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Synthesis and β-Adrenergic Properties of (*Z*)-*N*-[3-(Alkylamino)-2-hydroxypropylidene](aryl-methyloxy)amines: Effects of the Configuration Around the Methyloxyiminomethyl (MOIM) Double Bond on the Biopharmacological Properties of MOIM-type β-Blocking Agents

Aldo Balsamo,[a]* Maria C. Breschi,[b] Grazia Chiellini,[b] Annalina Lapucci,[a] Nicola Lazzeri,[b] Marco Macchia,[a] Adriano Martinelli,[a] Eugenio Micali,[a] Susanna Nencetti[a] and Armando Rossello[a]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 6, 56126, Pisa, Italy*

[b]*Dipartimento di Psichiatria Neurobiologia Farmacologia e Biotecnologie, Università di Pisa, via Bonanno 6, 56126, Pisa, Italy*

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Abstract—The *N*-isopropyl- (**3a–g**) and *N*-*tert*-butyl-substituted (**4a–g**) (*Z*)-*N*-(3-(amino)-2-hydroxypropylidene)-(arylmethyloxy)amines were synthesized in order to compare their β_1- and β_2-adrenergic properties with those of their previously studied corresponding analogues with the *E* configuration (**1a–g** and **2a–g**). Compounds **3** and **4** were tested for their affinity for β_1- and β_2-adrenoceptors by radioligand binding experiments, and the compounds with the highest affinity were also assayed for their activity towards the same types of β-adrenoceptors by functional tests on isolated preparations. The *Z*-methyloxyiminomethyl (*Z*-MOIM) compounds **3** and **4** proved to possess, on the whole, affinity (K_i) and activity (pIC_{50}) indices similar to those of the *E* isomers **1** and **2**, thus indicating that for the MOIM-type β-adrenergic antagonists **1–4**, the type of configuration around the MOIM double bond does not have any appreciable effect either on the affinity or on the activity towards β-adrenoceptors. These results are rationalized on the basis of the steric and electronic analogies existing between the MOIM groups of **1–4** in the two types of configurations (*E* and *Z*).

Introduction

As part of an on-going study[1–8] on the stereoelectronic requirements for the interaction of adrenergic drugs with β-receptors, we examined the possibility of effectively replacing the aryl (Ar) of type **A** β-blocking drugs with non-aromatic substituents which were believed to be capable of simulating an Ar. It was thus found that the methyleneaminoxymethyl moiety ($C{=}NOCH_2$, MAOMM) of type **B** compounds can act as a bioisoster of the Ar of type A β-blocking agents.[1] This result was explained by a comparison of the chemical reactivity of the two types of compounds (**A** and **B**). In a subsequent study, it was found that the replacement of the MAOMM of type **B** compounds with the methyloxyiminomethyl moiety ($CH_2ON{=}C$, MOIMM) with the *E* configuration leads to compounds (**C**) possessing β-adrenergic properties similar to those of compounds **B**.[3,5] In the design of compounds **C**, the type of configuration (*E*) of the MOIMM was chosen because in this configuration, the atomic sequence $CH_2ON{=}C$ appeared to present greater steric and electronic analogies with an Ar than in the *Z* configuration.[3] Theoretical

Key words: adrenergic drug; β-blocking agent; [(methyloxy)-imino]methyl moiety; (*Z*)-N-[3-(amino)-2-hydroxypropylidene]-(arylmethyloxy)amine.

*Corresponding author. Tel.: 39 50 500 209; fax: 39 50 40517.

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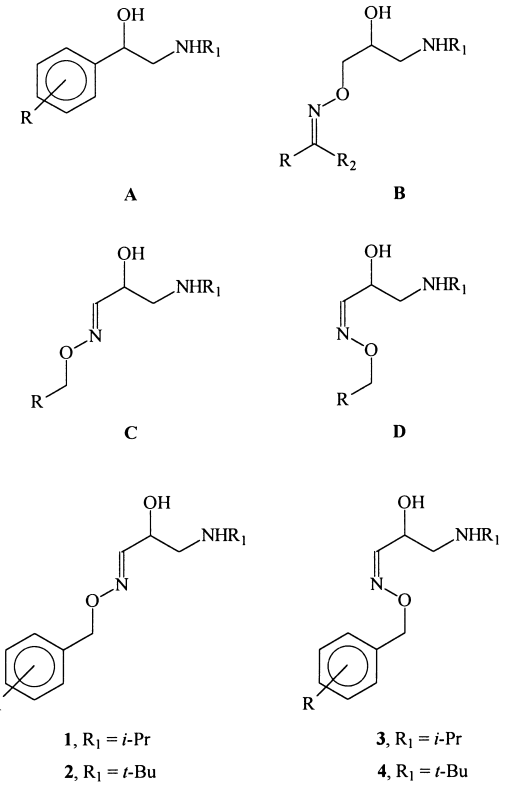
studies carried out on model compounds of types **B** and **C** showed clear spatial correspondences and electronic analogies between the *E*-MOIMM present in compounds **C** and the MAOMM of compounds **B**, and therefore the Ar of drugs of type **A**.[3]



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

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

Furthermore, the results obtained with the previously studied type **C** derivatives (**1** and **2**) do not exclude the possibility that, at least in the field of β-adrenergic antagonists, the MOIM group may be bioequivalent to the MAOMM, even if its atomic sequence is arranged in a different way from the one shown by the MOIMM with the *E* configuration. On the basis of this consideration, and as an extension of our earlier research, we decided to study the compounds of type **D** which differ from those of type **C** previously studied (**1** and **2**) in their configuration around the imino double bond of the MOIMM, which is of type *Z* instead of *E*. This paper describes the synthesis and complete characterization of the (*Z*)-*N*-[3-(amino)-2-hydroxypropylidene](arylmethyloxy)amines **3a–g** and **4a–g** (see Table 1), together with their biopharmacological β-adrenergic properties, evaluated by radioligand binding experiments and, for the compounds with the highest affinity, by functional tests on isolated preparations.

Chemistry

The (*Z*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene]-(**3a–g**) and (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](arylmethyloxy)amines (**4a–g**) were prepared following the synthetic procedure shown in Scheme 1. Condensation of the *O*-arylmethylhydroxylamines (**5a–g**),[9] as hydrochlorides, with acrolein yielded mixtures of the corresponding *Z* (**6a–g**) and *E* (**7a–g**) unsaturated oxime ethers in a ratio of about 3:7,[5] from which the *Z* compounds (**6a–g**) were isolated by column chromatography. Epoxidation[10] of **6a–g** with potassium peroxymonosulfate (oxone) in heterogeneous phase at pH 7.5 in the presence of 18-crown-6, afforded almost exclusively the corresponding *Z* epoxides (**8a–g**) which were purified by column chromatography. Subsequent aminolysis of **8a–g** with *i*-$PrNH_2$ or *t*-$BuNH_2$ yielded the crude *Z* amino-alcohols which, after purification by column chromatography, were transformed into the corresponding neutral oxalate salts (**3a–g**.$1/2H_2C_2O_4$, **4a–g**.$1/2H_2C_2O_4$).

The structure and configuration of the intermediate (**6a–g** and **8a–g**) and final (**3a–g** and **4a–g**) compounds were assigned by comparison of their ^{1}H NMR spectral data (for **3** and **4** see Table 2) with those obtained for the same compounds in the crude E/Z mixtures,[5] with the only exception of the unsaturated oxime ether with the *Z* configuration **6g**, which was also previously separated from the *E* isomer **7g**.[5]

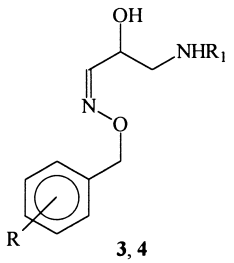
In order to obtain information about the configurational stability of the *Z*-MOIM derivatives, one of them (**4g**$\frac{1}{2}H_2C_2O_4$) was placed in the experimental conditions of the biopharmacological tests and was found to be configurationally stable (^{1}H NMR).

Radioligand binding assays

The β-adrenergic affinity of the *Z*-MOIM derivatives (**3, 4**) and of the reference drugs dichloroisoproterenol and propranolol (Table 3) was checked by binding tests on rat brain and bovine lung membrane preparations for β_1- and β_2-adrenoceptors, respectively. 1-[[2-(3-carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]-phenoxy]-2-propanol ([^{3}H]-CGP 26505)[11] was used as a specific tritiated ligand for rat brain β_1-adrenoceptors. [^{3}H]-Dihydroalprenolol ([^{3}H]-DHA)[12] was utilized in the presence of 50 nM CGP 26505 to label bovine lung β_2-adrenoceptors. The reason for the presence of CGP 26505 in this latter type of test was that of displacing [^{3}H]-DHA binding from the bovine lung β_1-adrenoceptor subpopulation (17%).[13]

Rat brain β_1-adrenoceptors. The *Z*-MOIM derivatives **3** and **4** proved to possess a certain binding affinity

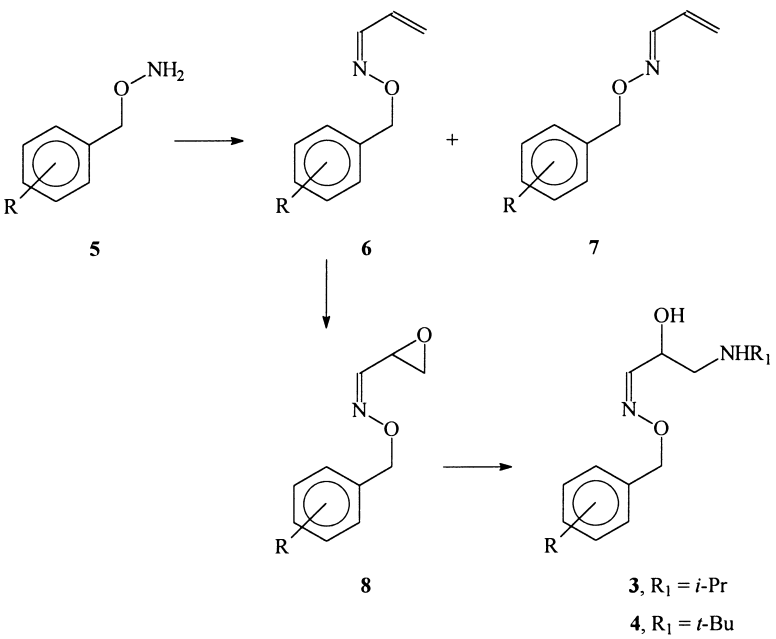
Table 1. Analytical and chemical data of (*Z*)-*N*-[3-(isopropylamino)-(**3a–g**) and (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene]-(arylmethyloxy)amines (**4a–g**) as oxalates



3, **4**

Compound	R	R_1	mp (°C)	Yield[a] (%)	Formula[b]
3a ½$H_2C_2O_4$	H	*i*-Pr	144–146	43	$C_{14}H_{21}N_2O_4$
4a ½$H_2C_2O_4$	H	*t*-Bu	168–170	48	$C_{15}H_{23}N_2O_4$
3b ½$H_2C_2O_4$	*o*-MeO	*i*-Pr	113–118	48	$C_{15}H_{23}N_2O_5$
4b ½$H_2C_2O_4$	*o*-MeO	*t*-Bu	162–164	46	$C_{16}H_{25}N_2O_5$
3c ½$H_2C_2O_4$	*m*-MeO	*i*-Pr	148–150	48	$C_{15}H_{23}N_2O_5$
4c ½$H_2C_2O_4$	*m*-MeO	*t*-Bu	156–158	46	$C_{16}H_{25}N_2O_5$
3d ½$H_2C_2O_4$	*p*-MeO	*i*-Pr	140–143	47	$C_{15}H_{23}N_2O_5$
4d ½$H_2C_2O_4$	*p*-MeO	*t*-Bu	170–172	36	$C_{16}H_{25}N_2O_5$
3e ½$H_2C_2O_4$	*o*-Cl	*i*-Pr	121–124	39	$C_{14}H_{20}N_2O_4Cl$
4e ½$H_2C_2O_4$	*o*-Cl	*t*-Bu	170–172	43	$C_{15}H_{22}N_2O_4Cl$
3f ½$H_2C_2O_4$	*m*-Cl	*i*-Pr	145–148	37	$C_{14}H_{20}N_2O_4Cl$
4f ½$H_2C_2O_4$	*m*-Cl	*t*-Bu	170–173	45	$C_{15}H_{22}N_2O_4Cl$
3g ½$H_2C_2O_4$	*p*-Cl	*i*-Pr	146–148	40	$C_{14}H_{20}N_2O_4Cl$
4g ½$H_2C_2O_4$	*p*-Cl	*t*-Bu	178–180	46	$C_{15}H_{22}N_2O_4Cl$

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



5 **6** **7**

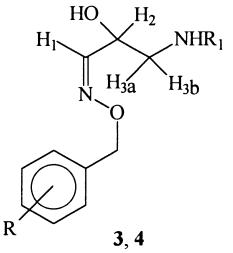
8

3, R_1 = *i*-Pr

4, R_1 = *t*-Bu

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 2. ^{1}H NMR data of (*Z*)-*N*-[3-(isopropylamino)-(**3a–g**) and (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](arylmethyloxy)amines (**4a–g**) as free bases



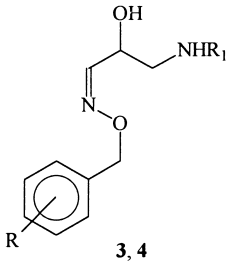
3, 4

Compd	R	R_1	H_1	H_2	H_{3a}	H_{3b}	CH_2O
3a	H	*i*-Pr	6.80 d (J=4.9)	4.72 ddd (J=8.0, 4.9, 3.9)	2.96 dd (J=12.2, 3.9)	2.63 dd (J=12.2, 8.0)	5.1 s
4a	H	*t*-Bu	6.81 d (J=4.9)	4.67 ddd (J=8.0, 4.9, 4.1)	2.93 dd (J=12.0, 4.1)	2.57 dd (J=12.0, 8.0)	5.09 s
3b	*o*-MeO	*i*-Pr	6.82 d (J=4.8)	4.71 ddd (J=8.1, 4.8, 4.1)	2.96 dd (J=12.2, 4.1)	2.65 dd (J=12.2, 8.1)	5.16 s
4b	*o*-MeO	*t*-Bu	6.82 d (J=4.7)	4.66 ddd (J=8.0, 4.7, 4.1)	2.93 dd (J=12.0, 4.1)	2.59 dd (J=12.0, 8.0)	5.15 s
3c	*m*-MeO	*i*-Pr	6.80 d (J=5.0)	4.73 ddd (J=8.1, 5.0, 4.1)	2.97 dd (J=12.2, 4.1)	2.63 dd (J=12.2, 8.1)	5.07 s
4c	*m*-MeO	*t*-Bu	6.81 d (J=5.1)	4.67 ddd (J=8.1, 5.1, 4.1)	2.94 dd (J=12.0, 4.1)	2.57 dd (J=12.0, 8.1)	5.07 s
3d	*p*-MeO	*i*-Pr	6.78 d (J=4.9)	4.69 ddd (J=8.2, 4.9, 4.2)	2.93 dd (J=12.2, 4.2)	2.61 dd (J=12.2, 8.2)	5.02 s
4d	*p*-MeO	*t*-Bu	6.79 d (J=5.0)	4.63 ddd (J=8.0, 5.0, 4.0)	2.90 dd (J=12.1, 4.0)	2.55 dd (J=12.1, 8.0)	5.02 s
3e	*o*-Cl	*i*-Pr	6.82 d (J=4.9)	4.75 ddd (J=8.1, 4.9, 3.9)	3.00 dd (J=12.2, 3.9)	2.65 dd (J=12.2, 8.1)	5.21 s
4e	*o*-Cl	*t*-Bu	6.83 d (J=4.9)	4.69 ddd (J=8.2, 4.9, 4.0)	2.98 dd (J=12.1, 4.0)	2.59 dd (J=12.1, 8.2)	5.21 s
3f	*m*-Cl	*i*-Pr	6.81 d (J=5.0)	4.73 ddd (J=8.2, 5.0, 3.9)	2.97 dd (J=12.2, 3.9)	2.63 dd (J=12.2, 8.2)	5.06 s
4f	*m*-Cl	*t*-Bu	6.81 d (J=4.9)	4.67 ddd (J=8.2, 4.9, 4.1)	2.94 dd (J=12.1, 4.1)	2.57 dd (J=12.1, 8.2)	5.06 s
3g	*p*-Cl	*i*-Pr	6.82 d (J=4.9)	4.74 ddd (J=8.1, 4.9, 3.9)	2.98 dd (J=12.2, 3.9)	2.64 dd (J=12.2, 8.1)	5.15 s
4g	*p*-Cl	*t*-Bu	6.80 d (J=5.1)	4.65 ddd (J=8.0, 5.1, 4.0)	2.92 dd (J=12.1, 4.0)	2.55 dd (J=12.1, 8.0)	5.05 s

towards β_1-adrenoceptors, with K_i values ranging from the 10 μM of **3b** to the 0.2 μM of **4c**. Among the *N*-isopropyl derivatives **3**, while the *m*-substituted compounds (**3c,f**) exhibited K_i values similar to that of the unsubstituted oxime ether **3a**, the *o*-(**3b**) and *p*-MeO-substituted (**3d**) compounds showed affinity indices substantially higher than that of **3a**; the K_i values of the *o*-(**3e**) and *p*-Cl-substituted (**3g**) were found to be a bit lower or higher, respectively, than the K_i value of **3a**. For all the *N*-isopropyl-substituted compounds, the ratio between the K_i values obtained with the compounds with the *Z* configuration and those obtained with the corresponding *E* configuration isomers[5] was between 2.0 (i.e. the value indicating half the affinity with respect to that of the *E* isomer) and 0.5 (i.e. the value indicating twice the affinity of the *E* isomer), with the only exception of **3g**, for which the *Z*/*E* ratio was slightly higher than **2**. As regards the N-*tert*-butyl derivatives **4**, the *o*-Cl-substituted compound (**4e**) showed a K_i value slightly higher than that of **4a**, while the *o*-MeO-compound (**4b**) and both the *p*-substituted ones (**4d,g**) were among the compounds with the highest affinity indices; the *m*-MeO-(**4c**) and the *m*-Cl-substituted (**4f**) compounds appeared to possess K_i values slightly lower than or analogous to that of **4a**, respectively. Among the *N*-*tert*-butyl-substituted compounds, while **4b,d,f** exhibited *Z*/*E* ratios between 2.0 and 0.5, **4c,g** showed values appreciably outside this range and **4a,e** values slightly below 0.5.

Bovine lung β_2-adrenoceptors. All the *Z*-MOIM compounds (**3,4**) showed a good degree of affinity towards this type of receptor, with K_i values which, with the only exceptions of **3b** and **3c**, were in the nanomolar range. Within the series of N-isopropyl-substituted compounds (**3a–g**), the *p*-Cl-derivative (**3g**) appeared to possess the lowest K_i value, while the *o*-(**3b**) and the *m*-MeO-(**3c**) compounds exhibited the highest affinity indices; the phenyl-unsubstituted derivative (**3a**) and its *p*-MeO-(**3d**) and *o*-(**3e**) and *m*-Cl (**3f**) analogs showed very similar K_i values. The *Z*-MOIM compounds **3a,b,d,g** showed K_i values similar to those obtained for the corresponding *E*-MOIM isomers,[5] with *Z*/*E* ratio values between 2.0 and 0.5, while **3c,e,f** exhibited *Z*/*E* ratio values slightly lower than 0.5 (**3e**) or slightly higher than 2.0 (**3c,f**). As for the N-*tert*-butyl-substituted compounds, (**4**) the one unsubstituted on the phenyl ring (**4a**) and the *p*-MeO-(**4d**) and *m*-Cl-substituted (**4f**) analogues showed practically identical affinity indices, while the *o*-Cl-(**4e**) and *p*-Cl-substituted (**4g**) compounds exhibited K_i values slightly lower or slightly higher, respectively, than those of **4a,d,f**; the *o*-(**4b**) and the *m*-MeO (**4c**) derivatives were

Table 3. Radioligand binding affinity of *Z*-MOIM derivatives **3** and **4**



3, 4

			β-Adrenergic binding affinity			
			Rat brain (β_1)		Bovine lung (β_2)	
Compd	R	R_1	K_i (nM)[a]	Z/E[b]	K_i (nM)[a]	Z/E[b]
3a ½$H_2C_2O_4$	H	*i*-Pr	3800 (3400–4200)	1.86	680 (610– 760)	0.83
3b ½$H_2C_2O_4$	*o*-MeO	*i*-Pr	11000 (9300–12000)	0.71	1700 (1500–1800)	0.70
3c ½$H_2C_2O_4$	*m*-MeO	*i*-Pr	3000 (2700–3300)	0.64	1500 (1300–1600)	3.42
3d ½$H_2C_2O_4$	*p*-MeO	*i*-Pr	9200 (8200–10300)	1.42	460 (400–530)	1.10
3e ½$H_2C_2O_4$	*o*-Cl	*i*-Pr	2100 (1800–2300)	0.84	480 (430–530)	0.42
3f ½$H_2C_2O_4$	*m*-Cl	*i*-Pr	3100 (2700–3500)	1.44	940 (820–1100)	2.21
3g ½$H_2C_2O_4$	*p*-Cl	*i*-Pr	4900 (4300–5600)	2.13	310 (270–350)	1.00
4a ½$H_2C_2O_4$	H	*t*-Bu	690 (630–760)	0.33	120 (100–130)	0.32
4b ½$H_2C_2O_4$	*o*-MeO	*t*-Bu	1900 (1700–2200)	1.91	490 (440–540)	0.70
4c ½$H_2C_2O_4$	*m*-MeO	*t*-Bu	220 (200–230)	0.11	490 (430–560)	1.32
4d ½$H_2C_2O_4$	*p*-MeO	*t*-Bu	5800 (5100–6400)	0.87	130 (120–140)	0.97
4e ½$H_2C_2O_4$	*o*-Cl	*t*-Bu	1200 (1100–1300)	0.36	77 (76–78)	0.31
4f ½$H_2C_2O_4$	*m*-Cl	*t*-Bu	760 (700–830)	0.54	140 (130–160)	0.67
4g ½$H_2C_2O_4$	*p*-Cl	*t*-Bu	2400 (2200–2600)	7.74	210 (190–230)	2.14
dichloroisoproterenol			58 (49–67)		150 (140–170)	
propranolol			5.1 (3.9–5.9)		2.0 (1.7–2.2)	

[a]Geometric means of five separate determinations with confidence limits in parentheses.
[b]Ratio between the K_i value shown by the *Z* (**3** and **4**) and by the corresponding *E* isomer (**1** and **2**) (see ref. 5).

found to possess the lowest affinity. Among the N-*tert*-butyl-substituted compounds, **4b,c,d,f** exhibited affinity indices similar to those of the corresponding *E*-MOIM isomers,[5] with *Z*/*E* ratio values between 2.0 and 0.5, while **4a,e,g** showed slightly different K_i values, with *Z*/*E* ratio values slightly lower (**4a,e**) or higher (**4g**) than 0.5 or 2.0, respectively.

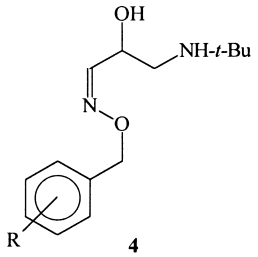
Functional tests

The β-adrenergic activity of the *Z*-MOIM derivatives that proved to possess the highest affinity ($K_i < 1500$ nM) for β_1-adrenoceptors was evaluated by functional tests on guinea-pig atria and guinea-pig tracheal strips for β_1- and β_2-adrenoceptors, respectively. Table 4 shows the pIC_{50} values obtained on these receptors for *Z*-MOIM compounds **4a,c,e,f**, for the *Z*-MOIM derivative **4g** which is the analogue of the *E*-MOIM compound possessing the best affinity on both β-adrenoceptors (**2g**),[5] and for dichloroisoproterenol and propranolol.

Guinea pig atria β_1-adrenoceptors. All the *Z*-MOIM compounds examined (**4a,c,e–g**) antagonized the stimulating effects of isoprenaline to a lower degree than the reference drugs. The highest pIC_{50} values were shown by the *m*-MeO-(**4c**) and *o*-Cl-(**4e**) derivatives, while the *m*-Cl-(**4f**) and the *p*-Cl-substituted (**4g**) compounds exhibited a slightly lower pIC_{50} value. For the *Z*-MOIM compounds for which also the corresponding *E* isomers were submitted to the same functional test (**4a,c,g**), the activity indices were somewhat lower (**4a,g**) or slightly higher (**4c**) than those of the corresponding analogs (**2a,c,g**).[5] None of the *Z*-MOIM compounds **4** shown in Table 4 exhibited any stimulating activity on this β-adrenoceptor.

Guinea pig tracheal strip β_2-adrenoceptors. The *Z*-MOIM compounds **4a,c,e–g** showed a good β_2-blocking activity, with similar pIC_{50} indices which in some cases (**4a,e,g**) were higher than that of dichloroisoproterenol. The highest activity index was shown by the *o*-Cl

Table 4. β-Adrenergic activity of selected *Z*-MOIM derivatives (**4**)



4

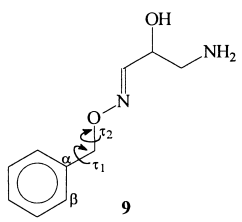
Compd	R	β-Adrenergic activity[a] pIC_{50}[b]	
		Isolated guinea pig atria (β_1)	Isolated guinea pig tracheal strips (β_2)
4a ½$H_2C_2O_4$	H	4.39 ± 0.16[c]	6.51 ± 0.02[d]
4c ½$H_2C_2O_4$	*m*-MeO	4.78 ± 0.11[e]	6.04 ± 0.18[f]
4e ½$H_2C_2O_4$	*o*-Cl	4.80 ± 0.02	6.72 ± 0.02
4f ½$H_2C_2O_4$	*m*-Cl	4.52 ± 0.07	6.03 ± 0.14
4g ½$H_2C_2O_4$	*p*-Cl	4.31 ± 0.14[g]	6.53 ± 0.24[h]
dichloroisoproterenol		6.82 ± 0.18	6.09 ± 0.28
propranolol		7.42 ± 0.15	7.60 ± 0.12

[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]For **2a** (ref. 5): pIC_{50} 5.10 ± 0.17.
[d]For **2a** (ref. 5): pIC_{50} 6.89 ± 0.10.
[e]For **2c** (ref. 5): pIC_{50} 4.60 ± 0.01.
[f]For **2c** (ref. 5): pIC_{50} 6.48 ± 0.26.
[g]For **2g** (ref. 5): pIC_{50} 5.13 ± 0.08.
[h]For **2g** (ref. 5): pIC_{50} 6.77 ± 0.31.

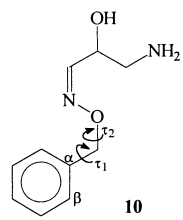
derivative (**4e**), while the analogue unsubstituted on the phenyl ring (**4a**) and the *p*-Cl-derivative (**4g**), and, in decreasing order, the *m*-substituted compounds (**4c,f**) appeared to be slightly less active. For *Z*-MOIM compounds **4a,c,g**, the activity indices were found to be slightly lower than those shown in the same type of test by the corresponding *E* analogs **2a,c,g**.[5] The *Z*-MOIM compounds of type **4** shown in Table 4 (**4a,c,e–g**) were devoid of any agonistic activity also on β_2-adrenoceptors.

Theoretical calculations

In order to gain a better understanding of the experimental results obtained for *Z*-MOIM derivatives (**D**) in comparison with those previously obtained for *E*-MOIM derivatives (**C**), the conformational and reactivity properties of model compound **10** were studied and compared with those of the previously investigated[5] model compound **9**. Model compounds **9** and **10** correspond to *E*-MOIM (**1a** and **2a**) and *Z*-MOIM (**3a** and **4a**) drugs respectively, the only difference being the lack of the N-substituent, in accordance with a simplification already tested and used by us in previous papers.[1a,2,3]



9



10

As regards model compound **9** (the one possessing the *E* configuration), a systematic search, performed by means of molecular mechanics calculations, showed[5] that in its preferred conformation, its C(α)-C-O-N=C moiety is planar, and the torsion angles τ_1 and τ_2 have values of 90° and 180°, respectively; however, it had also been found that the benzylic portion possesses a large conformational freedom.

An analogous conformational analysis was carried out on model compound **10** (the one possessing the *Z* configuration). Also for this compound in its preferred conformation, the torsion angles τ_1 and τ_2 have values of 90° and 180°, respectively, and the C(α)-C-O-N=C

moiety is planar; furthermore, **10** proves to have a large degree of freedom like **9**.

The superimposition of model compounds **9** and **10** in their preferred conformations (Fig. 1) shows that there is an optimal spatial correspondence of the N=CH-CHOH-CH_2NH_2 portions, and that the remaining benzyloxy moieties occupy very different spatial regions; however, the MOIMM of both molecules are almost coplanar. As regards the phenyl-substituted *Z*-MOIM derivatives (**3b–g, 4b–g**), no influence of the phenyl substituent on the conformation was observed; this is in agreement with previous findings[5] for the corresponding phenyl-substituted *E*-MOIM derivatives (**1b–g**, **2b–g**).

Figure 2 shows the molecular electrostatic potential (MEP) of model compounds **9** and **10** in their preferred conformations. A very similar MEP trend is found in the region of the MOIMM in spite of the different spatial arrangements of the moieties.

For both **9** and **10**, the MEP trend is characterized by three negative regions. One of them is isolated and generated by the common aminic nitrogen atom in both compounds; the other two regions are close to each other: the more extended one is generated in **9** by the alcoholic and the ethereal oxygens and in **10** by the alcoholic oxygen and the iminic nitrogen, whereas the less extended one is generated by the iminic nitrogen in **9** and by the ethereal oxygen in **10**.

It was also found that the MEP in the region of the CH_2-O-N=CH-CHOH-CH_2-NH_2 region of *Z*-MOIM derivatives **3b–g**, **4b–g** is not significantly influenced by the phenyl substituent, in agreement with previous findings for *E*-MOIM compounds **1b–g**, **2b–g**.[5]

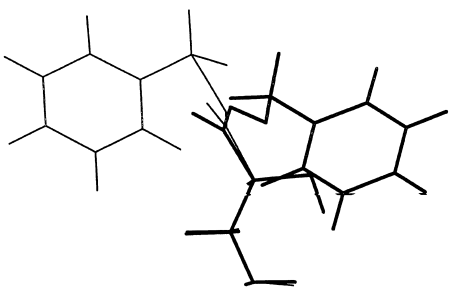


Figure 1. Compounds **9** (thinner line) and **10** (thicker line) in their preferred conformations.

Discussion and Conclusions

An examination of the data reported in Table 3 shows that the *Z*-MOIM compounds studied (**3, 4**) possess affinity indices towards β_1-adrenoceptors ranging from 0.2 to 11 μM. A very similar range of K_i values (0.3–14 μM) was found for the corresponding analogues with the *E* configuration previously studied (**1** and **2**). This indicates that, on the whole, the MOIM derivatives of types **1–4** possess an ability to interact with β_1-receptors which seems to be independent of the configuration around the double bond of the MOIM group. This result is confirmed by the values of the ratio (*Z*/*E*) between the K_i of the pairs of isomers with opposite configurations, which, in most cases, are between 2.0 and 0.5, i.e. within the range indicating an affinity of *Z* compounds for β_1-receptors equal to half or twice that of the corresponding *E* isomers, respectively.

As regards the *Z*-MOIM derivatives submitted to the functional tests on β_1-adrenoceptors (**4a, c, e–g**), the trend of their activity indices (pIC_{50}) appeared to be in line with that of their affinity indices (K_i), with the exception of the compound not substituted on the phenyl (**4a**), which was among the best in terms of affinity and among the worst in terms of activity. The activity indices for β_1-adrenoceptors obtained for the *Z*-MOIM compounds studied are within a range of values (4.31–4.80) which is not very different from the one (4.35–5.13) shown by the analogues with the *E* configuration previously submitted to the same type of test.

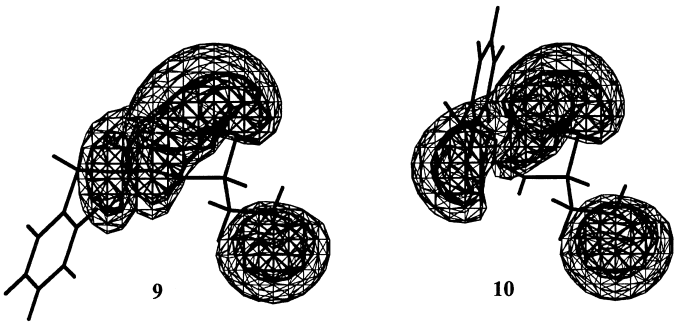


Figure 2. Compounds **9** and **10** in their preferred conformations. The MEP contours corresponding to values of −10 (thinner network) and −20 kcal/mol (thicker network) are shown.

As regards β_2-adrenoceptors, the results obtained in the binding tests indicate that the *Z*-MOIM compounds **3** and **4** possess affinity indices ranging from 0.08 to 1.7 μM, that is, a range of K_i values very similar to those shown by the corresponding isomers with the *E* configuration **1** and **2** (0.1–5.6 μM). This indicates that for these types of aminoalcohols (**1–4**), the configuration around the double bond of the MOIM does not substantially influence their ability to interact also with β_2-adrenoceptors. Confirmation of these findings is offered by an examination of the values of the ratio between the K_i of the couples of configurational isomers (*Z*/*E*), which are all between 2 and 0.5, also for this type of β-adrenoceptor.

The activity indices obtained for the *Z*-MOIM derivatives **4a, c, e–g** in the functional tests carried out on tracheal β_2-adrenoceptors appear to be in good agreement with the affinity indices obtained in the binding tests, apart from the *m*-chloro-substituted compound (**4f**) which exhibits the lowest pIC_{50} value, even if it possesses an affinity practically identical to that of **4a**, one of the more active *Z*-MOIM derivatives on this adrenoceptor. The pIC_{50} values of **4a, c, e–g** are between 6.03 and 6.72, a range of values that is very similar to those obtained for the analogues with the opposite configuration previously evaluated for β_2 antagonist activity in the same kind of test (6.01–6.89).

A comparison of the results obtained for the *Z*-MOIM derivatives **3** and **4** with those previously obtained for their corresponding isomers with the *E* configuration (**1** and **2**) demonstrates that for the MOIM-type aminoalcohols **1–4** studied, the β-adrenergic properties, as far as both the affinity and the activity are concerned, are substantially independent of the configuration around the MOIM double bond (*E* or *Z*).

A comparison of the conformational and electronic properties of the model compounds **9** and **10** of the *E*-MOIM (**1,2**) and *Z*-MOIM (**3,4**) analogues respectively, determined by means of molecular calculations, reveals that, in spite of the differences in the steric characteristics due to the *Z* or *E* configuration, at least at the level of the spatial position of the arylmethyloxy group ($ArCH_2O$), the two kinds of compounds present some important analogies in their molecular reactivity. In particular, the reactivity pattern of the two configurational isomers is very similar not only in the ethanolaminic portion, but also at the level of the O-N=C atomic sequence. This fact suggests that for the interaction of the MOIM-type compounds **1–4** with β-adrenoceptors, the fundamental role may be played by the atomic sequence $O\text{-}N{=}CH(OH)CH_2NH$. Moreover, the *E*- and *Z*-MOIM analogues possess analogous adrenergic properties, even if they present different steric characteristic linked to the different spatial arrangement of the benzylic moiety with respect to the remaining portion of the molecule. This fact could be explained either by excluding the possibility of a direct interaction of the aryl with the appropriate receptor sites, or by hypothesizing the presence of two different binding sites for the aryl groups of the two types (*Z* or *E*) of MOIM compounds.

Experimental

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. ^1H NMR spectra of all 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. Analytical TLCs were carried out on 0.25 mm layer silica gel plates containing a fluorescent indicator (Macherey–Nagel Alugram® SilG/UV254 Art. Nr. 81813); spots were detected under UV light (254 nm). Column chromatographies were performed using 230–400 mesh silica gel (Macherey–Nagel Silica Gel 60 Art. Nr. 81538). Glc analyses were performed on a Carlo Erba model 4200 apparatus with a flame ionization detector, using a 1.6 m×2.6 mm column packed with neopentylglycolsuccinate 10% on chromosorb W silanised 80/100 mesh. Evaporations were made 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%.

Synthesis of (*Z*)-*N*-Propenylidene(arylmethyloxy)amines (6a–g). A heterogeneous mixture of the appropriate *O*-(arylmethyl)hydroxylamine hydrochloride[9] **5a–f** (0.094 mol) and acrolein (6.3 mL, 0.094 mol) in a 1:1 $H_2O/CHCl_3$ mixture (600 mL), was treated as previously reported[5] to yield a crude residue consisting of the *Z* (**6a–f**) and *E* (**7a–f**) unsaturated oxime ethers in a ratio of about 3/7, which was chromatographed on silica gel, eluting with a 7/1/0.15 hexane/CH_2Cl_2/methyl ethyl ketone mixture and collecting 15 mL fractions. The first fractions afforded pure **7a–f**, whereas the subsequent ones yielded pure **6a–f** as oils, whose ^1H NMR spectral data were in agreement with those previously detected by us for the same compounds in the crude *E*/*Z* mixtures.[5] **6a** (3.8 g, 25%). Anal. $C_{10}H_{11}NO$ (C, H, N). **6b** (4.3 g, 24%). Anal. $C_{11}H_{13}NO_2$ (C, H, N). **6c** (4.1 g, 22%). Anal. $C_{11}H_{13}NO_2$ (C, H, N). **6d** (4.1 g, 22%).

Anal. $C_{11}H_{13}NO_2$ (C, H, N). **6e** (4.3 g, 23%). Anal. $C_{10}H_{10}NOCl$ (C, H, N). **6f** (3.1 g, 17%). Anal. $C_{10}H_{10}NOCl$ (C, H, N). Compound **6g** was synthesized and purified as previously reported.[5]

General procedure for the preparation of (*Z*)-*N*-(2,3-epoxypropylidene)(arylmethyloxy)amines (8a–g). A vigorously stirred heterogeneous mixture of the appropriate unsaturated oxime ether **6a–g** (0.015 mol), 18-crown-6 (0.680 mg, 0.0026 mol) and acetone (17 mL) in freshly distilled benzene (85 mL) and saturated phosphate aqueous pH 7.5 buffer solution (45 mL) containing an undissolved quantity of solid KH_2PO_4 (prepared by adding portionwise with stirring KH_2PO_4 to 100 mL of freshly distilled water until saturation and then treating with solid KOH until the pH was 7.5, being careful to maintain an undissolved quantity of solid KH_2PO_4), was treated dropwise with a freshly prepared solution of 0.4 M potassium peroxymonosulfate (oxone) (51 mL), being careful to keep the pH constant (7.5) by the addition of solid KOH. The mixture was left to react at rt under vigorous stirring, further adding 10 mL of 0.4 M potassium peroxymonosulfate (oxone) and 5 mL of acetone every 24 h (again keeping the pH at 7.5 with solid KOH) until the ratio of the amounts of unsaturated oxime ether and (*Z*)-epoxide was constant (glc) (from 4 to 9 days). The final percentage of epoxide in the reaction mixture varied from 50 to 82% (GLC). The two phases were then separated and the aqueous one was extracted with benzene (2×100 mL). The organic layers were collected, dried and evaporated to give a crude residue which after purification by column chromatography on silica gel, eluting with hexane/Et_2O mixture (40:1 for **8a**, **8e–g** and 40:3 for **8b–d**), yielded the pure (*Z*)-epoxides **8a–g** as oils, whose ^{1}H NMR spectral data were in agreement with those previously detected for the same compounds in the crude mixtures of **8a–g** together with their corresponding *E* isomers.[5] **8a** (0.89 g, 33%). Anal. $C_{10}H_{11}NO_2$ (C, H, N). **8b** (1.5 g, 48%). Anal. $C_{11}H_{13}NO_3$ (C, H, N). **8c** (1.3 g, 42%). Anal. $C_{11}H_{13}NO_3$ (C, H, N). **8d** (1.0 g, 32%). Anal. $C_{11}H_{13}NO_3$ (C, H, N). **8e** (1.3 g, 41%). Anal. $C_{10}H_{10}NO_2Cl$ (C, H, N). **8f** (1.3 g, 41%). Anal. $C_{10}H_{10}NO_2Cl$ (C, H, N). **8g** (1.6 g, 51%). Anal. $C_{10}H_{10}NO_2Cl$ (C, H, N).

General procedure for the preparation of (*Z*)-*N*-[3-(isopropylamino)-2-hydroxypropylidene](arylmethyloxy)-amine oxalates (3a-g½$H_2C_2O_4$). A stirred solution of the appropriate epoxide **8a–g** (0.0019 mol) in anhydrous benzene (7 mL) and *i*-$PrNH_2$ (0.97 mL, 0.0114 mol) was kept at 90 °C for 4 days. The resulting mixture was evaporated to give a residue, which, after purification by column chromatography eluting with a 60:5:4 hexane/AcOEt/*i*-$PrNH_2$ mixture, yielded pure **3a–g** as free bases. **3a** (0.25 g, 56%). **3b** (0.29 g, 57%). **3c** (0.30 g, 59%). **3d** (0.31 g, 61%). **3e** (0.31 g, 60%). **3f** (0.29 g, 56%). **3g** (0.30 g, 58%). For ^{1}H NMR spectral data, see Table 2.

The appropriate aminoalcohols **3a–g** (0.001 mol) were dissolved in anhydrous Et_2O (2 mL) and treated in portions at 0 °C, under stirring, with a solution of oxalic acid (0.072 g, 0.0008 mol) in anhydrous MeOH (1 mL). Addition of anhydrous Et_2O gave a solid precipitate consisting of pure **3a–g**½$H_2C_2O_4$. For analytical and chemical data, see Table 1. ^{1}H NMR data for N=CH proton (δ, Hz). **3a**½$H_2C_2O_4$: 6.90 (d, J=4.8); **3b**½$H_2C_2O_4$: 6.88 (d, J=4.8); **3c**½$H_2C_2O_4$: 6.92 (d, J=4.9); **3d**½$H_2C_2O_4$: 6.89 (d, J=4.7); **3e**½$H_2C_2O_4$: 6.90 (d, J=4.8); **3f**½$H_2C_2O_4$: 6.92 (d, J=4.8); **3g**½$H_2C_2O_4$: 6.92 (d, J=4.8).

General procedure for the preparation of (*Z*)-*N*-[3-(*tert*-butylamino)-2-hydroxypropylidene](arylmethyloxy)amine oxalates (4a–g½$H_2C_2O_4$). A stirred solution of the appropriate epoxide **8a–g** (0.0019 mol) in anhydrous benzene (7 mL) and *t*-$BuNH_2$ (1.2 mL, 0.0114 mol) was kept at 90 °C for 8 days and then treated, as described in the preparation of **3a–g**, to yield pure **4a–g** as free bases. **4a** (0.28 g, 59%). **4b** (0.30 g, 56%). **4c** (0.31 g, 58%). **4d** (0.33 g, 62%). **4e** (0.35 g, 65%). **4f** (0.32 g, 59%). **4g** (0.30 g, 56%). For ^{1}H NMR spectral data, see Table 2.

The appropriate aminoalcohols **4a–g** (0.001 mol) were dissolved in anhydrous Et_2O (2 mL) and treated with oxalic acid (0.072 g, 0.0008 mol) as described in the preparation of **3a–g**½$H_2C_2O_4$, to give a solid precipitate consisting of pure **4a–g**½$H_2C_2O_4$. For analytical and chemical data, see Table 1. ^{1}H NMR data for N=CH proton (δ, Hz). **4a**½$H_2C_2O_4$: 6.91 (d, J=4.7); **4b**½$H_2C_2O_4$: 6.92 (d, J=4.6); **4c**½$H_2C_2O_4$: 6.90 (d, J=4.8); **4d**½$H_2C_2O_4$: 6.91 (d, J=4.7); **4e**½$H_2C_2O_4$: 6.90 (d, J=4.7); **4f**½$H_2C_2O_4$: 6.92 (d, J=4.8); **4g**½$H_2C_2O_4$: 6.91 (d, J=4.8).

Stability test for 4g½$H_2C_2O_4$. The *Z* aminoalcohol **4g**½$H_2C_2O_4$ (50 mg) was incubated for 60 min with 25 mL of either 50 mM Tris-HCl buffer at pH 8 (used in the radioligand binding experiments) or Tyrode solution (used in the pharmacological tests), at the temperature used in the biopharmacological tests. After alkalinization with 5% aqueous K_2CO_3 solution, the usual work-up made it possible to recover the unaltered *Z*-MOIM oxime **4g** (^{1}H NMR).

Theoretical calculations: conformational analysis. All calculations were made by means of the molecular mechanics program Discover,[14] using the CVFF forcefield, and a dielectric constant equal to 4 and distance-dependent. The results were reported for molecules considered as free bases because no significant difference was

observed with respect to the *N*-protonated forms. The starting conformation of **10** was built from the preferred one of **9**;[5] the rotational freedom of its hydroxyl group was limited in order to prevent the formation of an intramolecular H bond between this group and the ethereal oxygen, which should be unfavoured in the biological environment.[15]

A full geometry optimization was performed on the compounds **3a–g** and **4a–g**, starting from the preferred geometry of **10**.

Molecular electrostatic potential. The MEP was calculated by using the STO3G wave functions of **9** and **10** considered in the conformations of Figure 2 in a three-dimensional grid with a step of 0.5 Å. The grid was then contoured at levels of −10 and −20 kcal/mol using the molecular modelling program InsightII.[14]

An analogous MEP calculation was performed on compounds **3b–g** and **4b–g** considered in their preferred conformations.

Radioligand binding methods: rat brain β_1-receptors. β_1-Receptors were assayed in rat cortical membranes, as previously described,[3] using [^{3}H]CGP 26505[11] (1-[[2-(3-carbamoyl-4-hydroxyphenoxy)ethyl]amino]-3-[4-[1-methyl-4-(trifluoromethyl)-2-imidazolyl]phenoxy]-2-propanol) as the specific ligand (DuPont de Nemours, New England Nuclear Division; specific activity 2.5 Ci/mmol).

Bovine lung β_2-receptors. β_2-Receptor binding was studied in bovine lung, as previously described,[3] using [^{3}H]dihydroalprenolol (DHA)[12] as the ligand (DuPont de Nemours, New England Nuclear Division; specific activity 48.1 Ci/mmol), in the presence of CGP 26505.

Pharmacological methods: guinea pig atria and guinea pig tracheal strips. The activity of compounds **4a, c, e–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 compounds tested on β_1- and β_2-adrenoceptors was experimented on preparations of isolated guinea-pig atria and of tracheal smooth musculature respectively, following the methods previously described.[3] For both β_1 and β_2 preparations, the antagonistic activity of the compounds towards β_1- and β_2-adrenoceptors was expressed as pIC_{50}, (i.e. the negative log of the molar concentration that reduced the response to isoprenaline by 50%).[16] All compounds were tested at a concentration ranging from 10^{-9} M to 10^{-3} M. Each antagonistic activity index was obtained from at least five active concentrations.

Acknowledgements

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