basis of their dose-dependent inhibitory effect on the response of this musculature to the agonist isoprenaline at a dose that corresponds to the ED_{75} of the reference agonist in this tissue. The incubation time was 15 min. For both preparations, the antagonistic activity of the compounds tested towards β_1- and β_2-receptors was expressed as pIC_{50}, ie, the negative log of the molar concentration that reduced the response to isoprenaline by 50% [5].

Isoprenaline was used as hydrochloride, while **1b–4b** and **1d–4d** were used as maleate, compound **5d** as fumarate and compound **6d** as hydrochloride.

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