The [(Methyloxy)imino]methyl Moiety as a Bioisoster of Aryl. A Novel Class of Completely Aliphatic β-Adrenergic Receptor Antagonists

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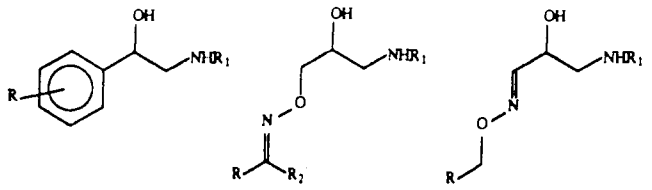
Previous studies in the field of β-adrenergic drugs had supported the hypothesis of the existence of a bioisosterism between the [(methyleneamino)oxy]methyl moiety (C=NOCH$_2$, MAOMM) of type B β-blocking drugs and the aryl (Ar) of type A β-blocking agents. In the MAOMM, however, the carbon of the CH$_2$ linked to the oximic oxygen possesses a hybridization (sp^3) and a geometry different from those of the corresponding carbon of Ar which possesses an sp^2 hybridization. Furthermore, in the MAOMM, in its preferred conformation, the unsaturated portion (C=N) is situated in a spatial area which does not correspond exactly to the area occupied by Ar. 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, with respect to the MAOMM. On the basis of these observations, some completely aliphatic (*E*)-*N*-(3-amino-2-hydroxypropylidene)(alkyloxy)amino derivatives of type C (**11a,b** and **12a,b**) were synthesized, the their β-adrenergic properties were compared with those of the corresponding [(methyleneamino)oxy]methyl isomers of type B (**19a,b** and **20a,b**). The similar β-adrenergic properties of **11,12** and **19,20** evaluated in vitro both by radioligand binding assays and by functional tests on isolated preparations, are discussed on the basis of considerations regarding the spatial correspondences and electronic analogies between the MOIMM and the MAOMM.

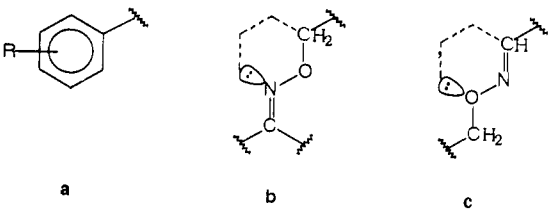
Previous papers[1-3] from this laboratory described the synthesis and the pharmacological properties of completely aliphatic adrenergic β-blocking agents (B) designed as analogs of arylethanolaminic β-adrenergic drugs (A) where the aryl moiety (Ar) is replaced by a [(methyleneamino)-oxy]methyl moiety (C=NOCH$_2$, MAOMM).



A B C

The potent β-blocking properties of these compounds (B) and a comparison of their chemical reactivity with that of type A drugs supported the hypothesis of the existence of a bioisosterism between Ar and the MAOMM (see a and b of Figure 1), at least in the field of adrenergic drugs.[1-3] This possible bioisosterism was then successfully verified for non-adrenergic drugs in which the aryl moiety seems to be important for activity, such as antiinflammatory arylacetic acids,[4] β-lactam antibiotics,[5] and antidepressant agents.[6]

In the MAOMM (see b of Figure 1), the carbon of the CH$_2$ linked to the oximic oxygen possesses a hybridization (sp^3) and a geometry different from those of the corresponding carbon of Ar (see a of Figure 1) which possesses an sp^2 hybridization. Furthermore, in the MAOMM, in its preferred conformation shown in b of Figure 1, the unsaturated portion (C=N) is situated in a spatial area which does not correspond exactly to the area occupied by Ar. An examination of Figure 1b indicates 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 (see c of Figure 1), appears to present greater steric and electronic analogies with an Ar, with respect to the MAOMM.



a b c

Figure 1. Representation of the spatial correspondences existing between molecular portions of Ar (a), MAOMM (b), and MOIMM (c) which may account for their bioisosteric relationship.

In the MOIMM, the carbon of the CH involved in the building of the possible Ar bioisoster presents the same type of hybridization and geometry as the corresponding carbon of the Ar group which the MOIMM should mimic. In the MOIMM of *E* configuration, furthermore, the unsaturated portion (N=C) comes to coincide spatially with a part of the unsaturated system of Ar (see a–c of Figure 1).

On the basis of these observations, some completely aliphatic (*E*)-*N*-(3-amino-2-hydroxypropylidene)(alkyloxy)-amino derivatives of type C (**11a,b** and **12a,b**) (Table 1) were synthesized, as analogs of type A or B β-adrenergic drugs, in which the Ar or the MAOMM, respectively, are substituted by the MOIMM. In addition, for the purposes of comparison, the type B derivatives **19a,b** and **20a,b**, which are isomers of the new compounds **11a,b** and **12a,b**, were also synthesized.

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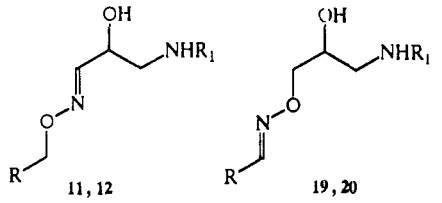
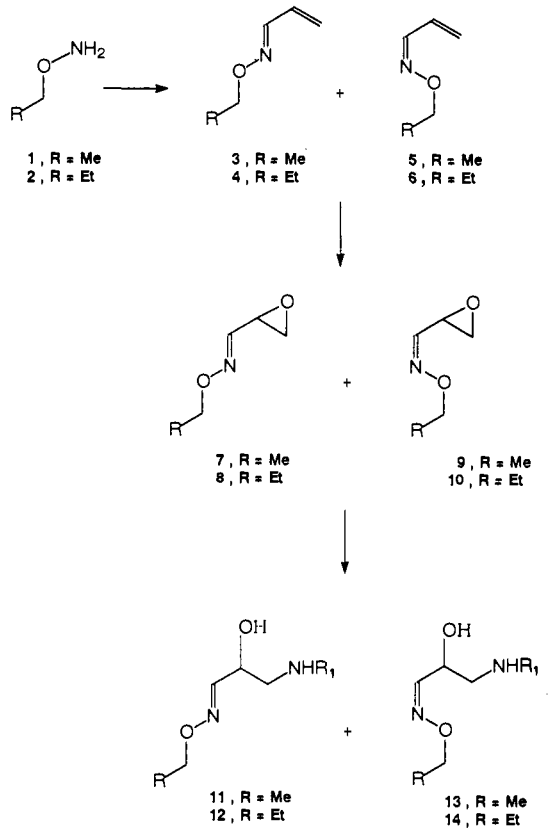
Table 1. Chemical Data of MOIM (11, 12) and MAOM (19, 20) Derivatives



compd	R	R_1	mp (°C)	crystn solvent[a]	yield (%)	formula[c]
11a·$H_4C_4O_4$	Me	*i*-Pr	72–73	A	30[d]	$C_{12}H_{22}N_2O_6$
11b·$H_4C_4O_4$	Me	*t*-Bu	126–127	A	19[d]	$C_{13}H_{24}N_2O_6$
12a·$H_4C_4O_4$	Et	*i*-Pr	78–79	A	25[d]	$C_{13}H_{24}N_2O_6$
12b·$H_4C_4O_4$	Et	*t*-Bu	122–124	A	23[d]	$C_{14}H_{26}N_2O_6$
19a·$H_4C_4O_4$	Me	*i*-Pr	83–85	B	70	$C_{12}H_{22}N_2O_6$
19b·$H_4C_4O_4$	Me	*t*-Bu	131–132	B	60	$C_{13}H_{24}N_2O_6$
20a·$H_4C_4O_4$	Et	*i*-Pr	85–87	B	78	$C_{13}H_{24}N_2O_6$
20b·$H_4C_4O_4$	Et	*t*-Bu	127–129	B	62	$C_{14}H_{26}N_2O_6$

[a] A = *i*-Pr_2O–hexane; B = EtOH–Et_2O. [b] For the epoxide aminolysis, no efforts were made to optimize yields. [c] All compounds were analyzed for C, H, and N. [d] Yield calculated only on the E epoxide present in the *E*–*Z* diastereoisomer mixture.

Scheme 1[a]

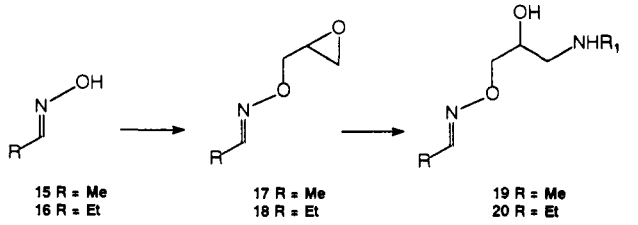


[a] **a**, R_1 = *i*-Pr; **b**, R_1 = *t*-Bu.

Chemistry

The (*E*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene]-(**11a**, **12a**) and (*E*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](alkyloxy)amines (**11b**, **12b**) (Table 1) were synthesized as outlined in Scheme 1. Base-catalyzed (KH_2PO_4) condensation of *O*-ethyl-[7] (1) or *O*-propylhydroxylamine[8] (2) with acrolein afforded mixtures of the corresponding *E* (**3**, 4) and *Z* (**5**, **6**) unsaturated oxime ethers in a ratio of approximately 7:3, which could not be separated by the usual fractioning techniques. Epoxidation with *m*-chloroperoxybenzoic acid of the crude mixtures of **3**,**5** and **4**,**6** yielded mixtures of the corresponding *E* (**7**, **8**) and *Z* (**9**, **10**) epoxides in a ratio of about 7:3, which proved to be too unstable to allow separation. Aminolysis of the mixtures of **7**,**9** and **8**,**10** with *i*-$PrNH_2$ or *t*-$BuNH_2$ afforded mixtures of the amino alcohols *E* (**11**, **12**) and *Z* (**13**, **14**) in the same ratio as the corresponding epoxides. From these crude mixtures, the *E* compounds **11** and **12** were isolated as maleate salts by fractional crystallization.

Scheme 2[a]



[a] **a**, R_1 = *i*-Pr; **b**, R_1 = *t*-Bu.

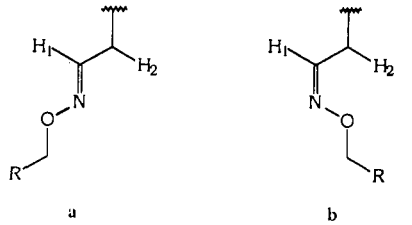


Figure 2. Drawing showing the spatial relationship between the H(1) and H(2) protons and the oximic oxygen in the *E* (a) and *Z* (b) MOIM derivatives.

The oxime ether derivatives **19** and **20** were obtained (see Scheme 2) by reaction of (*E*)-acetaldoxime (**15**)[9] or (*E*)-propionaldoxime (**16**)[10] with epichlorohydrin followed by aminolysis of the epoxides **17** and **18**. The amines **19** and **20** were isolated as maleate salts.

The *E* and *Z* configurations of the couples of unsaturated oxime ethers (**3**,**5** and **4**,**6**), epoxides (**7**,**9** and **8**,**10**), and *N*-isopropyl- (**11a**,**13a** and **12a**,**14a**) and *N*-*tert*-butyl-substituted (**11b**,**13b** and **12b**,**14b**) amino alcohols were assigned on the basis of a comparison of their ^1H NMR spectral data (see the Experimental Section). In the compounds of *E* configuration, the proton linked to the carbon of the iminomethyl portion (H(1) of Figure 2) resonates at lower fields (0.61–0.74 ppm) with respect to the same hydrogen of the corresponding *Z* isomers, due to the paramagnetic effect of the spatially proximal oximethereal oxygen. On the contrary, in the compounds with *Z* configuration, it is the proton linked to the carbon in the α position with respect to the N=C portion (H(2) of Figure 2), which being on the same side as the oximethereal oxygen, resonates at lower fields (0.52–0.72 ppm) than the same hydrogen of the corresponding *E* isomers. These results are consistent with the data reported in literature for similar oxime ether derivatives.[11]

The ^1H NMR spectral data of the amino alcohols **11** and **12**, as salts, provide information about their conformational situation around the C(2)–C(3) bond in solution. Figure 3 shows the Newman projections of the three classical staggered rotamers α, β, and γ around the C(2)–C(3) bond of **11** and **12**. In compounds **11** and **12**, the proton linked to C(2) shows two vicinal coupling constants with the protons linked to C(3), one of which reveals a fairly high value (9.5–9.9 Hz), while the other is relatively low (2.1–2.9 Hz). This tends to confirm the existence of a preferential conformer, in which the H(2) proton is in

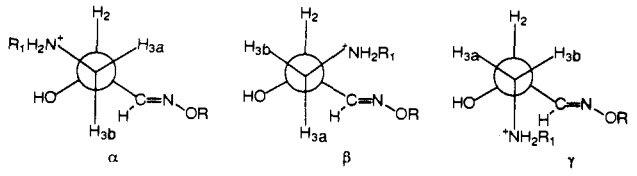


Figure 3. Newman projections along the C(2)–C(3) bond of the three classical staggered rotamers of 11 and 12.

a *trans* and *gauche* relationship, respectively, with the two protons linked to C(3), as happens in the case of conformers α and β, thus excluding the possibility of any appreciable participation in the conformation equilibrium of the γ rotamer, in which the H(2) proton is in a *gauche* relationship with both the protons linked to C(3). Among the α and β rotamers, then, it is possible to assign the largest population to rotamer α, which can be stabilized through an internal $^+$NH···O bonding[12] and possesses fewer steric interactions, and consequently, it is possible to attribute the relative vicinal coupling constants to the H(3a) and H(3b) protons, which cannot be directly identified in the spectra of 11 and 12. It is of interest to observe that type A adrenergic drugs in solution preferentially exist, both as free bases and salts, in the conformation corresponding to rotamer α of Figure 3, in which the amino nitrogen is in a *trans* and *gauche* relationship with the Ar and the OH group, respectively.[13]

The geometry of the (methyleneamino)oxy group of the MAOM derivatives **19** and **20** was assumed on the basis of the knowledge of the configuration of the starting oximes **15** and **16**. The configuration around the C=N double bond of acetaldoxime (**15**) and propionaldoxime (**16**) should remain unmodified both in the nucleophilic substitution of the chlorine of epichlorohydrin leading to epoxides **17** and **18** and in the subsequent aminolysis of **17** and **18** to **19** and **20**. Oximes **15** and **16** proved to be configurationally stable under the reaction conditions which lead from **15** and **16** to **17** and **18** and from **17** and **18** to **19** and **20**.

Radioligand Binding Assays

The β-adrenergic affinity of MOIM (**11**, **12**) and MAOM (**19**, **20**) derivatives and of dichloroisoproterenol, taken as the reference drug (Table 3), was checked by binding tests on rat brain and bovine lung membrane preparations for β_1- and β_2-receptors, respectively. 1-[[2-(3-Carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]phenoxy]-2-propanol ([^{3}H]-CGP 26505)[14] was used as a specific tritiated ligand for rat brain β_1-receptors. [^{3}H]Dihydroalprenolol ([^{3}H]DHA)[15] was utilized to label bovine lung β_2-receptors 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.[16]

Rat Brain β_1-Receptors. MOIM derivatives **11** and **12** presented an appreciable binding affinity, which was quite similar to that of the corresponding MAOM derivatives **19** and **20**. In the case of **11a** and **12a**, the affinity indices (K_i) were slightly lower than those of the corresponding isomers **19a** and **20a**, whereas in the case of **11b** and **12b**, K_i values were slightly higher than those of **19b** and **20b**. Among both MOIM and MAOM derivatives, the *N-tert*-butyl-substituted analogs (**11b**,**12b** and **19b**,**20b**) revealed a higher affinity for this type of β-receptor, compared with the corresponding *N*-isopropyl-substituted compounds (**11a**,**12a** and **19a**,**20a**). Moreover, among the *N-tert*-butyl-substituted MOIM and MAOM derivatives, the compounds with the better affinity proved to be those in which R is the ethyl group (**12b** and **20b**).

Bovine Lung β_2-Receptors. MOIM derivatives **11** and **12** showed appreciable affinities which, in the case of **11a**,**b** and **12b**, were higher than those of the corresponding MAOM derivatives (**19a**,**b** and **20b**); in the case of **12a**, the affinity was lower than that of **20a**. Also for this subtype of β receptor, the *N-tert*-butyl-substituted compounds revealed a greater affinity than the *N*-isopropyl-substituted analogs in both series of compounds. The lowest K_i values were exhibited by *N-tert*-butyl-substituted compounds in which R is an ethyl group.

Functional Tests

MOIM derivatives **11** and **12**, their MAOM analogs **19** and **20**, and the reference drug dichloroisoproterenol were tested on isolated guinea pig atria and on isolated guinea pig tracheal strips for their activity on β_1- and β_2-receptors, respectively (see Table 3).

Guinea Pig Atria β_1-Receptors. All the compounds tested (**11**, **12**, **19**, and **20**) displayed a β_1-blocking activity, as indicated by their ability to antagonize isoprenaline inotropic response. The activity indices (-log IC_{50}) shown by the MOIM derivatives were virtually identical (for **11b** and **12a**) or slightly higher (for **11a** and **12b**) than those of the corresponding MAOM isomers. Among the compounds **11**,**12** and **19**,**20**, the most active analog was found to be the *N-tert*-butyl-substituted MOIM derivative in which the R substituent is an ethyl group (**12b**). No stimulating properties were detected for the compounds examined.

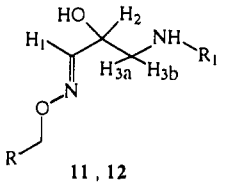
Guinea Pig Tracheal Strip β_2-Receptors. MOIM derivatives (**11**, **12**) showed an appreciable β_2-blocking activity with -log IC_{50} values ranging from 5.36 ± 0.01 of **12a** to 6.57 ± 0.30 of **12b**, which were similar to that of dichloroisoproterenol (6.01 ± 0.55). All these values were a little higher than those found for the corresponding MAOM isomers **19** and **20**. In both series of compounds (**11**,**12** and **19**,**20**) the *N-tert*-butyl-substituted analogs proved to be more active than the *N*-isopropyl-substituted ones. The MOIM *N-tert*-butyl-substituted derivative in which the R substituent is an ethyl group (**12b**) was the most active compound. None of the compounds examined displayed any stimulating properties on β_2 tracheal receptors.

Theoretical Calculations

In order to obtain information about the overall conformational situation of compounds **11**,**12** and **19**,**20** as isolated molecules, a conformational analysis was performed on the model compounds **21** and **22** (see Figures 4 and 5) which are the *N*-unsubstituted analogs of compounds **11** and **19**, respectively. The choice to use model compounds allows a simplification of the conformational analysis (and, later, of the MEP calculation) without conditioning the significance of the results and has already been discussed.[1,2,17]

The calculations were made on the salt forms of **21** and **22**, using the molecular mechanics program Discover;[18] the selected enantiomeric form possesses the geometry on the chiral carbon which corresponds to that of the natural catecholamines. In both molecules **21** and **22**, it is mainly the four torsion angles τ_1–τ_4 (see Figures 4 and 5) that

Table 2. ^{1}H NMR Data of MOIM Derivatives 11 and 12 as Salts[a]

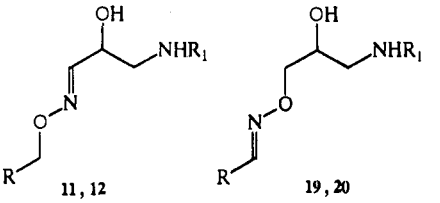


11, 12

compd	R	R'1	H_{3b}	H_{3a}	H_2	H_1
11a·$H_4C_4O_4$	Me	*i*-Pr	3.06 dd (J = 13.1, 9.9)	3.29 dd (J = 13.1, 2.1)	4.68 ddd (J = 9.9, 3.4, 2.1)	7.35 d (J = 3.4)
11b·$H_4C_4O_4$	Me	*t*-Bu	3.04 dd (J = 12.2, 9.5)	3.25 dd J = 12.2, 2.9)	4.69 ddd (J = 9.5, 3.8, 2.9)	7.38 d (J = 3.8)
12a·$H_4C_4O_4$	Et	*i*-Pr	3.16 dd (J = 11.3, 9.3)	3.35 dd (J = 11.3, 2.5)	4.75 ddd (J = 9.3, 3.3, 2.5)	7.42 d (J = 3.3)
12b·$H_4C_4O_4$	Et	*t*-Bu	3.08 dd (J = 12.3, 9.8)	3.32 dd (J = 12.3, 2.4)	4.63 ddd (J = 9.8, 3.8, 2.4)	7.44 d (J = 3.8)

[a] Values for J are reported in hertz.

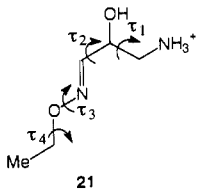
Table 3. β-Adrenergic Activity and Radioligand Binding Affinity of MOIM (11, 12) and MAOM (19, 20) Derivatives



11, 12 19, 20

			β-adrenergic binding affinity[a] K_i (nM)		β-adrenergic activity[b] −log IC_{50}[c]	
compd	R	R_1	rat brain (β_1)	bovine lung (β_2)	isolated guinea pig atria (β_1)	isolated guinea pig tracheal strips (β_2)
11a·$H_4C_4O_4$	Me	*i*-Pr	1040 (850–1200)	3500 (3170–3900)	4.91 ± 0.53	5.48 ± 0.13
11b·$H_4C_4O_4$	Me	*t*-Bu	1080 (1020–1140)	1400 (1300–1520)	4.86 ± 0.23	6.01 ± 0.14
12a·$H_4C_4O_4$	Et	*i*-Pr	1240 (1130–1340)	5600 (5190–6050)	4.81 ± 0.05	5.36 ± 0.01
12b·$H_4C_4O_4$	Et	*t*-Bu	850 (790–910)	850 (770–930)	5.08 ± 0.22	6.57 ± 0.30
19a·$H_4C_4O_4$	Me	*i*-Pr	1480 (1160–1800)	7200 (6870–7560)	4.52 ± 0.23	4.89 ± 0.20
19b·$H_4C_4O_4$	Me	*t*-Bu	760 (670–860)	1980 (1800–2140)	4.87 ± 0.23	5.39 ± 0.68
20a·$H_4C_4O_4$	Et	*i*-Pr	2200 (2000–2450)	4580 (4120–5030)	4.81 ± 0.24	4.92 ± 0.45
20b·$H_4C_4O_4$	Et	*t*-Bu	500 (470–530)	1260 (1140–1390)	4.58 ± 0.59	5.81 ± 0.09
dichloroisoproterenol			51 (44–57)	140 (120–160)	6.94 ± 0.23	6.01 ± 0.55

[a] Geometric means of five separate determinations with confidence limits in parentheses. [b] The values represent the mean of three to five experiments for each drug ± standard error in parentheses. [c] −log IC_{50} is the negative logarithm of the concentration that reduces the agonist response by 50%.



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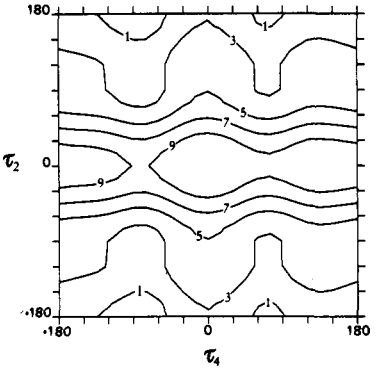
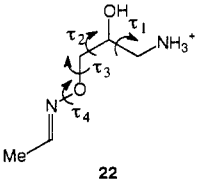


Figure 4. Compound 21. Contour plot of the conformational relative energy obtained by varying together torsion angles τ_2 and τ_4 by 15° steps. The isoenergy contours correspond to values of 1, 3, 5, 7, and 9 kcal/mol.



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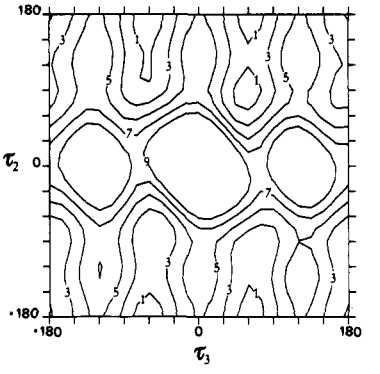


Figure 5. Compound 22. Contour plot of the conformational relative energy obtained by varying together torsion angles τ_2 and τ_3 by 15° steps. The isoenergy contours correspond to values of 1, 3, 5, 7, and 9 kcal/mol.

define the conformations. However, the conformational analysis did not need to take into account either the torsion angle O—C—C—N (τ_1 in both **21** and **22**) or the torsion angle C=N—O—C (τ_3 in **21** and τ_4 in **22**), seeing that the C=N—O—C portion had been found to show a marked preference for a planar conformation,[1,2,17] corresponding to a value of the torsion angle of 180°. The value of the torsion angle τ_1 (about −60°) is conditioned by a hydrogen bond between the cationic nitrogen and the alcoholic oxygen; this value corresponds to the one found in the "pharmacophoric" conformation of the ethanolaminic portion of type A adrenergic drugs.[19]

Only two torsion angles thus remained to be considered for both compounds: the torsion angles τ_2 and τ_4 for **21** and τ_2 and τ_3 for **22**. For each compound, the two torsion angles considered were varied together by 15° steps, thus

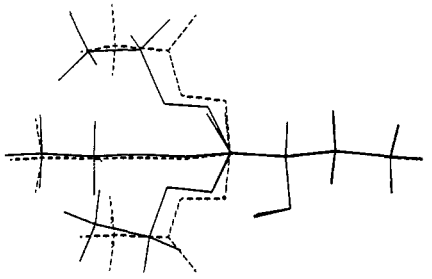


Figure 6. Superimposition of the three lowest energy conformers of **21** (solid line) and the three analogous ones of **22** (dashed line); the picture was obtained by superimposing the ethanolaminic portions of all conformers.

obtaining 576 conformations in which all other freedom degrees were fully optimized.

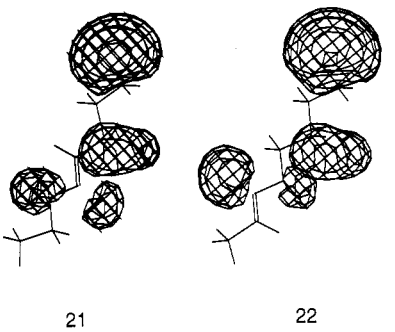
Figure 4 shows the conformational energy trend of **21** vs τ_2 and τ_4: the three conformations with $\tau_2 = 180°$ and $\tau_4 = 90°$, 180°, and 75° possess nearly the same energy. Moreover, the conformational energy remains slightly higher and fairly constant for τ_2 ranging between −180° and −90° and between 90° and 180°. The absolute minimum was found for $\tau_2 = 180°$ and $\tau_4 = -90°$, and it was less than 1.5 kcal/mol lower than the other two low-energy conformers.

Also in the case of compound **22**, as shown in Figure 5, a certain freedom of rotation exists, as in compound **21**. All staggered conformations around τ_3 (180°, 60°, −60°) and with $\tau_2 = 180°$ possess nearly the same energy, and the conformational energy remains almost unchanged with τ_2 ranging between −180° and −90° and between 60° and 180°. The absolute minimum was found for $\tau_2 = 180°$ and $\tau_3 = 60°$, and it was less than 1 kcal/mol lower than the other ones. The conformational results obtained for **22** were in very good agreement with those which had been found at ab initio STO3G level for another MAOM derivative.[1,2,17]

In Figure 6, the superimposition of the three lowest energy conformers of both **21** and **22** shows that the two compounds considered have fairly similar conformational profiles: each conformer of **21** displays close similarities with the corresponding one of **22**. A very good fit is obtained for the conformers in which τ_2 and τ_4 for **21** and τ_2 and τ_3 for **22** are 180°.

The electronic characteristics of **11**,**12** and **19**,**20** were evaluated by calculating the molecular electrostatic potential (MEP) of the model compounds **21** and **22**; the MEP was computed by using the ab initio STO3G wave functions of the free bases.[20] For **21** the low-energy conformer with $\tau_2 = \tau_4 = 180°$ and for **22** the one with $\tau_2 = \tau_3 = 180°$ were selected; these conformers, in which the molecular skeletons are planar, are those where the best fit was found, and therefore, they seem to be the most suitable for a comparison of their chemical reactivity.

In Figure 7, the solid contours corresponding to a value of −10 kcal/mol are shown for **21** and **22**. A close similarity clearly exists between their MEP trends. The ethanolaminic portion generates two negative regions near the alcoholic oxygen and the aminic nitrogen in the same way for **21** and **22**. Moreover, the other two negative regions which in **21** are generated by the oxygen and the nitrogen of the MOIMM are very similar, as regards both extension and position, to the two regions which in **22** are generated by the nitrogen and the oxygen of the MAOMM, respectively.



21 22

Figure 7. Compounds **21** (left) and **22** (right) and their isopotential surfaces corresponding to an MEP value of −10 kcal/mol.

Discussion and Conclusions

An examination of the results shown in Table 3 reveals a fairly good agreement between the affinity index values obtained for MOIM (**11**, **12**) and MAOM (**19**, **20**) derivatives in binding tests on both types of β-receptors and those obtained for the same compounds in functional tests. The limited quantitative differences sometimes observable between indices obtained in the two types of tests might be attributable to differences between the animal species and/or the different kinds of tissues used.[21]

A comparison of the K_i and −log IC_{50} values shown in Table 3 shows that the MOIM derivatives **11** and **12** possess a binding affinity and a blocking activity on β_1-receptors which are comparable to those of the corresponding MAOM derivatives **19** and **20** with regard to β_2-receptors. However, the MOIM derivatives **11** and **12** prove to possess affinity and activity levels that are slightly higher than those of the corresponding MAOM derivatives **19** and **20**, and which, in the case of **12b**, are comparable to those of dichloroisoproterenol.

These biopharmacological data indicate that in the field of β-adrenergic drugs, the MOIM moiety in the *E* configuration appears to reveal a bioisosterism with aryl groups which is analogous to the one already found for the MAOMM. Furthermore, the fact that the biopharmacological properties of the MOIM derivatives (**11**, **12**), which are similar to those of the MAOM derivatives (**19**, **20**) on β_1-receptors, are appreciably better on β_2-receptors would appear to indicate that, at least on β_2-receptors, the atomic sequence $CH_2ON{=}C$ of the MOIMM in the *E* configuration possesses a higher degree of bioisosterism with the aryl groups of adrenergic drugs, compared with the $C{=}NOCH_2$ sequence of the MAOMM.

The similar biopharmacological properties of the MOIM and MAOM derivatives may be rationalized by means of a comparison of their conformational and electronic characteristics. The conformational data indicate that the two types of compounds possess very similar conformational profiles. In particular, for the two model compounds **21** and **22**, which represent MOIM (**11**, **12**) and MAOM (**19**, **20**) derivatives, respectively, it is possible to obtain a good superimposition of their structures in the low-energy conformations (see Figure 6), thus obtaining, in the case of the totally planar conformations, a nearly perfect correspondence of the presumed pharmacophoric groups (MAOM and MOIM moieties, alcoholic oxygen, and amino nitrogen).[22] With regard to the electronic characteristics, an analysis of the molecular electrostatic potential of the two model compounds **21** and **22** at their MOIM and MAOM molecular portions does not reveal any substantial differences in chemical reactivity.

The results of theoretical studies appear to be capable of explaining, in terms of analogies in structural and electronic characteristics, the ability of the MOIMM to substitute the MAOMM effectively as a bioisostere of the Ar, at least in the field of β-adrenergic blocking drugs.

In conclusion, the MOIMM may be considered as an effective new tool for the study of nonaromatic β-blockers and therefore for the development of novel β-blocking drugs. The MOIMM might also prove to be useful in classes of non-adrenergic drugs, in which the presence of an Ar group would appear to be important for the activity.

Experimental Section

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 NMR spectra of all compounds were routinely detected with a Varian CFT-20 instrument operating at 80 MHz in a ca. 2% $CDCl_3$ solution, using Me_4Si as the internal standard. The ^{1}H NMR spectra of the mixtures of unsaturated oxime ethers (**3,5** and **4,6**), oxirane derivatives **7,9** and **8,10**, and pure amino alcohols **11a,b**, and **12a,b**, **19a,b**, and **20a,b**, as salts, were also detected with a Bruker AC-200 instrument; 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 (**3,5** and **4,6**), epoxides (**7,9** and **8,10**), and amino alcohols (**11,13**, and **12,14**) were evaluated on the basis of the integrals of the N=CH proton of **3**–**14** in the ^{1}H NMR spectra of the crude reaction mixtures. *E* oximes **15** and **16** were obtained by the method described in ref 23, whereas *O*-ethyl- (**1**) and *O*-propylhydroxylamine (**2**) were prepared following the procedure described in refs 7 and 8. Boiling points refer to the air-bath temperature of bulb-to-bulb distillation carried out by using a Buchi GKR 51 apparatus and are uncorrected. 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%.

(*E*)- (3) and (*Z*)-*N*-Propenylidene(ethyloxy)amine (5). A solution of *O*-ethylhydroxylamine (**1**)[7] (4.09 g, 0.067 mol) and KH_2PO_4 (11.08 g, 0.081 mol) in H_2O (15 mL) was cooled to 0 °C and treated dropwise, under stirring, with acrolein (3.76 g, 0.067 mol). The resulting mixture was stirred at room temperature for 45 min, and the organic phase was separated to yield a 7:3 mixture (^{1}H NMR) of **3** and **5** (6.24 g) which was immediately used in the subsequent transformation. **3**: ^{1}H NMR δ 1.20 (t, 3H, *J* = 7.1 Hz, CH_3), 4.06 (q, 2H, *J* = 7.1 Hz, OCH_2), 5.43 (m, 1H, *J* = 16.7, 1.6, 0.4 Hz, HC=CH*H* (*trans*)), 5.46 (m, 1H, *J* = 10.8, 1.6, 0.3 Hz, HC=C*H*H (*cis*)), 6.34 (m, 1H, *J* = 16.7, 10.8, 9.8 Hz, C*H*=CH_2), 7.65 (m, 1H, *J* = 9.8, 0.4, 0.3 Hz, N=CH). **5**: ^{1}H NMR δ 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 4.09 (q, 2H, *J* = 7.2 Hz, CH_2O), 5.48 (m, 1H, *J* = 9.4, 1.6, 0.3 Hz, HC=C*H*H (cis)), 5.50 (m, 1H, *J* = 15.9, 1.6, 0.4 Hz, HC=CH*H* (*trans*)), 6.88 (m, 1H, *J* = 15.9, 9.4, 9.1 Hz, C*H*=CH_2), 6.93 (m, 1H, *J* = 9.1, 0.4, 0.3 Hz, N=CH).

(*E*)- (4) and (*Z*)-*N*-Propenylidene(propyloxy)amine (6). An aqueous solution of *O*-propylhydroxylamine (**2**)[8] (5.03 g, 0.067 mol) was treated, as described for the preparation of **3** and **5**, with KH_2PO_4 (11.08 g, 0.081 mol) and acrolein (3.76 g, 0.067 mol) to yield a 7:3 mixture of **4** and **6** (7.10 g) which, without further purification, was immediately used for the following reaction. **4**: ^{1}H NMR δ 0.87 (t, 3H, *J* = 7.4 Hz, CH_3), 1.62 (m, 2H, CH_3CH_2-CH_2), 3.95 (m, 2H, $CH_3CH_2CH_2$), 5.44 (m, 1H, *J* = 16.7, 1.6 Hz, HC=CH*H* (*trans*)), 5.46 (m, 1H, *J* = 11.2, 1.6 Hz, HC=C*H*H (*cis*)), 6.35 (m, 1H, *J* = 16.7, 11.2, 9.8 Hz, C*H*=CH_2), 7.64 (d, 1H, *J* = 9.8 Hz, N=CH). **6**: ^{1}H NMR δ 0.86 (t, 3H, *J* = 7.4 Hz, CH_3), 1.62 (m, 2H, $CH_3CH_2CH_2$), 3.95 (m, $CH_3CH_2CH_2$), 5.48 (m, 1H, *J* = 9.4, 1.6 Hz, HC=C*H*H (*cis*)), 5.50 (m, 1H, *J* = 16.0, 1.6 Hz, HC=CH*H* (*trans*)), 6.88 (m, 1H, *J* = 16.0, 9.4, 9.3 Hz, *H*C=CH_2), 6.94 (d, 1H, *J* = 9.3 Hz, N=CH).

(*E*)- (7) and (*Z*)-*N*-(2,3-Epoxypropylidene)(ethyloxy)amine (9). A solution of a 7:3 mixture of **3** and **5** (8.92 g, 90 mmol) in CH_2Cl_2 (50 mL) was added to a 10% aqueous solution of $NaHCO_3$ (20 mL), and the resulting mixture was cooled at 0 °C and then treated dropwise under stirring with a solution of 70% *m*-chloroperoxybenzoic acid (24.7 g, 100 mmol) in CH_2Cl_2 (120 mL). The mixture was stirred in the dark at room temperature for 72 h, and the organic phase was separated, washed (5% aqueous K_2CO_3, 1 N aqueous $Na_2S_2O_3$, and H_2O), filtered, and evaporated to dryness to yield a crude residue (9.67 g) consisting almost exclusively of a 7:3 mixture of **7** and **9** which was used immediately for the following reaction. **7**: ^{1}H NMR δ 1.27 (t, 3H, *J* = 7.0 Hz, CH_3), 2.81 (dd, 1H, *J* = 5.0, 2.8 Hz, $\overline{OCHCH}H$), 3.04 (dd, 1H, *J* = 5.0, 4.3 Hz, $\overline{OCHC}H$H), 3.54 (ddd, 1H, *J* = 8.0, 4.3, 2.8 Hz, $\overline{OCH}CH_2$), 4.14 (q, 2H, *J* = 7.0 Hz, CH_2O), 6.93 (d, 1H, *J* = 8.0 Hz, N=CH). **9**: ^{1}H NMR δ 1.29 (t, 3H, *J* = 6.9 Hz, CH_3), 2.75 (dd, 1H, *J* = 5.4, 2.6 Hz, $\overline{OCHCH}H$), 3.06 (dd, 1H, *J* = 5.4, 5.1 Hz, $\overline{OCHC}H$H), 4.06 (ddd, 1H, *J* = 7.0, 5.1, 2.6 Hz, $\overline{OCH}CH_2$), 4.16 (q, 2H, *J* = 6.9 Hz, CH_2O), 6.32 (d, 1H, *J* = 7.0 Hz, N=CH).

(*E*)- (8) and (*Z*)-*N*-(2,3-Epoxypropylidene)(propyloxy)amine (10). A solution of a 7:3 mixture of **4** and **6** (10.18 g, 90 mmol) in CH_2Cl_2 (50 mL) was treated with a 10% aqueous solution of $NaHCO_3$ (20 mL) and then with a solution of 70% *m*-chloroperoxybenzoic acid (24.7 g, 100 mmol) in CH_2Cl_2 (120 mL), following the procedure described for the preparation of **7** and **9**. The crude residue (8.61 g), consisting almost exclusively of a 7:3 mixture of **8** and **10**, was directly used for the following aminolysis reactions. **8**: ^{1}H NMR δ 0.94 (t, 3H, *J* = 7.4 Hz, CH_3), 1.68 (m, 2H, $CH_3CH_2CH_2$), 2.82 (dd, 1H, *J* = 4.9, 2.6 Hz, $\overline{OCHCH}H$), 3.06 (dd, 1H, *J* = 4.5, 2.6 Hz, $\overline{OCHC}H$H), 3.55 (ddd, 1H, *J* = 8.0, 4.5, 2.6 Hz, $\overline{OCH}CH_2$), 4.06 (m, 2H, CH_2O), 6.95 (d, 1H, *J* = 8.0 Hz, N=CH). **10**: ^{1}H NMR δ 0.96 (t, 3H, *J* = 7.4 Hz, CH_3), 1.68 (m, 2H, $CH_3CH_2CH_2$), 2.76 (dd, 1H, *J* = 5.4, 2.6 Hz, $\overline{OCHCH}H$), 3.07 (dd, 1H, *J* = 5.2, 2.6 Hz, $\overline{OCHC}H$H), 4.06 (m, 2H, CH_2O), 4.27 (ddd, 1H, *J* = 7.0, 5.2, 2.6 Hz, $\overline{OCH}CH_2$), 6.34 (d, 1H, *J* = 7.0 Hz, N=CH).

(*E*)-*N*-[3-(Isopropylamino)-2-hydroxypropylidene](ethyloxy)amine Maleate (11a·$H_4C_4O_4$). A stirred solution of a 7:3 mixture of **7** and **9** (1.84 g, 16 mmol) in anhydrous EtOH (60 mL) and *i*-$PrNH_2$ (1.89 g, 32 mmol) was kept at room temperature at 48 h and then evaporated to dryness at 25 °C to yield an oily residue consisting almost exclusively of a 7:3 mixture of **11a** (**11a**: ^{1}H NMR δ 7.45 (d, 1H, *J* = 5.2 Hz, N=CH)) and (*Z*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene](ethyloxy)amine (**13a**) (**13a**: ^{1}H NMR δ 6.82 (d, 1H, *J* = 4.1 Hz, N=CH)). The crude mixture was submitted to column chromatography on silica gel, eluting with a 95:5 AcOEt–Et_3N mixture and collecting 20-mL fractions. Evaporation of the middle fractions yielded an oil (1.66 g) which was dissolved in anhydrous Et_2O (20 mL) and treated in portions at 0 °C under stirring with a solution of maleic acid (0.55 g, 4.8 mmol) in anhydrous MeOH (6 mL). Addition of anhydrous Et_2O gave a solid precipitate which was filtered and crystallized from *i*-Pr_2O–hexane to yield the pure maleate salt of **11a** (0.97 g) (for physical and analytical data, see Table 1).

When an etheral solution of the chromatographed mixture of **11a** and **13a** was treated with a molar equivalent of maleic acid in anhydrous MeOH, only decomposition products were obtained.

(*E*)-*N*-[3-(*tert*-Butylamino)-2-hydroxypropylidene](ethyloxy)amine (11b·$H_4C_4O_4$). A stirred solution of a 7:3 mixture of **7** and **9** (1.84 g, 16 mmol) in anhydrous EtOH (60 mL) and *t*-$BuNH_2$ (2.34 g, 32 mmol) was kept at room temperature for 48 h and then treated, as in the preparation of **11a**, to yield an oily residue (2.10 g) consisting mainly of a 7:3 mixture of **11b** (**11b**: ^{1}H NMR δ 7.57 (d, 1H, *J* = 4.8 Hz, N=CH)) and (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](ethyloxy)amine (**13b**) (**13b**: ^{1}H NMR δ 6.83 (d, 1H, *J* = 4.3 Hz, N=CH)) which was submitted to column chromatography, as in the purification of **11a**. Treatment of the oily residue (1.50 g) with maleic acid (0.524 g, 4.5 mmol) in anhydrous Et_2O–MeOH afforded a solid precipitate which was crystallized from *i*-Pr_2O–hexane to yield

the pure maleate salt of **11b** (0.662 g) (for physical and analytical data, see Table 1).

Treatment of a sample of the chromatographed mixture of **11b** and **13b** with a molar equivalent of maleic acid gave only decomposition products.

(*E*)-*N*-[3-(Isopropylamino)-2-hydroxypropylidene](propyloxy)amine Maleate (12a·$H_4C_4O_4$). A stirred solution of a 7:3 mixture of **8** and **10** (2.07 g, 16 mmol) in anhydrous EtOH (60 mL) and *i*-$PrNH_2$ (1.89 g, 32 mmol) was kept at room temperature for 48 h and then evaporated to dryness at 25 °C to yield an oily residue consisting mainly of a 7:3 mixture of **12a** (**12a**: ^{1}H NMR δ 7.45 (d, 1H, J = 5.0 Hz, N=CH)) and (*Z*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene](propyloxy)amine (**14a**) (**14a**: ^{1}H NMR δ 6.82 (d, 1H, J = 4.4 Hz, N=CH)). The crude mixture was submitted to column chromatography on silica gel, eluting with a 95:5 AcOEt–Et_3N mixture and collecting 20-mL fractions. Evaporation of the middle fractions yielded an oil (1.86 g) which was dissolved in anhydrous Et_2O (10 mL) and treated in portions at 0 °C under stirring with a solution of maleic acid (0.57 g, 4.9 mmol) in anhydrous MeOH (3 mL). Addition of anhydrous Et_2O gave a solid precipitate which was filtered and crystallized from *i*-Pr_2O–hexane to yield the pure maleate salt of **12a** (0.84 g) (for physical and analytical data, see Table 1).

Treatment of a sample of the chromatographed mixture of **12a** and **14a** with a molar equivalent of maleic acid yielded only a complex mixture of decomposition products.

(*E*)-*N*-[3-(*tert*-Butylamino)-2-hydroxypropylidene](propyloxy)amine Maleate (12b·$H_4C_4O_4$). A stirred solution of a 7:3 mixture of **8** and **10** (2.07 g, 16 mmol) in anhydrous EtOH (60 mL) and *t*-$BuNH_2$ (2.34 g, 32 mmol) was kept at room temperature for 48 h and then treated, as in the preparation of **12a**, to yield an oily residue (2.27 g) consisting mainly of a 7:3 mixture of **12b** (**12b**: ^{1}H NMR δ 7.44 (d, 1H, J = 5.2 Hz, N=CH)) and (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](propyloxy)amine (**14b**) (**14b**: ^{1}H NMR δ 6.83 (d, 1H, J = 4.2 Hz, N=CH)) which was submitted to column chromatography as in the purification of **12a**. Treatment of the oily residue (1.95 g) with maleic acid (0.56 g, 4.8 mmol) in anhydrous Et_2O–MeOH afforded a solid precipitate which was crystallized from *i*-Pr_2O–hexane to yield the pure maleate salt of **12b** (0.81 g) (for physical and analytical data, see Table 1).

Treatment of a sample of the mixture of **12b** and **14b** with a molar equivalent of maleic acid gave only decomposition products.

General Procedure for the Preparation of 19 and 20. The procedure is illustrated by the synthesis of 3-[(ethylideneamino)oxy]-1-(isopropylamino)-2-propanol (**19a**). A solution of (*E*)-acetaldoxime (**15**) (5.9 g, 100 mmol) in anhydrous MeOH (150 mL) was added dropwise to a stirred solution of MeONa, prepared from anhydrous MeOH (150 mL) and Na (2.3 g, 100 mmol), and the resulting mixture was refluxed for 1 h and then evaporated at reduced pressure. The solid residue was taken up in anhydrous DMF (200 mL), and the mixture was added dropwise to a cooled (0 °C) and stirred solution of epichlorohydrin (9.3 g, 100 mmol) in anhydrous DMF (50 mL). After stirring at room temperature for 72 h, the solution was diluted with H_2O and extracted with $CHCl_3$. The organic phase was washed three times with H_2O, filtered, and evaporated to yield practically pure crude 3-[(ethylideneamino)oxy]-1,2-epoxypropane (**17**) which was directly used for the following reaction. An analytical sample of **17** was obtained by distillation (82%): bp 67–69 °C (0.1 mmHg); ^{1}H NMR δ 1.86 (d, 3H, J = 5.2 Hz, CH_3), 2.77 (m, 2H, $\overline{OCHCH_2}$), 3.2 (m, 1H, CHO), 3.57 (d, 2H, J = 5.0 Hz, $NOCH_2$), 7.14 (q, 1H, J = 5.6 Hz, CH=N). Anal. ($C_5H_9NO_2$) C, H, N. **18** (80%): bp 78–80 °C (0.1 mmHg); ^{1}H NMR δ 1.07 (t, 3H, J = 7.0 Hz, CH_3), 2.6 (m, 2H, CH_3CH_2), 2.7 (m, 2H, $\overline{OCHCH_2}$), 3.27 (m, 1H, CHO), 3.6 (d, 2H, J = 5.0 Hz, $NOCH_2$), 7.46 (t, 1H, J = 5.0 Hz, CH=N). Anal. ($C_6H_{11}NO_2$) C, H, N.

For the stability tests, the oximes of acetaldehyde (**15**) and propionaldehyde (**16**) were treated, under the conditions described above for the preparation of **17**, with MeONa in anhydrous MeOH and DMF. The usual workup made it possible to recover the starting oximes, unaltered.

A stirred solution of the crude epoxide **17** (11.5 g, 100 mmol) in anhydrous EtOH (60 mL) was treated with *i*-$PrNH_2$ (29.5 g, 500 mmol), and the solution was left at room temperature for 72 h. The solution was evaporated to dryness, and the oily residue was dissolved in a 7:3 mixture of anhydrous Et_2O–MeOH and then treated with a solution of maleic acid (11.6 g, 100 mmol) in anhydrous MeOH (8 mL). The crude precipitate was filtered and crystallized to yield the pure maleate salt of **19a**. **19a·$H_4C_4O_4$**: ^{1}H NMR δ 3.08 (d, 2H, J = 13.0 Hz, CH_2NH), 4.14 (m, 1H, CHOH), 4.23 (d, 2H, J = 11.7 Hz, $NOCH_2$), 7.44 (q, J = 6.05 Hz, 1H, CH=N). Anal. ($C_{12}H_{22}N_2O_6$) C, H, N. **20a·$H_4C_4O_4$**: ^{1}H NMR δ 3.16 (d, 2H, J = 12.7 Hz, CH_2), 4.20 (m, 1H, CHOH), 4.34 (d, 2H, J = 10.8 Hz, $NOCH_2$), 7.35 (t, J = 5.83 Hz, 1H, CH=N). Anal. ($C_{13}H_{24}N_2O_6$) C, H, N. **19b·$H_4C_4O_4$**: ^{1}H NMR δ 3.05 (d, 2H, J = 12.8 Hz), 4.18 (m, 1H, CHOH), 4.30 (d, 2H, J = 11.4 Hz, $NOCH_2$), 7.35 (q, 1H, J = 6.03 Hz, CH=N). Anal. ($C_{13}H_{24}N_2O_6$) C, H, N. **20b·$H_4C_4O_4$**: ^{1}H NMR δ 3.04 (d, 2H, J = 12.3 Hz, CH_2), 4.12 (m, 1H, CHOH), 4.30 (d, 2H, J = 11 Hz, $NOCH_2$), 7.40 (t, 2H, J = 5.80 Hz, CH=N). Anal. ($C_{14}H_{26}N_2O_6$) C, H, N.

For other physical and microanalytical data of **19** and **20**, see Table 1.

For the stability tests, acetaldoxime (**15**) and propionaldoxime (**16**) were dissolved in EtOH and treated with *i*-$PrNH_2$ in the conditions used for the aminolysis reaction of **17**. Evaporation of the organic solution yielded the starting oximes in a practically pure state.

Radioligand Binding Methods. Rat Brain β_1-Receptors. β_1-receptors were assayed in rat cortical membranes, as previously described,[24] using [^{3}H]CGP 26505[14] (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 28.5 Ci/mmol).

Rat cortices were rapidly isolated and homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer at pH 8. The homogenates were centrifuged at 48000*g* for 15 min at 4 °C. This step was repeated four times, resuspending the pellets in 10 volumes of fresh Tris buffer. The final crude membranes were suspended in Tris-HCl buffer containing 0.1% ascorbic acid. Protein concentration, as assayed by the method of Lowry et al.,[25] amounted to 4 mg/mL for displacement studies.

Routine [^{3}H]CGP binding assays were run by incubating 0.1 mL of crude rat brain membrane suspensions at 25 °C for 60 min with 1 nM [^{3}H]CGP in a total volume of 0.5 mL of Tris-HCl buffer. Incubations were terminated by rapid vacuum filtration through Whatman GF/B fiberglass filters. Filters were washed with 3 × 5-mL portions of ice-cold Tris-HCl buffer, dried, and added to 8 mL of Ready Protein Beckman scintillation cocktail. The specific binding was determined as the excess over blanks containing 30 μM *l*-isoprenaline.

Bovine Lung β_2-Receptors. β_2-Receptor binding was studied in bovine lung using [^{3}H]dihydroalprenolol (DHA)[15] as the ligand (DuPont de Nemours, New England Nuclear Division; specific activity 48.1 Ci/mmol).

Membranes were obtained by lung homogenization in 1:20 volumes of 0.32 M sucrose and centrifugation at 800*g* for 10 min at 5 °C. The supernatant was centrifuged at 3000*g* for 10 min at 5 °C. The resulting pellet was suspended in 50 mM phosphate buffer at pH 7.4 containing 0.02% ascorbic acid and then centrifuged. This step was repeated twice. Crude lung membranes 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/B fiberglass filters and washed with 3 × 5 mL of phosphate buffer, dried, and added to 8 mL of Ready Protein Beckman scintillation cocktail. No specific binding was measured in the presence of 35 μM *l*-isoprenaline.

Inhibition of the [^{3}H]CGP 26505 and [^{3}H]DHA specific binding was determined in the presence of various concentrations of the compounds studied. The affinity of the compounds for the specific binding sites was expressed as the molar concentration inhibiting the specific binding by 50% (IC_{50}). These values were calculated from the displacement curves by log probit analysis. The inhibition constant (K_i) was derived in accordance with the equation of Cheng and Prusoff.[26] The dissociation constants (K_d) of [^{3}H]CGP 26505 and [^{3}H]DHA were 0.7 and 1.0 nM, respectively.

Pharmacological Methods. The activity of compounds 11,12 and 19,20 on β-adrenoceptors was evaluated on isolated preparations obtained from adult male Dunkin-Hartley guinea pigs, weighing 300–350 g.

Guinea Pig Atria. Isolated guinea pig atria were employed to determine the activity of the compounds on β_1-adrenoceptors, in accordance with ref 13. The extremities of the strip consisting of both atria were tied with an inextensive thread. The first thread was used to tie the organ to a muscle holder fixed in place in a muscle chamber of an isolated organ bath; the second thread was used to connect atria to an isometric force-displacement transducer (Basile Model 7005) situated above the muscle chamber and connected to a microdynamometer (Basile Model 7050). The bathing fluid was Tyrode solution at 37 °C gassed with pure O_2. The atria were left at rest for 45 min before starting the experiments, and then, they were submitted to increasing doses of *l*-isoprenaline (at least five concentrations) to obtain dose–response curves with the method of single doses.

The agonistic action of the compounds under test was checked by evaluating their ability to increase the inotropic activity of spontaneously beating atria. Antagonistic properties were evaluated as the progressive reduction of submaximal agonist responses to the increasing concentrations of the drugs. The contact period for each antagonistic dose was 30 min.

Guinea Pig Tracheal Strips. Isolated guinea pig tracheal strips were used to evaluate the action of the compounds on β_2-adrenoceptors. The organs were excised, cleared of adhering fat and connective tissue, and prepared following the method of Emmerson and Mackay.[27] Zig-zag tracheal strips were tied at each end and suspended in a muscle chamber as described for atria. The perfusion fluid of the organs was Krebs maintained at 37 °C and aerated with 95% O_2–5% CO_2. The tracheae were attached to an isotonic force-displacement transducer (Basile Model 7006) and left to equilibrate for 1 h before administering drugs. Responses were registered by a microdynamometer (Basile Model 7050). In order to evaluate the relaxant properties of β-agonists, the preparations were contracted by carbachol (5.5 × 10^{-6} M). A dose–effect curve to *l*-isoprenaline was obtained in each organ, and then, the antagonistic activities of the compounds under test were assessed. The dose–response curves were obtained using the method of cumulative doses. The agonistic action was assessed as the ability of the compounds under test to relax the tracheal smooth musculature precontracted with carbachol; the antagonistic effects of the same compounds were evaluated after a 30-min incubation as their inhibitor properties on the relaxant effect of a submaximal dose of *l*-isoprenaline.

Antagonism of the above-cited drugs was expressed as −log IC_{50}, that is, the negative logarithm of the drug molar concentration able to reduce by 50% the stimulating effect of *l*-isoprenaline. Dichloroisoproterenol was taken as the reference antagonist.

The following drugs were used as salts: *l*-isoproterenol and dichloroisoproterenol as hydrochlorides, carbachol as chloride, and the MOIM (11, 12) and MAOM (19, 20) derivatives as maleates.

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References

(1) Macchia, B.; Balsamo, A.; Lapucci, A.; Martinelli, A.; Macchia, F.; Breschi, M. C.; Fantoni, B.; Martinotti, E. An Interdisciplinary Approach to the Design of New Structures Active at the β-Adrenergic Receptor. Aliphatic Oxime Ether Derivatives. *J. Med. Chem.* **1985**, *28*, 153–160.

(2) Balsamo, A.; Breschi, M. C.; Chini, M.; Doniano, P.; Giannaccini, G.; Lucacchini, A.; Macchia, B.; Macchia, M.; Manera, C.; Martinelli, A.; Martini, C.; Martinotti, E.; Nieri, P.; Rossello, A. Conformationally Restrained β-Blocking Oxime Ethers: Synthesis and β-Adrenergic Properties of Diastereoisomeric *Anti* and *Syn* (2-(5′-Isoxazolidinyl)-ethanolamines. *Eur. J. Med. Chem.* **1992**, *27*, 751–764.

(3) Balsamo, A.; Dell'Omodarme, G.; Lapucci, A.; Macchia, M.; Orlandini, E.; Ferni, G.; Pinza, M. Synthesis and β-Adrenergic Activity of New Completely Aliphatic 3-(Methyleneaminoxy)-propanolamine Derivatives. XVIIth National Meeting of the Italian Chemical Society, Genova, 1992.

(4) Macchia, B.; Balsamo, A.; Lapucci, A.; Macchia, F.; Martinelli, A.; Nencetti, S.; Orlandini, E.; Baldacci, M.; Mengozzi, G.; Soldani, G.; Domiano, P. Molecular Design, Synthesis, and Antiinflammatory Activity of a Series of β-Aminoxypropionic Acids. *J. Med. Chem.* **1990**, *33*, 1423–1430.

(5) Balsamo, A.; Broccali, G.; Lapucci, A.; Macchia, B.; Macchia, F.; Orlandini, E.; Rossello, A. Synthesis and Antimicrobial Properties of Substituted β-Aminoxypropyonyl Penicillins and Cephalosporins. *J. Med. Chem.* **1989**, *32*, 1398–1401.

(6) Balsamo, A.; Lapucci, A.; Macchia, M.; Martinelli, A.; Nencetti, S.; Rossello, A.; Cristina, T. 2-(Methyleneaminoxy)methylmorpholine Derivatives. Synthesis and Antidepressant Activity. *Farmaco* **1994**, *49*, 77–82.

(7) Jones, L. W.; Neuffer, L. The Preparation of Alkylhydroxyurea Chlorides and Their Relation to Esters of Carbon Dioxide Oxime, R—O—N=C=O. *J. Am. Chem. Soc.* **1917**, *39*, 652–659.

(8) Chimiak, A.; Kolasa, T. *O*-alkylhydroxylamines. *Bull. Acad. Pol. Sci., Ser. Sci., Chim.* **1974**, *22* (23), 195–199.

(9) Wieland, H. Über Acethydroxamsäurechlorid. (on Acethydroxamyl Chloride.) *Ber. Dtsch. Chem. Ges.* **1907**, *40*, 1676–1679.

(10) Beech, W. F. Preparation of Aromatic Aldehydes and Ketones from Diazonium Salts. *J. Chem. Soc.* **1954**, *1*, 1297–1302.

(11) Karabatsos, G. J.; Hsi, N. Structural Studies by Nuclear Magnetic Resonance. XI. Conformations and Configurations of Oxime O-Methyl Ethers. *Tetrahedron* **1967**, *23*, 1079–1095.

(12) See, for example: Portoghese, P. S. Stereochemical Studies on Medicinal Agents. IV. Conformational Analysis of Ephedrine Isomers and Related Compounds. *J. Med. Chem.* **1967**, *10*, 1057–1063.

(13) See, for example: (a) Balsamo, A.; Ceccarelli, G.; Crotti, P.; Macchia, B.; Macchia, F.; Tognetti, P. NMR Studies of β-Adrenergic Blocking Agents: Molecular Conformation of IMPEA and Dichloroisoproterenol in Solution. *Eur. J. Med. Chem.* **1982**, *17*, 471–478. (b) Macchia, B.; Balsamo, A.; Epifani, E.; Lapucci, A.; Nencetti, S.; Macchia, F.; Breschi, M. C.; Martinotti, E.; Ceserani, R. Conformational Effects on the Activity of Drugs. 11. Stereostructural Models For the Direct Activation of the α- and β-Adrenergic Receptor. *J. Med. Chem.* **1986**, *29*, 740–747.

(14) Dooley, D. J.; Bittiger, H.; Reymann, N. C. CGP 20712A: A Useful Tool for Quantitating β_1- and β_2-Adrenoceptors. *Eur. J. Pharmacol.* **1986**, *130*, 137–139.

(15) Nahorski, S. R.; Richardson, A. Pitfalls in the Assessment of the Specific Binding of (−)[3H]-Dihydroalprenolol to β-Adrenoceptors. *Br. J. Pharmacol.* **1979**, *66*, 469–470.

(16) Minneman, K.; Hegstrand, L. R.; Molinoff, P. B. Simultaneous Determination of Beta-1 and Beta-2 Adrenergic Receptors in Tissues Containing Both Receptors Subtype. *Mol. Pharmacol.* **1979**, *16*, 34–46.

(17) Lapucci, A.; Macchia, M.; Martinelli, A.; Nencetti, S.; Orlandini, E.; Rossello, A.; Baldacci, M.; Mengozzi, G.; Soldani, G. Synthesis, Antiinflammatory Activity and Molecular Orbital Studies of a Series of Benzylideneaminoxypropionic Acids Substituted on the Phenyl Ring. *Eur. J. Med. Chem.*, in press.

(18) INSIGHT II. Version 2.0.0; DISCOVER, Version 2.7.0. Biosym Technologies, San Diego.

(19) Macchia, B.; Balsamo, A.; Breschi, M. C.; Lapucci, A.; Lucacchini, A.; Macchia, F.; Manera, C.; Martinelli, A.; Martini, C.; Martinotti, E.; Nencetti, S. Conformational Effects on the Activity of Drugs. 13. A Revision of Previously Proposed Models for the Activation of α- and β-Adrenergic Receptors. *J. Med. Chem.* **1992**, *35*, 1009–1018.

(20) The salt form is the preferred one in the biological environment and was used for the conformational analysis; however, it is unsuitable for MEP calculations because the positive net charge of the protonated nitrogen completely hides any trend on the rest of the molecule.

(21) Willias, M.; Sills, M. *Comprehensive Medicinal Chemistry*; Emmet, J. C., Ed.; Pergamon Press: Oxford, 1990; Vol. 3, pp 45–80 and references cited therein.

(22) See ref 13 and references cited therein.

(23) Vogel, A. I. *Practical Organic Chemistry*; Longmans, Green & Co., Ltd.: London, 1956; p 719.

(24) Balsamo, A.; Breschi, M. C.; Giannaccini, G.; Lapucci, A.; Lucacchini, A.; Macchia, B.; Manera, C.; Martini, C.; Martinotti, E.; Nencetti, S.; Rossello, A.; Scatizzi, R. Synthesis and β-Adrenergic Properties of Tetrahydronaphthalene Analogs of Dichloroisoproterenol. *Eur. J. Med. Chem.* **1993**, *28*, 735–741.

(25) Lowry, D. H.; Rosemburg, N. J.; Farr, A. L.; Randal, R. J. *J. Biol. Chem.* **1951**, *193*, 265–275.

(26) Cheng, Y.; Prusoff, W. H. Relation Between the Inibition Constant Ki and the Concentration of Inhibitor which Causes Fifty per Cent Inhibition (Ic_{50}) of an Enzymic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(27) Emmerson, J.; Mackay, D. The zig-zag Tracheal Strip. *J. Pharm. Pharmacol.* **1979**, *31*, 798.