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Synthesis and β-adrenergic properties of (*E*)-*N*-[3-(alkylamino)-2-hydroxypropylidene](methyloxy)amines substituted with an aromatic group on their [(methyloxy)imino]methyl moiety (MOIMM): an investigation into the biopharmacological effects of an aryl substitution in the class of MOIM β-blocking drugs

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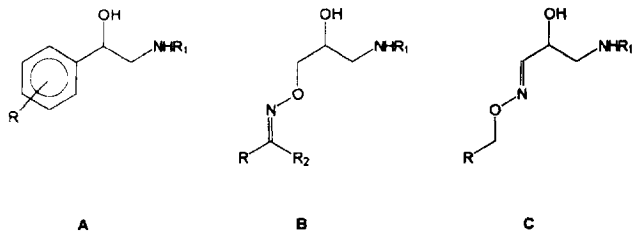
Summary — *N*-Isopropyl-(**5a–g**) and *N*-*t*-butyl-substituted (**6a–g**) (*E*)-*N*-[3-(amino)-2-hydroxypropylidene](arylmethyloxy)amines, which present an aromatic ring (Ar) linked to the CH_2 carbon of the [(methyloxy)imino]methyl moiety (MOIMM), were synthesized with the aim of comparing their β-adrenergic properties with those of the previously studied completely aliphatic analogs **1,2** and **3,4**. Compounds **5** and **6** were tested for their affinity towards β_1- and β_2-adrenoceptors by radioligand binding experiments; the compounds with the highest affinity were also assayed for their β_1- and β_2-adrenergic activity by functional tests on isolated preparations. The biopharmacological results show that, for the MOIM derivatives studied (**1–6**), the presence of an Ar substituent linked to the MOIM, as in **5** and **6**, does not have any appreciable effect on the β_1-adrenergic properties in terms of affinity and activity; this type of substituent, on the contrary, appears to be capable of improving the β_2-adrenergic properties, as far as the receptor affinity is concerned. These results are discussed on the basis of a comparison of the conformational and electronic characteristics.

adrenergic drug / β-blocking agent / [(methyloxy)imino]methyl moiety / (*E*)-*N*-[3-(amino)-2-hydroxypropylidene](arylmethyloxy)amine

Introduction

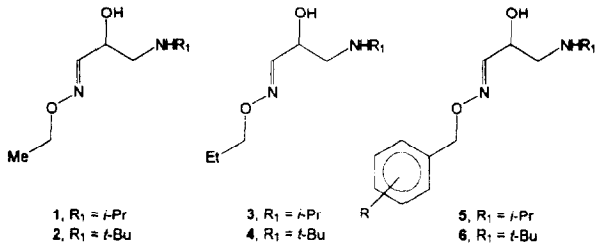
During studies on the role played by the various molecular factors in the interaction of β-adrenergic drugs with their receptor, we found that compounds of type **B**, which are either completely aliphatic or substituted on the imino carbon by an aromatic ring, maintain the β-blocking properties of type **A** compounds [1–3]. On the basis of these results, the existence of a bioisosteric relationship between the Ar and the [(methyleneamino)oxy]methyl moiety (C=NOCH$_2$, MAOMM) was hypothesized in the field of adrenergic drugs. This Ar/MAOMM bioisosterism was then succesfully verified in non-adrenergic drugs in which the Ar seems to be important for the activity, such as antiinflammatory arylacetic acids [4, 5], β-lactam antibiotics [6, 7], biogenic amine uptake inhibitors [8], and antidepressant drugs [9].

More recently, starting from the consideration that the formal inversion of the atomic sequence C=NOCH$_2$ of the MAOMM leads to a different type of group, the [(methyloxy)imino]methyl moiety (CH$_2$ON=C, MOIMM), which, in the *E* configuration, appears to present greater steric and electronic analogies with an Ar rather than the MAOMM, some completely aliphatic type **C** compounds, in which the



A B C

MAOMM is substituted by the MOIMM, were designed [10] as analogs of type **B** β-adrenergic drugs. The similar β-adrenergic properties of aliphatic type **C** compounds **1–4** compared with those of the corresponding type **B** isomers indicate the ability of the MOIMM to substitute the MAOMM effectively as a bioisoster of Ar, at least in the field of β-adrenergic drugs [10].



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

Overall, the results obtained for the MAOM derivatives studied reveal that while an aromatic substituent linked to the iminic carbon of the MAOMM is not essential for the activity, it may be capable of improving it [4, 5, 8, 9]. In the case of adrenergic drugs, an examination of our data [2] and those reported in literature [11] for other type **B** derivatives shows that compounds with an aromatic substituent linked to the MAOMM possess β-adrenergic properties that are often better than those of completely aliphatic compounds, especially as far as the affinity is concerned.

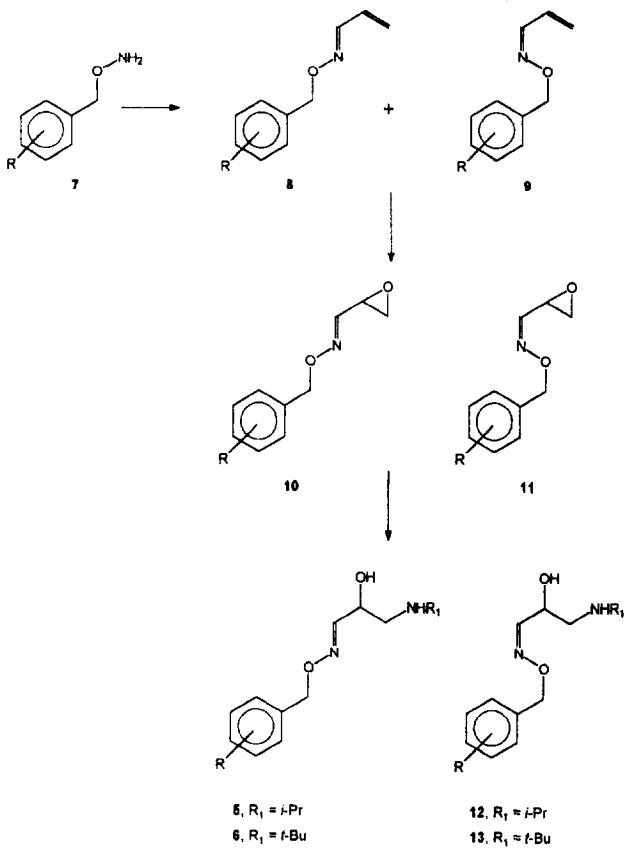
This observation suggested the idea of examining whether also in the case of the MOIM derivatives of type **C**, the presence of an aromatic nucleus linked to the CH_2 carbon of the MOIMM might have a positive influence on the β-adrenergic properties of these compounds.

This work reports the synthesis and the biopharmacological β-adrenergic properties of a series of (*E*)-*N*-[3-(amino)-2-hydroxypropylidene](arylmethyloxy)-amines of type **C** (**5a–g** and **6a–g**) in which the phenyl ring is unsubstituted (**5a** and **6a**), or substituted in the *ortho*, *meta*, or *para* position by substituents that can exercise different electronic effects, such as the methoxy group (**5b–d** and **6b–d**) or the chlorine atom (**5e–g** and **6e–g**).

Chemistry

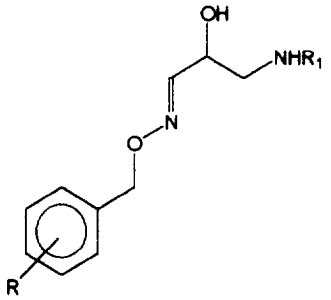
The (*E*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene] (**5a–g**) and (*E*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](arylmethyloxy)amines (**6a–g**) (table I) were prepared using the synthetic procedure shown in scheme 1.

Condensation of the hydrochloride salts of *O*-arylmethylhydroxylamines [12] (**7a–g**) with acrolein afforded mixtures of the corresponding *E* (**8**) and *Z* (**9**) unsaturated oxime ethers in a ratio of approximately 7:3, which were separated by column chromatography only in the cases of **8g** and **9g**. Epoxidation with *m*-chloroperoxybenzoic acid of both the crude isomeric mixtures of **8** and **9** and of the isolated compounds in the case of **8g** and **9g**, yielded mixtures of the corresponding *E* (**10**) and *Z* (**11**) epoxides in a ratio of about 4:1, which could not be separated by the usual fractioning techniques. Aminolysis of the mixtures of **10** and **11** with *i*-$PrNH_2$ or *t*-$BuNH_2$ and subsequent treatment of the crude product with oxalic acid, afforded mixtures of the oxalate salts of the aminoalcohols *E* (**5** and **6**) and *Z* (**12** and **13**) in the same ratio as the corresponding epoxides, from which the *E* compounds **5a–g**·$H_2C_2O_4$ and **6a–g**·$H_2C_2O_4$ were isolated by fractional crystallization.



Scheme 1. **a**, R = H; **b**, R = *o*-MeO; **c**, R = *m*-MeO; **d**, R = *p*-MeO; **e**, R = *o*-Cl; **f**, R = *m*-Cl; **g**, R = *p*-Cl.

Table I. Analytical and chemical data of (*E*)-*N*-[3-(isopropylamino)-(**5a–g**·$H_2C_2O_4$) and (*E*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](arylmethyloxy)amine oxalates (**6a–g**·$H_2C_2O_4$).



5a-g, 6a-g

Compound	*R*	R_1	*Mp (°C)*	*Yield*[a] *(%)*	*Formula*[b]
5a·$H_2C_2O_4$	H	*i*-Pr	75	40	$C_{15}H_{22}N_2O_6$
6a·$H_2C_2O_4$·1/4 H_2O	H	*t*-Bu	107–109	32	$C_{16}H_{24}N_2O_6$·1/4 H_2O
5b·$H_2C_2O_4$·1/4 H_2O	*o*-MeO	*i*-Pr	116–118	58	$C_{16}H_{24}N_2O_7$·1/4 H_2O
6b·$H_2C_2O_4$	*o*-MeO	*t*-Bu	118–119	60	$C_{17}H_{26}N_2O_7$
5c·$H_2C_2O_4$·1/2 H_2O	*m*-MeO	*i*-Pr	104–106	65	$C_{16}H_{24}N_2O_7$·1/2 H_2O
6c·$H_2C_2O_4$	*m*-MeO	*t*-Bu	95–97	58	$C_{17}H_{26}N_2O_7$
5d·$H_2C_2O_4$	*p*-MeO	*i*-Pr	128–129	61	$C_{16}H_{24}N_2O_7$
6d·$H_2C_2O_4$·1/2 H_2O	*p*-MeO	*t*-Bu	95–96	61	$C_{17}H_{26}N_2O_7$·1/2 H_2O
5e·$H_2C_2O_4$·1/2 H_2O	*o*-Cl	*i*-Pr	111–112	45	$C_{15}H_{21}N_2O_6Cl$·1/2 H_2O
6e·$H_2C_2O_4$	*o*-Cl	*t*-Bu	109–111	51	$C_{16}H_{23}N_2O_6Cl$
5f·$H_2C_2O_4$	*m*-Cl	*i*-Pr	107–109	35	$C_{15}H_{21}N_2O_6Cl$
6f·$H_2C_2O_4$	*m*-Cl	*t*-Bu	108–109	50	$C_{16}H_{23}N_2O_6Cl$
5g·$H_2C_2O_4$	*p*-Cl	*i*-Pr	107–109	45	$C_{15}H_{21}N_2O_6Cl$
6g·1/2 $H_2C_2O_4$	*p*-Cl	*t*-Bu	179–181	55	$C_{15}H_{22}N_2O_4Cl$

[a]For the epoxide aminolysis; no efforts were made to optimize yields; [b]all compounds were analyzed for C, H, and N.

The configurations around the N=C double bond of the couples of unsaturated oxime ethers (**8** and **9**), epoxides (**10** and **11**), and *N*-isopropyl- (**5**·$H_2C_2O_4$ and **12**·$H_2C_2O_4$), and *N*-*tert*-butyl-substituted (**6**·$H_2C_2O_4$ and **13**·$H_2C_2O_4$) aminoalcohols were assigned on the basis of a comparison of their ^{1}H-NMR spectral characteristics (see table II and *Experimental protocols*) with those of the previously studied aliphatic analogs **1–4** and the intermediates obtained during their synthesis, together with their corresponding *Z*-isomers [10]. These configurations were compared with reports in the literature for similar oxime derivatives [13]. In the compounds with the *E* configuration (**8**, **10**, **5**·$H_2C_2O_4$ and **6**·$H_2C_2O_4$), the proton linked to the carbon of the iminomethyl portion (H(1) of fig 1), resonates at lower fields (0.61–0.73 ppm) with respect to the same hydrogen of the corresponding *Z*-isomers (**9**, **11**, **12**·$H_2C_2O_4$ and **13**·$H_2C_2O_4$), due to the paramagnetic effect of the spatially proximal oximethereal oxygen. On the other hand, in the compounds with the *Z* configuration (**9**, **11**, **12**·$H_2C_2O_4$ and **13**·$H_2C_2O_4$), it is the proton linked to the carbon in the alpha position with respect to the C=N portion ((H2) of fig 1) which, being on the same side as the oximethereal oxygen, resonates at lower fields (0.52–0.63 ppm) than the same hydrogen of the corresponding *E* isomers (**8**, **10**, **5**·$H_2C_2O_4$ and **6**·$H_2C_2O_4$) [10, 13].

The analogies that exist between the spectral parameters of the aminoalcohols **5**·$H_2C_2O_4$ and **6**·$H_2C_2O_4$ and those of the corresponding previously studied aliphatic analogs **1,3** and **2,4** [10] make it possible to extend the considerations already expressed for compounds **1–4**, to the new compounds (**5**·$H_2C_2O_4$ and **6**·$H_2C_2O_4$), as far as their conformational situation around the C(2)-C(3) bond in solution is concerned. In particular, in the spectra of salts of the compounds **5** and **6**, the presence of two coupling constants between the proton linked to C(2) [H(2)] and the two protons linked to C(3) [H(3)], one with a fairly high value (8.5–8.9 Hz) and the other with a relatively low value

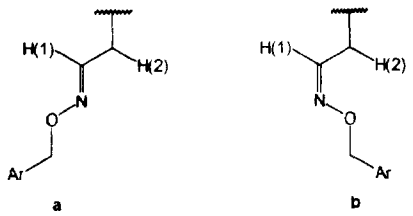


Fig 1. Drawing showing the spatial relationship between the H(1) and H(2) protons and the oximic oxigen in the *E* (**a**) and *Z* (**b**) MOIM derivatives.

(3.4–4.6 Hz), indicates the existence of a preferential conformer in which the H(2) proton is in a *trans* and *gauche* relationship with the two protons linked to C(3), as is found only in conformers α and β of figure 2. This excludes the possibility of any appreciable participation in the conformational equilibrium of conformer γ, in which the H(2) proton is in a *gauche* relationship with both the H(3) protons. Of the α and β rotamers, it is possible to assign the more important role in the conformational equilibrium to rotamer α which can be stabilized by the formation of an internal hydrogen bond between the cationic group and the hydroxyl oxygen [14]. Consequently, the relative vicinal coupling constants can be attributed to the H(3a) proton and the H(3b) proton, which cannot be directly identified in the spectra of **5**·$H_2C_2O_4$ and **6**·$H_2C_2O_4$.

Results

Radioligand binding assays

The affinity towards β-adrenoceptors of the aryl-substituted MOIM derivatives **5** and **6** and dichloroisoproterenol and propranolol, as the reference drugs, was checked by binding tests on rat brain membranes for β_1-adrenoceptors and on bovine lung membranes for β_2-adrenoceptors (table III). [^{3}H] CGP 26505 [15] was used as the specific tritiated ligand for rat brain β_1-adrenoceptors. [^{3}H]Dihydroalprenolol [16] 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 [17]. Table III also shows the results previously obtained by us in the same types of tests with the completely aliphatic MOIM derivatives **1,3** and **2,4** [10].

Rat brain β_1-adrenoceptors

All MOIM derivatives **5** and **6** exhibited an appreciable binding affinity towards this type of β-receptor. Among the *N*-isopropyl-substituted compounds **5a–g**, no compound showed an affinity higher than that of the completely aliphatic MOIM derivatives **1** and **3**. The affinity indices, which for the phenyl derivative **5a** and for all the chlorophenyl-substituted compounds **5e–g** were slightly higher than those of **1** and **3**, increased on passing from *m*-MeO- (**5c**) and *p*-MeO- (**5d**) to *o*-MeO-phenyl (**5b**) compounds.

As regards the *N-tert*-butyl-substituted **6a–g**, the *p*-Cl- (**6g**) and the *p*-MeO-substituted (**6d**) compounds presented a higher and a lower affinity, respectively, than the aliphatic *N-tert*-butyl substituted analogs **2** and **4**. The other MOIM derivatives, showed affinity indices only slightly lower than those of **2** and **4**.

Bovine lung β_2-adrenoceptors

Both *N*-isopropyl- (**5a–g**) and *N-tert*-butyl-substituted (**6a–g**) MOIM derivatives showed an affinity higher than that of the completely aliphatic *N*-isopropyl- (**1,3**) and *N-tert*-butyl-substituted (**2,4**) compounds, respectively. As regards the *N*-isopropyl-substituted derivatives **5a–g**, both the *meta* (**5c,f**) and *para* (**5d,g**) phenyl-substituted compounds exhibited slightly lower K_i values than that of the unsubstituted compound, while the *ortho*-substituted compounds (**5b,e**) showed slightly higher K_i values. Among the *N-tert*-butyl-substituted compounds (**6a–g**), only the *o*-MeO-phenyl derivative (**6b**) showed a K_i value higher than that of the phenyl-unsubstituted compound. All the other MOIM derivatives **6c–g** exhibited affinity indices slightly lower than that of **6a**. The best affinity was found for the *p*-chlorophenyl-substituted compound **6g**, whose K_i value was a quarter of that of **6a**.

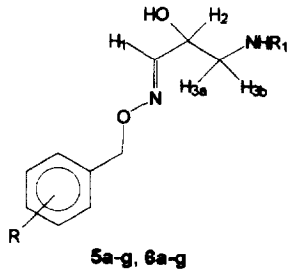
Functional tests

MOIM derivatives that showed an affinity index of 2000 nM or lower in the binding tests on rat brain β_1-adrenoceptors (**6a–c** and **6f,g**) and the reference drugs dichloroisoproterenol and propranolol 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 IV, together with those previously obtained in the same types of tests with the completely aliphatic *N-tert*-butyl-substituted analogs **2** and **4** [10].

Guinea-pig atria β_1-adrenoceptors

All the compounds examined (**6a–c,f,g**) exhibited a β_1-blocking activity, revealed by their ability to antagonize the stimulating effects of isoprenaline. The compound unsubstituted on the phenyl ring (**6a**) and

Table II. ^{1}H-NMR data of (*E*)-*N*-[3-(isopropylamino)-(**5a–g**•$H_2C_2O_4$) and (*E*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene]-(arylmethyloxy)amine oxalates (**6a–g**•$H_2C_2O_4$).



5a-g, 6a-g

Compound	*R*	R_1	H_1	H_2	H_{3a}	H_{3b}	CH_2O
5a•$H_2C_2O_4$	H	*i*-Pr	7.56 d (*J* = 4.9)	4.58 ddd (*J* = 8.8, 4.9, 4.3)	3.30 dd (*J* = 13.2, 4.3)	3.19 dd (*J* = 13.2, 8.8)	5.14 s
6a•$H_2C_2O_4$	H	*t*-Bu	7.57 d (*J* = 4.9)	4.55 ddd (*J* = 8.7, 4.9, 3.6)	3.30 dd (*J* = 12.8, 3.6)	3.16 dd (*J* = 12.8, 8.7)	5.15 s
5b•$H_2C_2O_4$	*o*-MeO	*i*-Pr	7.50 d (*J* = 4.9)	4.56 ddd (*J* = 8.5, 4.9, 4.2)	3.29 dd (*J* = 13.3, 4.2)	3.18 dd (*J* = 13.3, 8.5)	5.14 s
6b•$H_2C_2O_4$	*o*-MeO	*t*-Bu	7.52 d (*J* = 4.9)	4.56 ddd (*J* = 8.8, 4.9, 3.7)	3.30 dd (*J* = 12.8, 3.7)	3.16 dd (*J* = 12.8, 8.8)	5.15 s
5c•$H_2C_2O_4$	*m*-MeO	*i*-Pr	7.56 d (*J* = 4.9)	4.56 ddd (*J* = 8.9, 4.9, 4.4)	3.28 dd (*J* = 13.4, 4.4)	3.18 dd (*J* = 13.4, 8.9)	5.10 s
6c•$H_2C_2O_4$	*m*-MeO	*t*-Bu	7.58 d (*J* = 4.9)	4.56 ddd (*J* = 8.6, 4.9, 3.8)	3.30 dd (*J* = 12.8, 3.8)	3.16 dd (*J* = 12.8, 8.6)	5.12 s
5d•$H_2C_2O_4$	*p*-MeO	*i*-Pr	7.53 d (*J* = 4.9)	4.58 ddd (*J* = 8.6, 4.9, 4.1)	3.30 dd (*J* = 13.2, 4.1)	3.19 dd (*J* = 13.2, 8.6)	5.07 s
6d•$H_2C_2O_4$	*p*-MeO	*t*-Bu	7.53 d (*J* = 4.9)	4.55 ddd (*J* = 8.8, 4.9, 3.4)	3.30 dd (*J* = 12.8, 3.4)	3.16 dd (*J* = 12.8, 8.8)	5.07 s
5e•$H_2C_2O_4$	*o*-Cl	*i*-Pr	7.56 d (*J* = 4.9)	4.57 ddd (*J* = 8.5, 4.9, 4.3)	3.29 dd (*J* = 13.2, 4.3)	3.18 dd (*J* = 13.2, 8.5)	5.24 s
6e•$H_2C_2O_4$	*o*-Cl	*t*-Bu	7.56 d (*J* = 4.8)	4.54 ddd (*J* = 8.8, 4.8, 3.5)	3.29 dd (*J* = 12.8, 3.5)	3.14 dd (*J* = 12.8, 8.8)	5.26 s
5f•$H_2C_2O_4$	*m*-Cl	*i*-Pr	7.58 d (*J* = 4.7)	4.59 ddd (*J* = 8.8, 4.7, 4.6)	3.30 dd (*J* = 13.7, 4.6)	3.20 dd (*J* = 13.7, 8.8)	5.11 s
6f•$H_2C_2O_4$	*m*-Cl	*t*-Bu	7.59 d (*J* = 4.8)	4.56 ddd (*J* = 8.6, 4.8, 3.9)	3.30 dd (*J* = 12.9, 3.9)	3.16 dd (*J* = 12.9, 8.6)	5.12 s
5g•$H_2C_2O_4$	*p*-Cl	*i*-Pr	7.57 d (*J* = 4.9)	4.59 ddd (*J* = 8.5, 4.9, 4.2)	3.30 dd (*J* = 13.1, 4.2)	3.18 dd (*J* = 13.1, 8.5)	5.11 s
6g•$H_2C_2O_4$	*p*-Cl	*t*-Bu	7.56 d (*J* = 4.9)	4.55 ddd (*J* = 8.8, 4.9, 3.8)	3.30 dd (*J* = 13.0, 3.8)	3.16 dd (*J* = 13.0, 8.8)	5.11 s

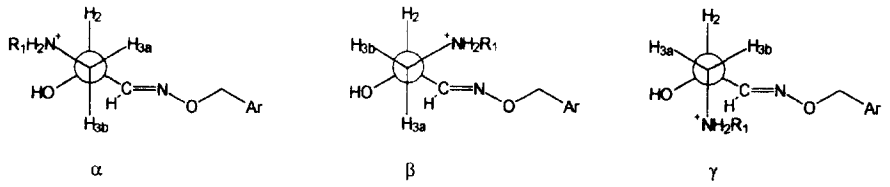
the *m*-Cl- (**6f**) and *p*-Cl-substituted derivatives (**6g**) showed an antagonistic activity index (pIC_{50}) which was practically equal to those of the completely aliphatic MOIM analogs **2** and **4**, while the methoxy-substituted compounds (**6b,c**) showed slightly lower pIC_{50} values. None of the new MOIM compounds shown in table IV proved to possess stimulating properties on atrial β_1-adrenoceptors.

Guinea-pig tracheal strip β_2-adrenoceptors

On β_2-adrenoceptors the compounds tested (**6a–c,f,g**) displayed an antagonistic activity towards isoprenaline-induced responses, with generally similar activity indices. The compound unsubstituted on the phenyl ring (**6a**) and the *p*-Cl-substituted compound (**6g**) exhibited pIC_{50} values slightly higher than that of the most active aliphatic analog **4**, while *o*-MeO- (**6b**), *m*-MeO- (**6c**) and the *m*-Cl-substituted (**6f**) compounds showed activity indices slightly lower than that of **4**. None of the compounds examined displayed any stimulating activity on tracheal β_2-adrenoceptors.

Theoretical calculations

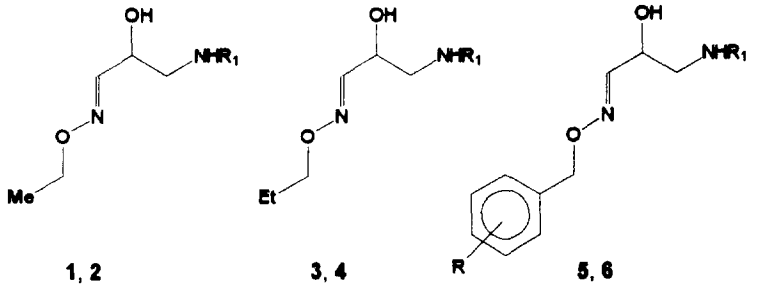
With the aim of attempting to rationalize the biopharmacological data obtained for the aryl-substituted



α β γ

Fig 2. Newman projections along the C(2)-C(3) bond of the three classical staggered rotamers of **5** and **6**.

Table III. Radioligand binding affinity of MOIM derivatives **1–6**.

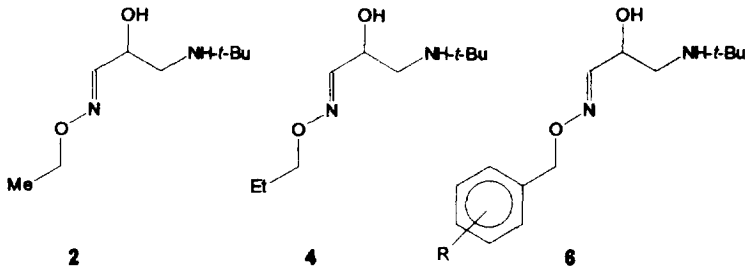


1, 2 **3, 4** **5, 6**

			β-Adrenergic binding affinity[a] K_i *(nM)*	
Compound	*R*	R_1	*Rat brain* (β_1)	*Bovine lung* (β_2)
1·$H_4C_4O_4$[b]		*i*-Pr	1040 (850–1200)	3500 (3170–3900)
3·$H_4C_4O_4$[b]		*i*-Pr	1240 (1130–1340)	5600 (5190–6050)
5a·$H_2C_2O_4$	H	*i*-Pr	2040 (1830–2250)	830 (740–920)
5b·$H_2C_2O_4$	*o*–MeO	*i*-Pr	14900 (13600–16200)	2400 (2140–2660)
5c·$H_2C_2O_4$	*m*–MeO	*i*-Pr	4700 (4180–5220)	430 (380–480)
5d·$H_2C_2O_4$	*p*–MeO	*i*-Pr	6480 (5860–7100)	420 (390–490)
5e·$H_2C_2O_4$	*o*–Cl	*i*-Pr	2440 (2180–2700)	1140 (1010–1270)
5f·$H_2C_2O_4$	*m*–Cl	*i*-Pr	2160 (1950–2370)	420 (370–470)
5g·$H_2C_2O_4$	*p*–Cl	*i*-Pr	2300 (1910–2690)	310 (270–350)
2·$H_4C_4O_4$[b]		*t*-Bu	1080 (1020–1140)	1400 (1300–1520)
4·$H_4C_4O_4$[b]		*t*-Bu	850 (790–910)	850 (770–930)
6a·$H_2C_2O_4$	H	*t*-Bu	2000 (1800–2190)	380 (330–430)
6b·$H_2C_2O_4$	*o*–MeO	*t*-Bu	1010 (880–1140)	700 (610–790)
6c·$H_2C_2O_4$	*m*–MeO	*t*-Bu	1870 (1610–2130)	370 (320–420)
6d·$H_2C_2O_4$	*p*–MeO	*t*-Bu	6580 (5730–7030)	130 (120–140)
6e·$H_2C_2O_4$	*o*–Cl	*t*-Bu	3300 (2910–3690)	250 (230–270)
6f·$H_2C_2O_4$	*m*–Cl	*t*-Bu	1400 (1200–1590)	210 (180–230)
6g·$H_2C_2O_4$	*p*–Cl	*t*-Bu	310 (270–350)	98 (85–110)
Dichloroisoproterenol			53 (45–60)	150 (130–170)
Propranolol			6.0 (3.7–6.2)	1.8 (1.5–2.0)

[a]Geometric means of five separate determinations with confidence limits in parentheses; [b]from reference [10].

Table IV. β-Adrenergic activity of selected MOIM derivatives.



2 **4** **6**

Compound	*R*	*β-Adrenergic activity*[a] *pIC_{50}*[b]	
		Isolated guinea-pig atria (β_1)	*Isolated guinea-pig tracheal strips (β_2)*
2·$H_4C_4O_4$[c]		4.86 ± 0.23	6.01 ± 0.14
4·$H_4C_4O_4$[c]		5.08 ± 0.22	6.57 ± 0.30
6a·$H_2C_2O_4$	H	5.10 ± 0.17	6.89 ± 0.10
6b·$H_2C_2O_4$	*o*-MeO	4.35 ± 0.04	6.10 ± 0.07
6c·$H_2C_2O_4$	*m*-MeO	4.60 ± 0.01	6.48 ± 0.26
6f·$H_2C_2O_4$	*m*-Cl	4.90 ± 0.01	6.38 ± 0.14
6g·$H_2C_2O_4$	*p*-Cl	5.13 ± 0.08	6.77 ± 0.31
Dichloroisoproterenol		6.89 ± 0.20	6.11 ± 0.50
Propranolol		7.40 ± 0.12	7.61 ± 0.14

[a]The values represent the mean of three to five experiments for each drug ± standard error; [b]pIC_{50} is the negative logarithm of the molar concentration that reduces the response to isoprenaline by 50%; [c]from reference [10].

MOIM derivatives, theoretical calculations were performed on model compounds **15a–g** (see table V), which are *N*-unsubstituted analogs of compounds **5a–g** and **6a–g**. The conformational and reactivity properties thus obtained were compared with those previously found [10] for the *N*-unsubstituted analog of compounds **1** and **2** (**14**). In previous studies [1, 2, 10] it had been verified that the structural simplification adopted here did not alter the results significantly.

The conformational analysis of **15a** was performed using the molecular mechanics program Discover [18]. The starting geometry selected for the ON=CHCH(OH)CH_2NH_2 portion was previously found [10] to be preferred for compound **14**. The torsion angles C(β)-C(α)-C-O (τ_1) and C(α)-C-O-N (τ_2), shown in figure 3, were varied together by 10° steps, thus obtaining 1296 conformations in which all other freedom degrees were fully optimized. Figure 3 shows the conformational energy trend of **15a** *vs* τ_1 and τ_2. In the preferred conformation, τ_1 and τ_2 are 90° and 180°, respectively; the C(α)-C-O-N=C portion is planar and the phenyl ring is perpendicular to this plane. This conformation corresponds to the fully extended one previously found to be preferred for the aliphatic model compound **14** (see fig 4) [10].

For the model compounds **15b–g**, the conformational study was carried out starting from a conformation corresponding to the preferred one of **15a** and then performing a full geometry optimization. The results showed that the minimal energy conformations of **15b–g** are very close to that of **15a**, thus indicating the negligible influence of the presence of a methoxy group or a chlorine atom on the aromatic ring on their conformational properties.

The molecular electrostatic potential (MEP) of **15a–g** was calculated at the *ab initio* STO-3G level, considering all the compounds in their optimized conformation. The results indicated that all the compounds **15a–g** possess MEP values which are practically identical on the CHCH(OH)CH_2NH_2 portion, while some minor differences can be found in the rest of the common part of the molecules. Greater MEP differences are found on the substituent on the aromatic ring.

Table V lists some representative MEP values for **15a–g**, calculated on the molecular surface, as defined by Connolly [19]. Table V also shows the corresponding MEP values for **14**. Generally, with respect to the phenyl-unsubstituted compound **15a**, the MEP values are slightly more negative or positive for the methoxy- (**15b–d**) or the chlorine-substituted derivatives (**15e–g**), respectively. The MEP values on the oxygen and nitrogen atoms of **14** are quite close to those found for the same atoms of **15a–g**.

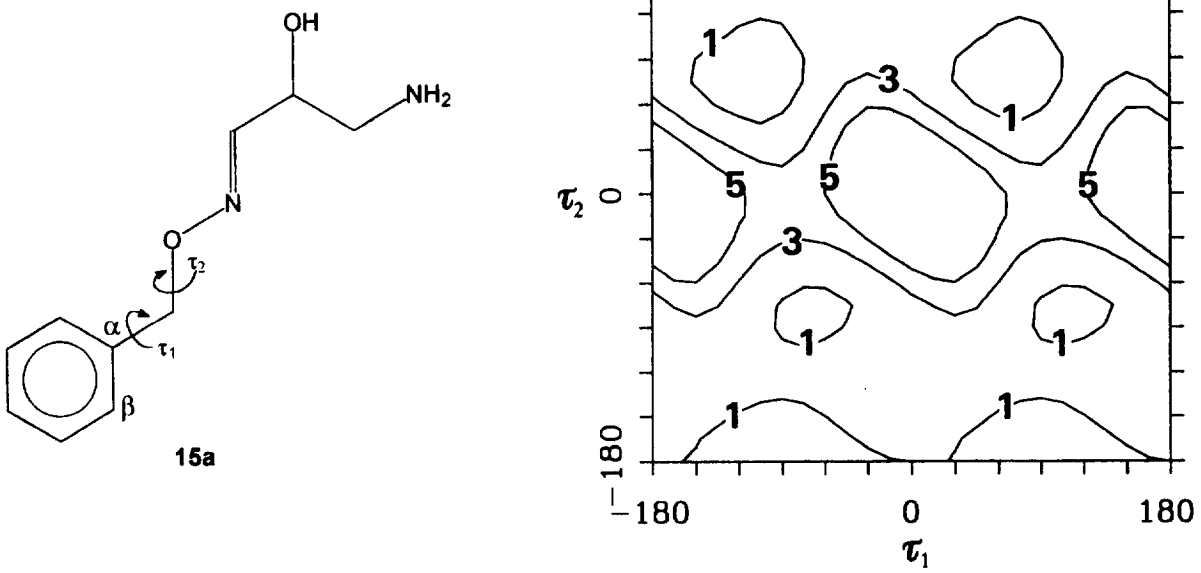


Fig 3. Contour plot of the conformational relative energy obtained by varying together torsion angles τ_1 and τ_2 by 10° steps. The isoenergy contours correspond to values of 1, 3 and 5 kcal/mol.

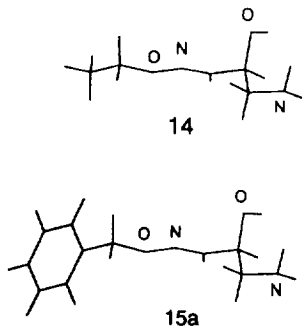
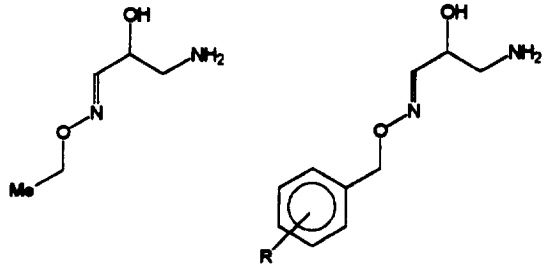


Fig 4. Preferred conformation of **14** and **15a**.

Finally, in order to compare the general MEP trend of aliphatic and aromatic MOIM derivatives, the solid contour corresponding to a value of −10 kcal/mol was calculated for **15a** and compared with the one previously obtained for **14** (see fig 5) [10]. A close similarity is evident between the negative MEP regions of the two compounds, as expected from the data reported in table V.

Table V. Molecular electrostatic potential values (V, kcal/mol) of compounds **14** and **15a–g** on the molecular surface, calculated for the optimized conformation.



14 **15**

Compound	*R*	*V(Φ)*[a]	*V(O)*[b]	*V(N)*[c]
14		–	−33.5	−32.8
15a	H	−1.6	−35.7	−34.4
15b	*o*-MeO	−2.8	−38.9	−37.6
15c	*m*-MeO	−3.5	−36.9	−35.5
15d	*p*-MeO	−3.1	−35.3	−32.2
15e	*o*-Cl	+3.7	−33.5	−32.6
15f	*m*-Cl	+3.5	−31.0	−30.0
15g	*p*-Cl	+3.6	−30.6	−28.9

[a]Mean MEP value on the phenyl ring; [b]minimum MEP value generated by the oxygen atom of the MOIMM; [c]minimum MEP value generated by the nitrogen atom of the MOIMM.

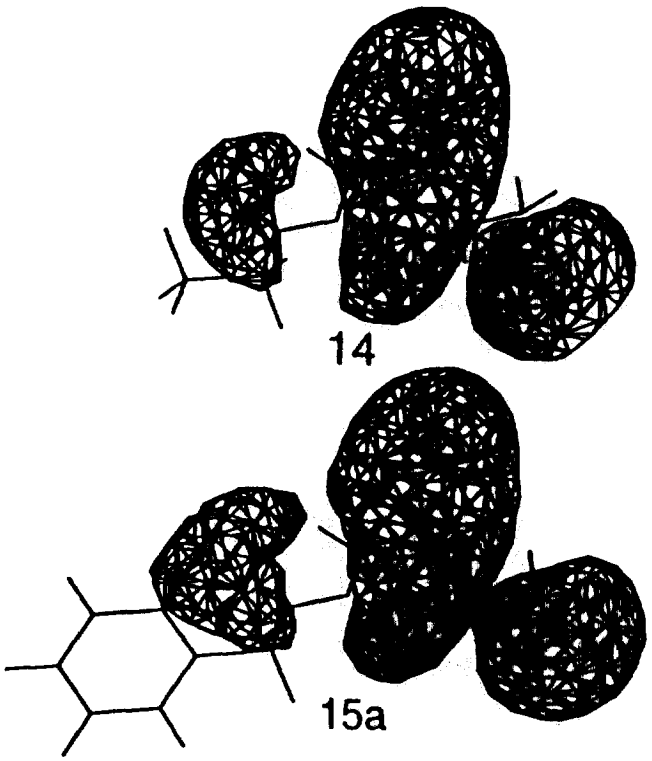


Fig 5. Compounds **14** and **15a** and their isopotential surfaces corresponding to an MEP value of −10 kcal/mol.

Discussion and conclusions

An examination of the binding data for β_1-adrenoceptors indicates that most of the aromatic MOIM derivatives **5** and **6** present an affinity for these receptors, which is similar or slightly lower than that of the completely aliphatic MOIM derivatives **1,3** and **2,4**. Only in the case of the *N-tert*-butyl-substituted *p*-chlorophenyl derivative **6g** did the affinity prove to be higher than that of **2** and **4**. In particular, as regards the *N*-isopropyl-substituted compounds (**5a–g**), a comparison of the affinity index values with those of the corresponding aliphatic analogs **1** and **3** indicates that the presence of an unsubstituted phenyl ring linked to the CH_2 carbon of the MOIMM, as in **5a**, does not substantially modify the ability of type **C** compounds to interact with β_1-adrenoceptors. Furthermore this ability remains practically unchanged when the phenyl ring of **5a** is substituted in the *ortho*, *meta* or *para* position by a chlorine atom. On the contrary, the K_i values of **5b–d** show that the introduction of a methoxy group on the phenyl ring of **5a** has a negative effect, leading to compounds which, especially in the case of the *ortho*-substituted **5b**, present a lower degree of affinity with respect to **5a**.

As regards the *N-tert*-butyl-substituted compounds (**6a–g**), the similarities observed between the K_i values on β_1-adrenoceptors of the aromatic compounds **6a–c,f** and the corresponding aliphatic analogs **2** and **4** indicate that the presence of a phenyl ring linked to the CH_2 carbon of the MOIMM as in **6a** and the substitution of this ring in the *ortho* position with a methoxy group as in **6b** or in the *meta* position with a methoxyl or a chlorine atom as in **6c** and **6f**, respectively, does not substantially influence the ability of these compounds to interact with the receptor. On the contrary, the K_i value of **6d** shows that the insertion of a methoxy group in the *para* position of **6a** has a negative effect, while the K_i value of **6g**, which is the lowest of the series, indicates that the insertion of a chlorine atom in the same *para* position of **6a** has an appreciable positive effect.

For the MOIM derivatives that were submitted to functional tests (**6a–c,f,g**), the activity indices for β_1-adrenoceptors are in quite good agreement with those obtained in the binding tests, apart from the *o*-methoxyphenyl derivative **6b** which, while possessing a higher affinity with respect to **6a,c,f**, exhibits a lower activity.

As regards β_2-adrenoceptors, the results obtained in the binding tests indicate that most of the aromatic MOIM derivatives **5** and **6** possess an affinity higher than that of the completely aliphatic analogs **1,3** and **2,4**. Among the *N*-isopropyl- (**5**) and the *N-tert*-butyl-substituted (**6**) MOIM compounds, the trend of the affinity was similar for both the methoxyphenyl- (**5b–d** and **6b–d**) and the chlorophenyl-substituted (**5e–g** and **6e–g**) compounds. In all cases, the affinity increases on passing from the *ortho*- to the corresponding *meta*- and then to the *para*-substituted isomers, among which the *p*-chloro MOIM derivative **6g** possesses the highest affinity. The compounds unsubstituted on the phenyl ring (**5a** and **6a**) present an intermediate affinity level, between those of the substituted compounds.

The results obtained for the MOIM derivatives **6a–c,f,g** in the functional tests carried out on guinea-pig tracheal β_2-adrenoceptors are in good agreement with the K_i values obtained in the binding tests, apart from the compound unsubstituted on the phenyl ring **6a**, which exhibits an activity similar to that of the *p*-chloro-substituted compound **6g**, even if it possesses a lower affinity.

The above results underline the fact that for type **C** compounds, the insertion of an aromatic system on the CH_2 carbon of the MOIMM, leads to different results in the case of β_1- or β_2-adrenergic adrenoceptors. While this type of substitution does not have any appreciable effects on β_1-adrenergic properties, as

regards the affinity and the activity, it appears to be capable of improving the β_2-adrenergic properties, as far as the receptor affinity is concerned.

The theoretical studies on the conformational and electronic characteristics of model compounds of both completely aliphatic (**14**) and aryl-substituted (**15a–g**) type **C** drugs, reveal that the presence of an aliphatic portion or of an aromatic nucleus on the CH_2 carbon of the MOIMM does not influence either the conformational preferences or the molecular reactivity of the remaining molecular portion. The only differences that exist between the aliphatic (**14**) and the aromatic (**15a–g**) model compounds lie in the different steric characteristics and reactivity of the two kinds of substituent, *ie* the aliphatic substituent of **14** and the aryl of **15a–g**. These differences appear to have practically no influence on the modulation of the β_1-adrenergic properties of type **C** compounds. On the contrary, these same differences seem to be responsible for the variations in affinity towards β_2-adrenergic receptors that exist between completely aliphatic and aromatic type **C** compounds.

In conclusion, on the basis of the results obtained for compounds **1–6** at the level of β_1-adrenoceptors, it is only possible to underline the capacity of these receptors to receive type **C** compounds with more or less awkward groups linked to the CH_2 of the MOIMM, without any appreciable variations in the β_1-adrenergic properties.

As regards β_2-adrenoceptors, the improved adrenergic affinities of type **C** compounds substituted with an aromatic moiety (**5** and **6**) compared with those substituted with aliphatic groups (**1,3** and **2,4**) may be explained by hypothesizing that an aromatic system may play a role in the drug–receptor interaction of **5** and **6**. Moreover, the fact that an analogous affinity trend towards β_2-receptors exists for these compounds, depending on the position of the substituent on the aromatic nucleus and irrespective of the electronic characteristics of the substituent, would appear to exclude the possibility of any direct interaction of the system of the aromatic nucleus with appropriate receptor sites. The relationship that appears to exist between the affinity of compounds **5** and **6** on β_2-adrenoceptors and the position of the substituent on their aromatic moiety might rather be tentatively attributed to the different steric effects of the methoxy group and the chlorine atom in the three different positions of the aromatic systems of **5** and **6**.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films on a Mattson 1000 Series FTIR spectrometer. The ^{1}H-NMR spectra of all the compounds were obtained with a Bruker AC-200 instrument in *ca* 2% solution of $CDCl_3$ (for the neutral compounds) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The spectral parameters of the single compounds were refined by a MOLE (LAOCOON) program, using an Atari PC3 computer. The relative percentages of *E*- and *Z*-isomers of unsaturated oxime ethers (**8a–g** and **9a–g**), epoxides (**10a–g** and **11a–g**) and oxalate salts of aminoalcohols of **5a–g**, **12a–g**, **6a–g** and **13a–g** were evaluated on the basis of the integrals of the N=CH protons in the ^{1}H-NMR spectra of the crude reaction mixtures. Analytical TLCs were carried out on 0.25 mm layer silica-gel plates (Merck F_{254}) containing a fluorescent indicator; spots were detected under UV light (254 nm). The solvents were evaporated *in vacuo* (rotating evaporator); $MgSO_4$ was always used as the drying agent. Elemental analyses were performed in our analytical laboratory and agreed with the theoretical values to within ±0.4%.

General procedure for the preparation of (E)- and (Z)-N-propenylidene(arylmethyloxy)amines 8a–g and 9a–g

A mixture of the appropriate *O*-(arylmethyl)hydroxylamine hydrochloride [12] **7a–g** (0.046 mol) in H_2O (300 ml) and $CHCl_3$ (300 ml) was cooled to 0°C and treated dropwise, under stirring, with acrolein (2.57 g, 0.046 mol). The resulting mixture was stirred at room temperature for 24 h, and the two phases were separated. The aqueous layer was extracted with $CHCl_3$ (2 x 150 ml) and the organic extracts were evaporated to yield the appropriate 7:3 mixture of *E* and *Z* unsaturated oxime ethers (**8a–g** and **9a–g** respectively), which was immediately used in the subsequent transformation. For ^{1}H-NMR spectral data of **8** and **9**, see table VI.

The isomeric mixture of **8g** and **9g** (8.7 g) was submitted to column chromatography on silica gel (Merck 9385), eluting with a 45:6:1 hexane/CH_2Cl_2/methylethylketone mixture and collecting 15 ml fractions. The first fractions afforded pure **8g** (4.0 g, 44%), whereas the subsequent fractions yielded pure **9g** (1.7 g, 19%) as oils. Anal for $C_{10}H_{10}NOCl$ (C, H, N).

General procedure for the preparation of (E)- and (Z)-N-(2,3-epoxypropylidene)(arylmethyloxy)amines 10a–g and 11a–g

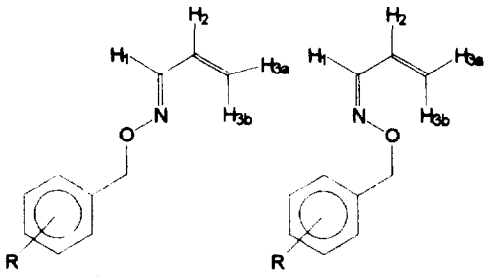
A solution of the appropriate 7:3 mixture of **8a–g** and **9a–g** (0.040 mol) in CH_2Cl_2 (120 ml) was added to a saturated solution of $NaHCO_3$ (120 ml), and the resulting mixture was cooled at 0°C and then treated dropwise under stirring with a solution of 70% *m*-chloroperoxybenzoic acid (20.7 g, 0.084 mol) in CH_2Cl_2 (120 ml). The mixture was stirred at 0°C for 1 h and then at rt for 1 h further. The organic phase was separated, washed (5% aqueous K_2CO_2, 1 N aqueous $Na_2S_2O_3$, and H_2O), filtered, and evaporated to dryness to yield a crude residue consisting almost exclusively of the appropriate 4:1 mixture of *E* and *Z* epoxides (**10a–g** and **11a–g**, respectively), which was used immediately for the following reactions. For ^{1}H-NMR spectral data of **10** and **11**, see table VII.

When the above reaction was carried out on the pure unsaturated oxime ethers **8g** and **9g**, only a 4:1 mixture of **10g** and **11g** was obtained (^{1}H-NMR).

General procedure for the preparation of (E)-N-[3-(isopropylamino)-2-hydroxypropylidene](arylmethyloxy)amine oxalates 5a–g·$H_2C_2O_4$

A stirred solution of the 4:1 mixture of **10a–g** and **11a–g** (4 mmol) in anhydrous benzene (12 ml) and *i*-$PrNH_2$ (1.42 g, 24 mmol) was kept at 90°C for 24 h. The resulting mixture was

Table VI. ^{1}H-NMR data of (*E*)- and (*Z*)-*N*-propenylidene(arylmethyloxy)amines **8a–g** and **9a–g** in the crude isomeric mixtures.



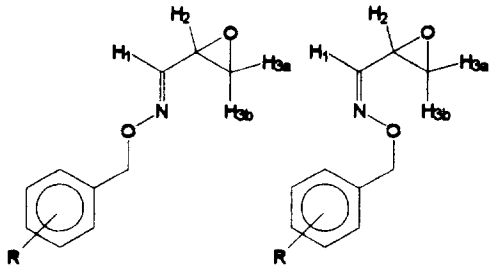
Compound	*R*	*Isomer*	H_1	H_2	H_{3a}	H_{3b}	CH_2O
8a	H	*E*	7.79 m (*J* = 9.9)	6.42 m (*J* = 17.0, 10.8, 9.9)	5.54 m (*J* = 10.8, 0.9)	5.52 m (*J* = 17.0, 0.9)	5.11 s
9a	H	*Z*	7.07 m (*J* = 9.4)	7.00 m (*J* = 16.0, 9.7, 9.4)	5.55 m (*J* = 9.7, 1.5)	5.58 m (*J* = 16.0, 1.5)	5.14 s
8b	*o*-MeO	*E*	7.80 m (*J* = 9.7)	6.43 m (*J* = 17.0, 10.7, 9.7)	5.52 m (*J* = 10.7, 1.7)	5.51 m (*J* = 17.0, 1.7)	5.18 s
9b	*o*-MeO	*Z*	7.07 m (*J* = 9.6)	7.00 m (*J* = 15.4, 8.7, 9.6)	5.54 m (*J* = 8.7, 1.5)	5.58 m (*J* = 15.4, 1.5)	5.21 s
8c	*m*-MeO	*E*	7.79 m (*J* = 9.7)	6.41 m (*J* = 16.8, 10.9, 9.7)	5.54 m (*J* = 10.9, 1.7)	5.52 m (*J* = 16.8, 1.7)	5.09 s
9c	*m*-MeO	*Z*	7.07 m (*J* = 9.6)	6.98 m (*J* = 15.9, 9.6, 9.6)	5.55 m (*J* = 9.6, 1.5)	5.58 m (*J* = 15.9, 1.5)	5.12 s
8d	*p*-MeO	*E*	7.75 m (*J* = 9.8)	6.42 m (*J* = 17.1, 10.5, 9.8)	5.53 m (*J* = 10.5)	5.50 m (*J* = 17.1)	5.04 s
9d	*p*-MeO	*Z*	7.05 m (*J* = 9.5)	6.96 m (*J* = 16.6, 9.7, 9.5)	5.52 m (*J* = 9.7, 1.5)	5.55 m (*J* = 16.6, 1.5)	5.07 s
8e	*o*-Cl	*E*	7.83 m (*J* = 9.8)	6.42 m (*J* = 16.7, 11.2, 9.8)	5.56 m (*J* = 11.2, 1.5)	5.54 m (*J* = 16.7 1.5)	5.23 s
9e	*o*-Cl	*Z*	7.12 m (*J* = 9.6)	7.03 m (*J* = 15.7, 9.9, 9.6)	5.58 m (*J* = 9.9, 1.4)	5.61 m (*J* = 15.7, 1.4)	5.27 s
8f	*m*-Cl	*E*	7.72 m (*J* = 9.8)	6.34 m (*J* = 16.7, 10.5, 9.8)	5.49 m (*J* = 10.5, 1.5)	5.47 m (*J* = 16.7, 1.5)	5.00 s
9f	*m*-Cl	*Z*	7.07 m (*J* = 9.5)	6.97 m (*J* = 16.1, 9.9, 9.5)	5.58 m (*J* = 9.9, 1.5)	5.61 m (*J* = 16.1, 1.5)	5.10 s
8g	*p*-Cl	*E*	7.77 m (*J* = 9.7)	6.40 m (*J* = 17.0, 10.7, 9.7)	5.56 m (*J* = 10.7, 1.7)	5.53 m (*J* = 17.0, 1.7)	5.06 s
9g	*p*-Cl	*Z*	7.06 m (*J* = 9.4)	6.96 m (*J* = 16.5, 9.8, 9.4)	5.56 m (*J* = 9.8, 1.5)	5.59 m (*J* = 16.5, 1.5)	5.09 s

evaporated to yield a residue, which was dissolved in anhydrous Et_2O (10 ml) and treated in portions at 0°C, under stirring, with a solution of oxalic acid (4 mmol) in anhydrous MeOH (5 ml). Addition of anhydrous Et_2O gave a solid precipitate consisting of a mixture of **5a–g**·$H_2C_2O_4$ and (*Z*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene](arylmethyloxy)-amine oxalates (**12a–g**·$H_2C_2O_4$) [^{1}H-NMR data for N=CH proton (δ, Hz); **12a**·$H_2C_2O_4$: 6.91 (d, *J* = 4.8); **12b**·$H_2C_2O_4$: 6.88 (d, *J* = 4.8); **12c**·$H_2C_2O_4$: 6.90 (d, *J* = 4.9); **12d**·$H_2C_2O_4$: 6.91 (d, *J* = 4.7); **12e**·$H_2C_2O_4$: 6.90 (d, *J* = 4.8); **12f**·$H_2C_2O_4$: 6.93 (d, *J* = 4.8); **12g**·$H_2C_2O_4$: 6.92 (d, *J* = 4.8)] in a ratio of about 4:1. Crystallization from MeOH/Et_2O yielded the appropriate pure oxalate salt of **5a–g**. For analytical and chemical data, see table I; for ^{1}H-NMR spectral data, see table II.

General procedure for the preparation of (E)-N-[3-(tert-butylamino)-2-hydroxypropylidene](arylmethyloxy)amine oxalates ***6a–g***·$H_2C_2O_4$

A stirred solution of the 4:1 mixture of **10a–g** and **11a–g** (4 mmol) in anhydrous benzene (12 ml) and *t*-$BuNH_2$ (1.75 g, 24 mmol) was kept at 90°C for 4 d and then treated, as in the preparation of **5a–g**·$H_2C_2O_4$, to yield a solid precipitate consisting of a mixture of **6a–g**·$H_2C_2O_4$ and (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](arylmethyloxy)amine oxalates (**13a–g**·$H_2C_2O_4$) [^{1}H-NMR data for N=CH proton (δ, Hz); **13a**·$H_2C_2O_4$: 6.92 (d, *J* = 4.7); **13b**·$H_2C_2O_4$: 6.91 (d, *J* = 4.6); **13c**·$H_2C_2O_4$: 6.93 (d, *J* = 4.8); **13d**·$H_2C_2O_4$: 6.91 (d, *J* = 4.7); **13e**·$H_2C_2O_4$: 6.90 (d, *J* = 4.7); **13f**·$H_2C_2O_4$: 6.94 (d, *J* = 4.8); **13g**·$H_2C_2O_4$: 6.92 (d, *J* = 4.8)] in a ratio of about 4:1.

Table VII. ^{1}H-NMR data of (*E*)- and (*Z*)-*N*-(2,3-epoxypropylidene)(arylmethyloxy)amines **10a–g** and **11a–g** in the crude isomeric mixtures.



10a-g **11a-g**

Compound	*R*	*Isomer*	H_1	H_2	H_{3a}	H_{3b}	CH_2O
10a	H	*E*	7.00 d (J = 8.2)	3.54 ddd (J = 8.2, 4.2, 2.7)	2.79 dd (J = 5.0, 2.7)	3.04 dd (J = 5.0, 4.2)	5.12 s
11a	H	*Z*	6.37 d (J = 7.0)	4.08 ddd (J = 7.0, 4.4, 2.7)	2.74 dd (J = 5.4, 2.7)	3.04 dd (J = 5.4, 4.4)	5.15 s
10b	*o*-MeO	*E*	7.01 d (J = 7.9)	3.55 ddd (J = 7.9, 4.1, 2.6)	2.79 dd (J = 5.0, 2.6)	3.04 dd (J = 5.0, 4.1)	5.19 s
11b	*o*-MeO	*Z*	6.37 d (J = 7.1)	4.11 ddd (J = 7.1, 4.3, 2.6)	2.74 dd (J = 5.4, 2.6)	3.05 dd (J = 5.4, 4.3)	5.22 s
10c	*m*-MeO	*E*	7.01 d (J = 7.9)	3.54 ddd (J = 7.9, 4.3, 2.7)	2.80 dd (J = 5.0, 2.7)	3.04 dd (J = 5.0, 4.3)	5.09 s
11c	*m*-MeO	*Z*	6.36 d (J = 7.1)	4.08 ddd (J = 7.1, 4.4, 2.5)	2.73 dd (J = 5.4, 2.5)	3.04 dd (J = 5.4, 4.4)	5.11 s
10d	*p*-MeO	*E*	6.97 d (J = 7.9)	3.53 ddd (J = 7.9, 4.3, 2.7)	2.79 dd (J = 5.0, 2.7)	3.03 dd (J = 5.0, 4.3)	5.05 s
11d	*p*-MeO	*Z*	6.35 d (J = 7.1)	4.05 ddd (J = 7.1, 4.4, 2.5)	2.73 dd (J = 5.4, 2.5)	3.03 dd (J = 5.4, 4.4)	5.08 s
10e	*o*-Cl	*E*	7.05 d (J = 8.1)	3.55 ddd (J = 8.1, 4.1, 2.7)	2.82 dd (J = 4.9, 2.7)	3.06 dd (J = 4.9, 4.1)	5.27 s
11e	*o*-Cl	*Z*	6.40 d (J = 6.9)	4.12 ddd (J = 6.9, 4.3, 2.6)	2.77 dd (J = 5.0, 2.6)	3.12 dd (J = 5.0, 4.3)	5.29 s
10f	*m*-Cl	*E*	7.01 d (J = 8.0)	3.53 ddd (J = 8.0, 4.1, 2.5)	2.81 dd (J = 4.9, 2.5)	3.05 dd (J = 4.9, 4.1)	5.08 s
11f	*m*-Cl	*Z*	6.38 d (J = 7.0)	4.08 ddd (J = 7.0, 4.3, 2.5)	2.76 dd (J = 5.4, 2.5)	3.08 dd (J = 5.4, 4.3)	5.11 s
10g	*p*-Cl	*E*	6.99 d (J = 8.0)	3.52 ddd (J = 8.0, 4.1, 2.6)	2.80 dd (J = 5.0, 2.6)	3.04 dd (J = 5.0, 4.1)	5.07 s
11g	*p*-Cl	*Z*	6.36 d (J = 7.1)	4.06 ddd (J = 7.1, 4.4, 2.7)	2.75 dd (J = 5.4, 2.7)	3.05 dd (J = 5.4, 4.4)	

Crystallization from MeOH/Et_2O yielded the appropriate pure oxalate salt of **6a–g**. For analytical and chemical data, see table I; for ^{1}H-NMR spectral data, see table II.

Radioligand binding methods

Rat brain β_1-receptors

β_1-Receptors were assayed in rat cortical membranes, as previously described [10], using [^{3}H]CGP 26505 [15] (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 [10], using [^{3}H]dihydroalprenolol (DHA) [16] 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 **5a–g**, **6a–g** on β-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

experimented on preparations of isolated guinea-pig atria and of tracheal smooth musculature respectively, following the method previously described [10].

For both β_1 and β_2 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% [20]. All compounds were tested at a concentration ranging from 10^{-9} M to 10^{-3} M. Each antagonistic activity index was obtained by at least five active concentrations.

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