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Synthesis and α-Adrenoreceptor Blocking Properties of Phenoxybenzamine-Related (2-Chloroethyl)-(2,3-dihydrobenzo-[1,4]dioxin-2-ylmethyl)-(2-phenoxyethyl) Amines

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Abstract—A series of β-chloroethylamines, structural hybrids of WB 4101, a competitive α_1-adrenoreceptor antagonist, and phenoxybenzamine, an irreversible α-adrenoreceptor antagonist, has been synthesized and tested in isolated rat vas deferens α-adrenoreceptors. Although, for all compounds, apparent blocking potency and α_1-selectivity are quite similar to those of phenoxybenzamine, affinity values calculated by taking into account the actual concentration of aziridinium ion in solution, reveal that compounds bearing a 1,4-benzodioxan-2-ylmethyl moiety, display a significantly higher potency for both α_1- and α_2-adrenoreceptors than compounds having a benzyl group. In addition, two of the compounds, having both methyl and methoxy groups in their structure, show a marked discontinuity in the α_1-adrenoreceptor concentration–inhibition curve, with a plateau in the range 30–100 nM. Stereochemical aspects are also shown to play an important role in the binding. The biological results suggest that the two irreversible antagonists may be able to discriminate between two α_1-adrenoreceptor subtypes, which are both involved in the noradrenaline-induced contraction of the epididymal portion of rat vas deferens.

Introduction

α-Adrenoreceptors have been classified into two main classes designated as α_1- and α_2.[1] Furthermore, a large body of pharmacological evidence has shown that both classes are composed of subtypes. At least two pharmacologically distinct α_1-adrenoreceptor subtypes, α_{1A} and α_{1B}, have been characterized on the basis of their affinity for the competitive antagonist WB 4101 and sensitivity to the alkylating agent CEC (chloroethylclonidine).[2] The α_{1A}-type is more sensitive, in a variety of tissues, to blockade by WB 4101 and resistant to inhibition by CEC, whereas the reverse applies to the α_{1B}-subtype.[2–4] In this regard, prazosin, another α_1-adrenoreceptor antagonist, does not discriminate between these two major subtypes.[5] In blood vessels, the situation appears more complex since α_1-adrenoreceptors have been classified into three subtypes designated as α_{1H}, α_{1L} and α_{1N} according to their antagonist affinity.[6] The α_{1H}-subtype has high affinity for prazosin, while both α_{1L} and α_{1N} types display lower affinity for prazosin.

To reconcile the two α_1-adrenoreceptor classifications (α_{1A} and α_{1B} vs α_{1H}, α_{1L} and α_{1N}), it was argued that α_{1A}- and α_{1B}-subtypes are a single site with high affinity for prazosin and, as a consequence, can be identified as the α_{1H}-subtype.[7,8] However, a general consensous on α_1-adrenoreceptor classification has not been reached yet.

On the other hand, the heterogeneity of α_1-adrenergic receptors has been clearly demonstrated since three distinct α_1-adrenoreceptor cDNAs have been cloned by molecular biology techniques, α_{1A} from rat cerebral cortex,[9] α_{1B} from hamster cells[10] and α_{1C} from bovine brain,[11] although controversy exists as to whether the clone termed α_{1A} is indeed the pharmacologically characterized α_{1A} or a novel α_{1D} subtype.[12]

It is evident that new selective and irreversible ligands for α_1-adrenoreceptor subtypes are needed for receptor characterization and classification. In this regard CEC proved to be a useful pharmacological tool that owes the irreversible α_{1B}-antagonistic property to the presence in its structure of a β-chloroethylamine moiety able to generate a reactive aziridinium ion, which is responsible for receptor inactivation by way of a covalent bond formation. Furthermore, phenoxybenzamine (**1**), another classical and irreversible α_1-adrenoreceptor antagonist, appears to block selectively α_{1B}-adrenoreceptors in both functional and binding experiments,[13–16] although a contradictory behaviour towards α_{1A}- and α_{1B}-subtypes has been also observed.[2]

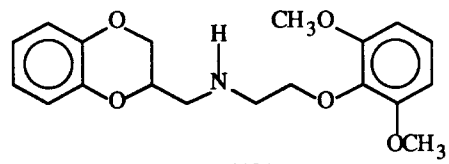
Thus, we thought that the synthesis of hybrid β-haloethylamines structurally related to **1** and WB 4101, prototypes of two classes of irreversible or competitive α_1-adrenoreceptor antagonists, respectively, could disclose irreversible blocking agents which would hopefully discriminate among α_1-adrenoreceptor subtypes. We report here the synthesis and α-adrenoreceptor blocking properties of compounds **2–8**. These compounds were designed to verify the effect on both affinity and selectivity of (a) replacing the benzyl group of **1** with a 1,4-benzodioxan-2-ylmethyl moiety, the main structural

peculiarity of WB 4101, (b) insertion of a 2-methoxy function, a group that contributes significantly to the affinity of WB 4101 for α_1-adrenoreceptors, on the phenoxy moiety of **1**, and (c) removal of the methyl group in the ethylenoxy chain of **1**.

Chemistry

The structures of the newly synthesized compounds (**4–8**) as well as those of the already reported **2**,[17] **3**[18] and **1** also used in the present study are given in Figure 1. These were synthesized by standard methods (Scheme 1) and characterized by ^{1}H NMR and elemental analysis.

The key intermediates were the secondary amines **9–13**. Compounds **11–13** were synthesized as previously described,[19,20] whereas **9** and **10** were obtained through a reductive amination in the presence of sodium cyanoborohydride of 1-(2-methoxyphenoxy)propan-2-one or 1-phenoxypropan-2-one with benzylamine or (2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)amine, respectively.



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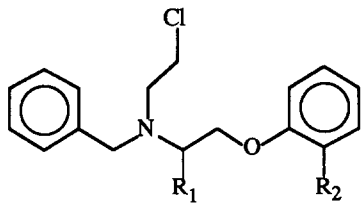
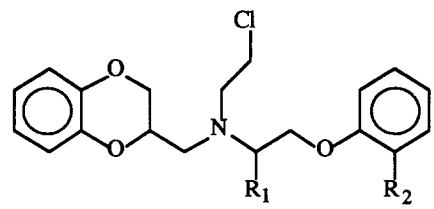




1: $R_1 = CH_3$, $R_2 = H$

2: $R_1 = H$, $R_2 = OCH_3$

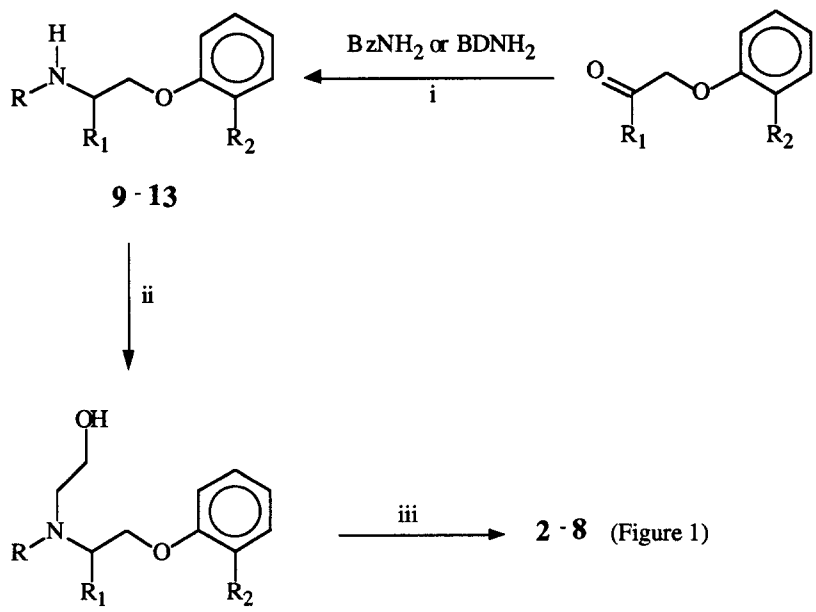
3: $R_1 = CH_3$, $R_2 = OCH_3$

4,5: $R_1 = CH_3$, $R_2 = H$

6: $R_1 = H$, $R_2 = OCH_3$

7,8: $R_1 = CH_3$, $R_2 = OCH_3$

Figure 1.



[a] Bz = benzyl; BD = 2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl; **9**, **14**: R = Bz, $R_1 = CH_3$, $R_2 = OCH_3$; **10**, **15**, **16**: R = BD, $R_1 = CH_3$, $R_2 = H$; **11**, **17**: R = BD, $R_1 = H$, $R_2 = OCH_3$; **12**, **18**, **19**: R = BD, $R_1 = CH_3$, $R_2 = OCH_3$; **13**, **20**: R = Bz, $R_1 = H$, $R_2 = OCH_3$. Reaction conditions; (i): HCl/EtOH, molecular sieves 4A, $NaBH_3CN$; (ii): $Br(CH_2)_2OH$, K_2CO_3, EtOH; (iii): $SOCl_2$, HCl (g), Benzene.

Scheme 1[a].

Alkylation of **9–13** with 2-bromoethanol afforded the corresponding *N,N*-disubstituted 2-aminoethanols **14**,[21] **17**, **20**[17] and the two diastereomeric pairs **15–16** and **18–19**. These were converted into the corresponding β-chloroethylamines **2–8** through a reaction with thionyl chloride in chloroform saturated with HCl gas following a reported procedure.[22]

Although a ^{1}H NMR spectral feature distinguished the two diastereomers in the couples **4/5** and **7/8**, their configuration remains to be assigned. In both cases, the signal at 4.73–4.82 or 4.70 ppm of one hydrogen of the methylene vicinal to the chiral center (CH_3CHCH_2) of diastereomers **4** and **7**, respectively, was isolated downfield from the multiplet including the other hydrogen of the methylene (4.24–4.37 and 3.10–4.48 ppm, respectively), whereas for **5** and **8** the signal of both hydrogens of the same methylene were included in a single multiplet at 3.00–4.80 and 4.00–4.45 ppm, respectively.

Kinetic Studies

Studies with phenoxybenzamine (**1**) and dibenamine gave evidence that their non-equilibrium blockade of α-adrenoreceptor is mediated by their corresponding aziridinium ions.[23] Furthermore, it was shown that differences in potency among β-haloalkylamines is primarily a result of transport and receptor affinity differences rather than differences in alkylating ability. Since the potency of these antagonists is strictly dependent on their receptor affinity, as well as the concentration of aziridinium ion, we determined, at pH 7.4 to approach physiological conditions, the rate of cyclization of **2–8** as well as the hydrolysis rate of the formed aziridinium ion in comparison to the kinetic profile of **1**. Aziridinium ion concentration was evaluated applying Gill and Rang's method.[24]

Rate constants for cyclization of **1–8** (k_1) and decay of the corresponding aziridinium ions (k_2) were estimated by fitting a kinetic model, based on the consecutive first-order reaction equation (1), $Q = [Q_o k_1/(k_2-k_1)](e^{-k1t}-e^{-k2t})$ (where Q is the concentration of aziridinium ion as a function of time t and Q_o is the initial concentration of β-chloroethylamine), to the experimental data by an unweighted Gauss–Newton non-linear regression routine[25] (Table 1). The new rate constants were used to calculate correct aziridinium ion concentrations as a time function (Fig. 2).

Results and Discussion

The biological profile of β-chloroethylamines **2–8** at α_1- and α_2-adrenoreceptors was assessed on isolated rat vas deferens[26, 27] and results are reported in Table 2 and Figures 3 and 4. In order to allow comparison of the results, phenoxybenzamine (**1**) was used as the standard compound.

Table 1. Rates of cyclization of β-chloroethylamines **1–8** and decay of relative aziridinium ions at 37 °C and pH = 7.4[a]

Compd.	k_1 (sec^{-1}) x 10^2	k_2 (sec^{-1}) x 10^2
1	11.12	1.00
2	27.30	2.82
3	35.71	3.14
4	2.33	26.80
5	9.50	124.52
6	3.22	8.72
7	2.19	13.10
8	2.23	22.63

[a]Experiments were performed at 0.4 mM concentration in MeOH:50 mM KH_2PO_4-Na_2HPO_4 buffer (pH 7.4), 8 : 2 (v/v).

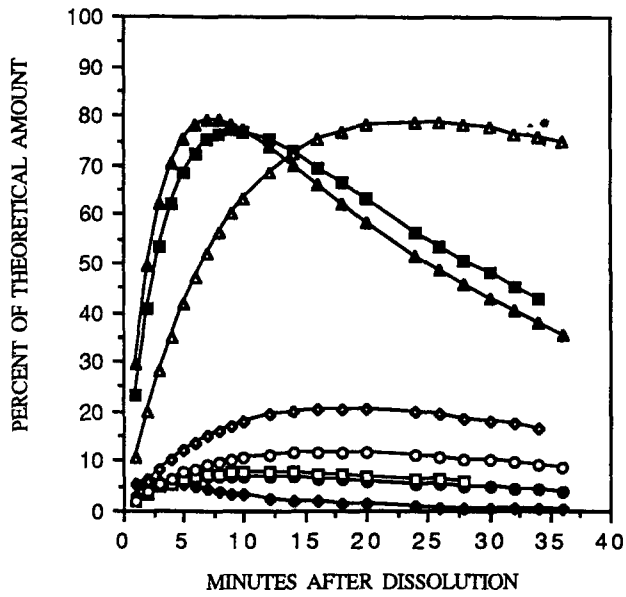
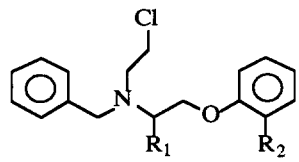


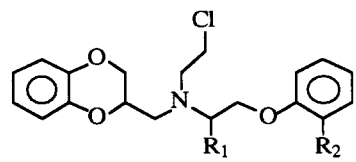
Figure 2. Aziridinium ion formation and decay at pH 7.4 and 37 °C for **1** (Δ), **2** (■), **3** (▲), **4** (●), **5** (◆), **6** (◊), **7** (○), **8** (□).

α_1-Adrenoreceptor blocking activity was assessed by antagonism of (–)-noradrenaline-induced contractions of the epididymal portion of the vas deferens. α_2-Adrenoreceptor blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of the vas deferens. The noncompetitive (irreversible) α_1- or α_2-antagonism was determined after a 30-min incubation followed by 30 min of washings. The decrease in maximum response was expressed as a percentage of the control value. Complete concentration–inhibition curves for α_1-adrenoreceptors were obtained for all compounds and are shown in Figures 3 and 4. Furthermore, the potency of each compound was expressed as IC_{50} values, the concentrations that produce 50% inhibition of the agonist maximal response (Table 2). In order to investigate a possible influence of the hydrolysis products of aziridinium ions on α-adrenoreceptor blocking properties of β-chloroethylamines **2–8**, aminoalcohols **14** and **19** were tested under the same conditions as parent compounds **3** and **8**.

Table 2 . α-Adrenoreceptor blocking activity of **1– 8** in the isolated rat vas deferens



1 - 3



4 - 8

No	R_1	R_2	α_1 pIC_{50}[a] vs noradrenaline		α_2 pIC_{50}[a] vs clonidine		Selectivity ratio[b] α_1/α_2
			Experimental[c]	Calculated[d]	Experimental[c]	Calculated[d]	
1	CH_3	H	7.27 ± 0.01	7.38 ± 0.01	6.28 ± 0.01	6.39 ± 0.01	10
2	H	OCH_3	7.13 ± 0.06	7.24 ± 0.06	6.15 ± 0.01	6.26 ± 0.01	10
3	CH_3	OCH_3	6.85 ± 0.05[e]	7.05 ± 0.05	6.77 ± 0.02	6.87 ± 0.02	1
4[f]	CH_3	H	6.71 ± 0.03	7.87 ± 0.03	5.36 ± 0.04	6.52 ± 0.04	22
5[f]	CH_3	H	6.44 ± 0.04	7.66 ± 0.04	4.91 ± 0.01	6.12 ± 0.01	34
6	H	OCH_3	6.86 ± 0.02	7.55 ± 0.02	5.64 ± 0.01	6.32 ± 0.01	17
7[f]	CH_3	OCH_3	6.77 ± 0.04	7.70 ± 0.04	6.19 ± 0.04	7.12 ± 0.04	4
8[f]	CH_3	OCH_3	6.82 ± 0.04[e]	7.93 ± 0.04	6.21 ± 0.03	7.33 ± 0.03	4

[a] pIC_{50} values represent the negative logarithm of the concentration that produces 50% inhibition of the agonist maximal response and are expressed as the mean value plus or minus standard error.
[b] The α_1/α_2 selectivity ratio is the antilog of the difference between the pIC_{50} or pA_2 values at α_1- and α_2-adrenoreceptors.
[c] pIC_{50} values were calculated assuming that β-chloroethylamines added to the bath are quantitatively transformed into the corresponding aziridinium ions.
[d] pIC_{50} values were calculated from the concentration of aziridinium ion which derives from β-chloroethylamines.
[e] These values represent an approximate affinity to block 50% of the total number of receptors. Assuming that the plateaus of curves shown in Figure 3 identify the line of separation in the blockade of two α_1-adrenoreceptor subtypes or subsites, it is possible to calculate the pIC_{50} value corresponding to 50% inhibition of both these sites: **3**, $pIC_{50(high)}$ 7.88 ± 0.03, $pIC_{50(low)}$ 6.85 ± 0.04; **8**, $pIC_{50(high)}$ 7.62 ± 0.01, $pIC_{50(low)}$ 6.83 ± 0.05.
[f] Compounds **4** and **5**, and **7** and **8** are diastereomers.

All compounds showed an irreversible blocking activity at both α_1- and α_2-adrenoreceptors since the response was not recovered after extensive washing, following 30 min of incubation. On the contrary, aminoalcohols **14** and **19** were inactive at α_1- and α_2-adrenoreceptors, both at a concentration (0.15 μM) near to IC_{50} values of corresponding chloro derivatives and to that (3 μM) causing a maximal inhibition of agonist-induced responses. No decrease of maximal response or shift to the right of the dose–response curve to noradrenaline or clonidine was observed for either compounds (results not shown).

An analysis of the results clearly shows that all compounds at α_1-adrenoreceptors were almost equiactive to each other while being slightly less potent than the standard **1**. A similar trend was observed for α_2-adrenoreceptors. However, compounds **4–6** were significantly less potent at α_2-adrenoreceptors than at α_1-adrenoreceptors and, as a consequence, they were able to discriminate between α_1- and α_2-adrenoreceptors as revealed by their selectivity ratios (Table 2). The most interesting aspect of the pharmacological profile of compounds **2–8** emerges from an analysis of their concentration–inhibition curves which reveals that **3** and **8**, as opposed to **2**, **4–7** and the standard **1** as well, did not inhibit α_1-adrenoreceptors of the epididymal portion of isolated rat vas deferens in a concentration-dependent fashion (Figs 3 and 4). Clearly, the concentration–inhibition plots of **3** and **8** have a marked discontinuity with a plateau in the range 30–100 nM. This finding suggests that a biphasic inhibition of α_1-adrenoreceptors is observed when both methyl and methoxy groups are present in the same structure. Furthermore, it appears that a 2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl moiety or a benzyl group have a similar influence not only on the affinity but also on the shape of the concentration–inhibition curves. Interestingly, compounds **3**, **7** and **8** which bear both methyl and methoxy functions in their structures are almost devoid of selectivity owing to an increase in affinity for α_2-adrenoreceptors. Furthermore, it appears that not only methyl and methoxy functions but also stereochemistry may play a role in receptor binding since of the two diastereomers **7** and **8** only the latter showed a biphasic saturation curve of α_1-adrenoreceptors. In this regard the enantiomers of **8** when available should give useful information.

The possible origin of the interesting marked discontinuity in the concentration–inhibition curves relative to functional assays of irreversible α_1-adrenoreceptor antagonists **3** and **8** deserves comment. Despite a large amount of pharmacological work, uncertainty still remains on the functional role of α_1-adrenoreceptor subtypes of rat vas deferens. Although binding studies and mRNA detection by Northern blot analysis evidenced in the whole rat vas deferens the presence of both α_{1A}- and α_{1B}-adrenoreceptors,[9,28] functional studies did not give definite results on the α_1-adrenoreceptor subtypes responsible for the contractile response since only α_{1A}-adrenoreceptors,[28,29] α_{1L},[30] or α_{1A} and α_{1B} subtypes[5,31] were found to be involved in noradrenaline-induced contractions. Similarly, studies performed in epididymal and prostatic

halves of isolated rat vas deferens, although affording useful information, did not clarify the number of α_1-adrenoreceptor subtypes involved in the contractile process.[30, 32]

Thus the biphasic nature of concentration–inhibition curves observed for **3** and **8** may well be related to the presence of two components or mechanisms in the functional contractile response evoked by noradrenaline-stimulated α_1-adrenoreceptors of the epididymal portion of rat vas deferens. These two components which are irreversibly and selectively inhibited by different concentration of **3** and **8** may reflect the selective inactivation of two α_1-adrenoreceptor subtypes. The proportions of the two sites which are selectively blocked by **3** and **8** can be obtained graphically from Figure 3. Thus, the high affinity site represents approximately 30%, whereas the low affinity site is about 70% of the total. Furthermore, an apparent affinity for the two sites could be calculated by deriving the concentration of β-chloroethyamine that inhibits 50% of each subtype. Thus compound **3** inhibited high and low affinity sites with pIC_{50} values of 7.88 ± 0.03 and 6.85 ± 0.04, respectively, whereas for **8** pIC_{50} values of 7.62 ± 0.01 and 6.83 ± 0.05, respectively, were obtained. Therefore, **3** and **8** are both capable of discriminating significantly between the two sites, as is revealed by their $IC_{50(high)}/IC_{50(low)}$ selectivity ratio of about 10.

An analysis of the results shown in Figure 2 reveals that the maximal aziridinium ion concentration is always lower than that of the relative β-chloroethylamine and a marked

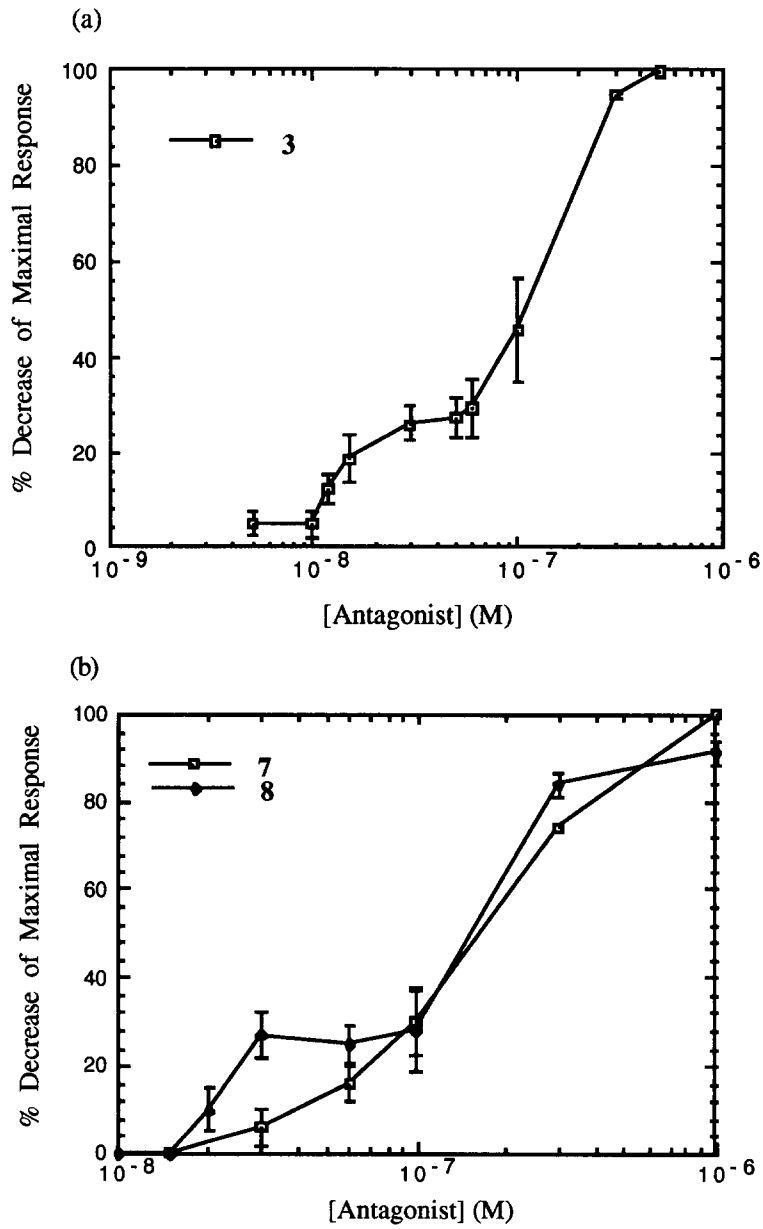


Figure 3. Epidydimal portion of rat vas deferens; course of α_1-adrenoreceptors covalent occupancy by **3** (a), and **7** and **8** (b). The per cent decrease of maximal response to noradrenaline was measured after a 30-min incubation for each concentration of antagonist followed by washing with the bath solution for 30 min. The results are expressed as the mean ± SEM of four to 10 indipendent observations.

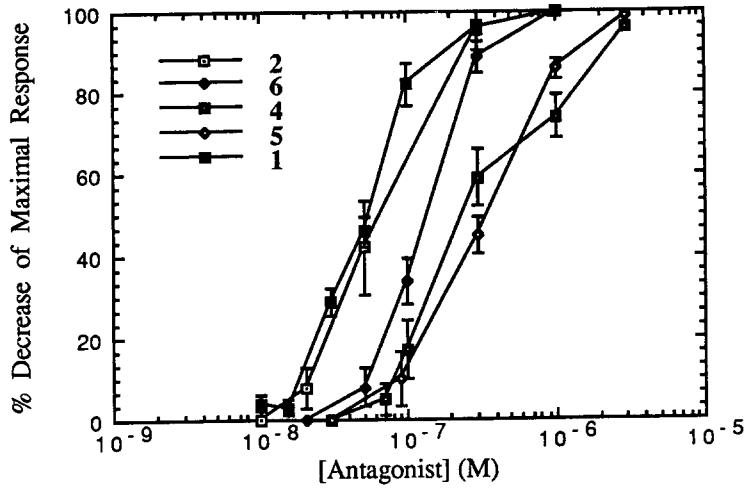


Figure 4. Epidydimal portion of rat vas deferens; course of α_1-adrenoreceptors covalent occupancy by **1**, **2** and **4–6**. The per cent decrease of maximal response to noradrenaline was measured after a 30-min incubation for each concentration of antagonist followed by washing with the bath solution for 30 min. The results are expressed as the mean ± SEM of four to 10 independent observations.

lower percentage is achieved with compounds **4–8**, which bear a 2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl moiety, in comparison to **1–3** having a benzyl group. Thus, in order to evaluate, for compounds **1–8**, the blocking potency related to the aziridinium ion species, we recalculated the pIC_{50} values from aziridinium ion concentration derived, by equation (1), from the β-chloethylamine concentration. It can be seen (Table 2) that, although selectivity ratios are not affected, calculated pIC_{50} values are significantly higher than apparent (experimental) ones for compounds **4–8**, whereas for compounds **1–3** there is only a slight difference between experimental and calculated values.

Compound **8** proved the most potent α_1-adrenoreceptor antagonist (pIC_{50} = 7.93 vs 7.38 for **1**) as well as the most potent α_2-adrenoreceptor antagonist of the series (pIC_{50} = 7.33 vs 6.39 for **1**).

These results suggest that the 2,3-dihydrobenzo-[1,4]dioxin-2-ylmethyl moiety meets the structural requisites of α-adrenoreceptor better than the benzyl group for interaction with the studied β-chloroethylamines.

Our future work with these compounds will include studies directed at gaining a better understanding of the intriguing trends noted above.

Experimental

Chemistry

Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian VXR 300 instruments, respectively. Although the IR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The elemental compositions of the compounds agreed to within ± 0.4% of the calculated value. Mass spectra were performed with a Hewlett Packard instrument consisting of mod. 5890 A for the separation section and mod. 5971 A for the mass section. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. R_f values were determined with silica gel TLC plates (Kieselgel 60 F_{254}, layer thickness 0.25 mm, Merck). The composition and volumetric ratio of eluting mixtures were: (A) cyclohexane:EtOAc (8:2); (B) EtOAc:cyclohexane:MeOH (2:5:0.1); (C) $CHCl_3$:EtOAc (8:2); (D) EtOAc:*n*-hexane:MeOH (5:2:0.1); (E) EtOAc:cyclohexane:MeOH (5:2:0.1); (F) EtOAc:*n*-hexane (5:2); (G) petroleum ether:EtOAc:MeOH:28% NH_3 in water (10:4:0.5:0.05); (H) petroleum ether:CH_2Cl_2:MeOH (8:5:1); (I) petroleum ether:EtOAc:MeOH (10:4:1); (J) petroleum ether:EtOAc-*i*-propanol:28% NH_3 in water (7:6:1.5:0.1); (K) *n*-hexane:EtOAc (6:4); (L) *n*-hexane:EtOAc (9.5:0.5). Petroleum ether refers to the fraction with a boiling point of 40–60 °C. The term 'dried' refers to the use of anhydrous sodium sulphate. Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic names in organic chemistry, Beilstein-Institut and Springer. Analytical HPLC separations were performed either on a Waters Associates liquid chromatograph, mod. 440, absorbance detector, equipped with a Beckman column, Ultrasphere ODS, 5μ, 4.6 × 250 mm or on a Hewlett Packard liquid chromatograph, 1090, serie II, DAD detector, provided with an NP-Hypersil column, 5μ, 2.1 × 100 mm.

*Benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (**9**).* A 2.53 M solution of HCl gas in EtOH (34.4 mL, 87 mmol) was added to a solution of benzylamine (27.97 g, 261.01 mmol) and 1-(2-methoxyphenoxy)propan-2-one (7.84 g, 43.5 mmol) in 300 mL EtOH, followed by the addition of $NaBH_3CN$ (2.19 g, 34.8 mmol) and molecular sieve 4Å. The mixture was stirred at room temperature for 72 h then acidified at pH 1 with 2 N HCl, filtered and evaporated. The residue was added with H_2O, basified with 6 N KOH and the mixture extracted with Et_2O. After drying, the solvent was

evaporated and the residue purified by chromatography. Eluting with mixture H yielded 7.65 g (65% yield) of **9** as the free base: oil; R_f 0.33; ^{1}H NMR ($CDCl_3$): δ 1.20 (*d*, *J* = 7.32 Hz, 3 H, CH_3), 2.13 (*br s*, 1H, NH), 3.18–3.28 (*m*, 1H, 1 ArCH_2), 3.80–4.03 (*m*, 7H, OCH_3, 1 ArCH_2, CH_3CH and CH_2O), 6.85–7.00 (*m*, 4H, OAr), 7,25–7.40 (m, 5H, Ar). The free base was transformed into the hydrochloride salt: mp 133–135 °C (from EtOAc). Anal. ($C_{17}H_{22}ClNO_2$) C, H, N.

(2,3-Dihydrobenzo[1,4]dioxin-2-ylmethyl)-(1-methyl-2-phenoxyethyl)amine hydrochloride (10). Prepared following the procedure described for **9** starting from 1-phenoxypropan-2-one and (2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)amine and purified by chromatography. Eluting with mixture I gave **10** as the free base: yield 66%; R_f 0.43; ^{1}H NMR ($CDCl_3$): δ 1.19 (*m*, 3H, CH_3), 1.74–1.83 (*br s*, 1H, NH), 2.92–3.07 (*m*, 2H, $NHCH_2$), 3.10–3.21 (*m*, 1H, CH_3CH), 3.81–3.95 (*m*, 2H, 1 H-3 of benzodioxan and 1 CH_2OAr), 4.01–4.11 (*m*, 1H, H-2 of benzodioxan), 4.25–4.35 (*m*, 2H, 1 H-3 of benzodioxan and 1 CH_2OAr), 6.80–6.99 (*m*, 7H, 4 aromatics of benzodioxan and 3 OAr), 7.26–7.32 (*m*, 2H, OAr). The free base was transformed into the hydrochloride salt; mp 157–160 °C (from EtOAc). Anal. ($C_{18}H_{22}ClNO_3 \cdot 0.5H_2O$) C, H, N.

General procedure for the synthesis of aminoalcohols 14–20. A mixture of appropriate amine (1 mmol), bromoethanol (1.1 mmol), and dry K_2CO_3 (2 mmol) in 10 mL EtOH was heated in a sealed glass tube at 110 °C for 72 h, then filtered and evaporated. The residue was dissolved in Et_2O and extracted with 2 N HCl. The acidic solution was basified with 2N NaOH and extracted with Et_2O. Removal of dried solvents gave a residue that was purified by chromatography. Eluting with the appropriate mixture gave **14–20**.

2-[Benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]amino]-ethanol (14). Prepared from **9**; eluting mixture, C; viscous oil; 52 % yield; R_f 0.43; MS *m/z* 315 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.13 (*d*, *J* = 6.67 Hz, 3H, CH_3), 2.63–2.70 (*m*, 1H, 1 CH_2CH_2OH), 2.80–2.89 (*m*, 1H, 1 NCH_2CH_2OH), 3.44–3.68 (*m*, 4, CH_2OH, 1 CH_2Ar and 1 CH_2OAr), 3.85–4.02 (*m*, 6H, OCH_3, 1 CH_2Ar, 1 CH_2OAr and CH_3CH), 6.80–6.93 (*m*, 4H, OAr), 7.20–7.39 (*m*, 5H, Ar).

2-[(2,3-Dihydrobenzo[1,4]dioxin-2-ylmethyl)-(1-methyl-2-phenoxyethyl)amino]ethanol (diastereomers 15 and 16). Prepared from **10** and purified by gravity column chromatography. Eluting with $CHCl_3$ gave the two diastereomeric aminoalcohols as oils. **15**: 20% yield; R_f 0.59 (eluting mixture, D); HPLC, R_t 4.28 (Waters Associates instrument; eluting mixture, K; flow rate, 2 mL min^{-1}); MS *m/z* 342 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.10 (*d*, *J* = 6.6 Hz, 3H, CH_3), 2.70–2.90 (*m*, 4H, $CH_2NCH_2CH_2OH$), 3.24–3.39 (*m*, 1H, $NCHCH_3$), 3.48–3.68 (*m*, 2H, CH_2OH), 3.81–4.07 (*m*, 3H, CH_2OAr and 1 H-3 of benzodioxan), 4.19-4.31 (*m*, 2H, 1 H-3 and H-2 of benzodioxan), 6.79–7.00 (*m*, 7H, aromatics of benzodioxan and 3 OAr), 7.26–7.33 (*m*, 2H, 2 OAr). **16**: 11% yield; R_f 0.54 (eluting mixture, D); HPLC, R_t 5.29 (Waters Associates instrument; eluting mixture, K; flow rate, 2 mL min^{-1}); MS *m/z* 342 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.13 (*d*, *J* = 6.38 Hz, 3H, CH_3), 2.71–2.97 (*m*, 4H, $CH_2NCH_2CH_2OH$), 3.23–3.37 (*m*, 1H, CH_3CH), 3.46–3.62 (*m*, 2H, CH_2OH), 3.74–3.82 (*m*, 1H, 1 CH_2OAr), 3.87–3.95 (*m*, 1H, 1 H-3 of benzodioxan), 4.02–4.10 (*m*, 1H, 1 CH_2OAr), 4.19–4.31 (*m*, 2H, 1 H-3 and H-2 of benzodioxan), 6.75–6.97 (*m*, 7H, aromatics of benzodioxan and 3 OAr), 7.22–7.30 (*m*, 2H, 2 OAr).

2-[(2,3-Dihydrobenzo[1,4]dioxin-2-ylmethyl)-[2-(2-methoxyphenoxy)ethyl)amino]ethanol (17). Prepared from **11**; eluting mixture, F; viscous oil; 43% yield; R_f 0.39; MS *m/z* 360 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.70 (*br s*, 1H, OH, exchangeable with D_2O), 2.80–3.08 [*m*, 6H, $N(CH_2)_3$], 3.50–3.70 (*m*, 2H, CH_2OH), 3.84 (s, 3H, OCH_3), 4.00–4.10 (*m*, 3H, CH_2OAr and 1 H-3 of benzodioxan), 4.28–4.38 (*m*, 2H, H-2 and 1 H-3 of benzodioxan), 6.78–6.97 (*m*, 8H, Ar).

2-[(2,3-Dihydrobenzo[1,4]dioxin-2-ylmethyl)-[2-(2-methoxyphenoxy)-1-methylethyl)amino]ethanol (diastereomers 18 and 19). Prepared from **12** and purified by gravity column chromatography. Eluting with chloroform gave the two diastereomeric aminoalcohols as oils. **18**: 13% yield; R_f 0.57 (eluting mixture, D); HPLC, R_t 0.73 min (Hewlett Packard instrument; eluting mixture, L; flow rate, 0.4 mL min^{-1}); MS *m/z* 373 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.07 (*d*, *J* = 6.28 Hz, 3H, CH_3), 1.58 (*br s*, 1H, OH, exchangeable with D_2O), 2.65–2.88 [*m*, 4H, $N(CH_2)_2$], 3.30–3.42 (*m*, 1H, CH_3CH), 3.43–3.72 (*m*, 2H, CH_2OH), 3.80–3.95 (*m*, 5H, OCH_3 and CH_2OAr), 4.00–4.10 (*m*, 1H, 1 H-3 of benzodioxan), 4.15–4.28 (*m*, 1H, H-2 of benzodioxan), 4.30–4.38 (*m*, 1H, 1 H-3 of benzodioxan), 6.78–6.97 (m, 8H, Ar). **19**: 20% yield; R_f 0.51 (eluting mixture, D); HPLC, R_t 0.81 min (Hewlett Packard instrument; eluting mixture, L; flow rate, 0.4 mL min^{-1}); MS *m/z* 373 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.06 (*d*, *J* = 5.66 Hz, 3H, CH_3), 1.62 (*br s*, 1H, OH, exchangeable with D_2O), 2.65–2.97 [*m*, 4H, $N(CH_2)_2$], 3.25–3.42 (*m*, 1H, CH_3CH), 3.48–3.65 (*m*, 2H, CH_2OH), 3.82 (*s*, 3H, OCH_3), 3.83–3.95 (*m*, 2H, CH_2OAr), 4.12–4.28 (*m*, 2H, H-2 and 1 H-3 of benzodioxan), 4.30–4.35 (*m*, 1H, H-3 of benzodioxan), 6.75–6.96 (*m*, 8H, Ar).

2-[Benzyl-[2-(2-methoxyphenoxy)ethyl]amino]ethanol (20). Prepared from **13**; eluting mixture, F; oil; 63% yield; R_f 0.29; MS *m/z* 301 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.62 (*br s*, 1H, OH, exchangeable with D_2O), 2.78 (*t*, *J* = 5.20 Hz, 2H, CH_2CH_2OH), 3.00 (*t*, *J* = 5.77 Hz, 2H, CH_2CH_2OAr), 3.61 (*t*, *J* = 5.27 Hz, 2H, CH_2OH), 3.76 (*s*, 2H, NCH_2Ar), 3.86 (*s*, 3H, OCH_3), 4.05 (*t*, *J* = 5.77 Hz, 2H, CH_2OAr), 6.80–6.98 (*m*, 4H, OAr), 7.20–7.38 (*m*, 5H, Ar).

General procedure for the synthesis of β-chloroethylamines hydrochlorides 2–8. HCl (g) was slowly bubbled for 15 min into a stirred and cooled (0 °C) solution of aminoalcohol (1 mmol) in dry benzene (25 mL), then $SOCl_2$ (1.26 mmol) in dry benzene (5 mL) was added dropwise and the mixture refluxed for 8 h. Removal of solvent and excess $SOCl_2$ gave a residue that was purified by crystallization or chromatography.

Benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)ethyl]amine hydrochloride (2). Prepared from aminoalcohol **20**; mp 112–114 °C (from *i*PrOH/Et_2O) (ref. 17, mp 127–129 °C); R_f 0.46 (eluting mixture, A); 27% yield; ^1H NMR ($CDCl_3$): δ 3.40–3.65 (*m*, 4H, CH_2Cl and CH_2CH_2OAr), 3.82 (*s*, 3H, OCH_3), 4.05–4.15 (*m*, 2H, CH_2OAr), 4.35–4.63 (*m*, 4H, $ArCH_2NCH_2CH_2Cl$), 6.84–6.93 (*m*, 3H, OAr), 6.95–7.06 (*m*, 1H, OAr), 7.38–7.51 (*m*, 3H, Ar), 7.72–7.80 (*m*, 2H, Ar), 13.15 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{18}H_{23}Cl_2NO_2$) C, H, N.

Benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (3). Prepared from aminoalcohol **14**; mp 144–146 °C (from *i*PrOH); R_f 0.49 (eluting mixture, A); 27% yield; ^1H NMR ($CDCl_3$, 30 °C): δ 1.60 (*d*, *J* = 6.40 Hz, 3H, CH_3CH), 3.30–3.76 (*m*, 2H, CH_2Cl), 3.80–4.06 (*m*, 6H, OCH_3, CH_3CH and CH_2CH_2Cl), 4.10–4.50 (*m*, 2H, 1 $ArCH_2N$ and 1 CH_2OAr), 4.54–4.78 (*m*, 2H, 1 $ArCH_2N$ and 1 CH_2OAr), 6.85–7.15 (*m*, 4H, OAr), 7.40–7.52 (*m*, 3H, Ar), 7.75–8.00 (*m*, 2H, Ar), 12.85 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{19}H_{25}Cl_2NO_2$) C, H, N.

(2-Chloroethyl)-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-(1-methyl-2-phenoxyethyl)amine hydrochloride (diastereomers 4 and 5). Prepared from aminoalcohols **15** and **16**, respectively; eluting mixture, A; transformed into the hydrochloride salts and recrystallized from EtOAc/Et_2O. **4**: mp 88–91 °C; R_f 0.50; 60% yield; ^1H NMR ($CDCl_3$, 40 °C): δ 1.56 (*d*, *J* = 6.41 Hz, 3H, CH_3CH), 3.26–3.50 (*m*, 2H, 1 $CHCH_2N$ and 1 CH_2Cl), 3.57–3.74 (*m*, 1H, 1 $CHCH_2N$), 3.87 (*br m*, 1H, 1 CH_2Cl), 4.03–4.22 (*m*, 4H, 1 H-3 of benzodioxan, CH_3CH and CH_2CH_2Cl), 4.24–4.37 (*m*, 2H, 1 H-3 of benzodioxan and 1 CH_2OAr), 4.73–4.82 (*m*, 1H, 1 CH_2OAr), 5.37 (*br s*, 1H, H-2 of benzodioxan), 6.86–6.94 (*m*, 6H, 4 aromatics of benzodioxan and 2 OAr), 6.97–7.06 (*m*, 1H, OAr), 7.28–7.35 (*m*, 2H, OAr), 13.50 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{20}H_{25}Cl_2NO_3$) C, H, N. **5**: mp 101–104 °C; R_f 0.44; 45% yield; ^1H NMR ($CDCl_3$): δ 1.62 (*br s*, 3H, CH_3CH), 3.00–4.80 (*br m*, 11H, H-3 of benzodioxan, $CH_2NCH_2CH_2Cl$, CH_3CH and CH_2OAr), 5.35 (*br s*, 1H, H-2 of benzodioxan), 6.56–7.10 (*m*, 7H, 3 OAr and 4 aromatics of benzodioxan), 7.20–7.38 (*m*, 2H, OAr), 13.25 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{20}H_{25}Cl_2NO_3$) C, H, N.

(2-Chloroethyl)-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-[2-(2-methoxyphenoxy)ethyl]amine hydrochloride (6). Prepared from aminoalcohol **17**; eluting mixture, A; transformed into the hydrochloride salt; mp 121–123 °C (from EtOAc); R_f 0.36; 52% yield; ^1H NMR ($CDCl_3$): δ 3.35–3.55 (*m*, 1H, 1 $CHCH_2N$), 3.60–3.88 (*m*, 8H, OCH_3, 1 $CHCH_2N$ and CH_2CH_2Cl), 3.90–4.27 (*m*, 3H, 1 H-3 of benzodioxan and CH_2CH_2OAr), 4.30–4.80 (*m*, 3H, 1 H-3 of benzodioxan and CH_2OAr), 5.28 (br s, 1H, H-2 of benzodioxan), 6.80–7.10 (*m*, 8H, Ar), 13.82 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{20}H_{25}Cl_2NO_4$) C, H, N.

(2-Chloroethyl)-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (diastereomers 7 and 8). Prepared from aminoalcohols **18** and **19**, respectively; eluting mixture, A; transformed into the hydrochloride salts. **7**: mp 125–127 °C (from EtOAc/petroleum ether); R_f 0.40; 19% yield; ^1H NMR ($CDCl_3$): δ 1.60 (*br s*, 3H, CH_3CH), 3.10–4.48 (*br m*, 13H, OCH_3, H-3 of benzodioxan, $CH_2NCH_2CH_2Cl$, CH_3CH and 1 CH_2OAr), 4.70 (*br s*, 1H, 1 CH_2OAr), 5.40 (*br s*, 1H, H-2 of benzodioxan), 6.82–7.10 (*m*, 8H, Ar), 13.40 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{21}H_{27}Cl_2NO_4 \cdot 0.25\ C_4H_8O_2$) C, H, N. **8**: mp 168–170 °C (from *i*PrOH); R_f 0.40; 54% yield; ^1H NMR ($CDCl_3$, 40 °C): δ 1.70 (*d*, *J* = 6.60 Hz, 3H, CH_3CH), 3.40–3.98 (*m*, 7H, OCH_3 and $CH_2NCH_2CH_2Cl$), 4.00–4.45 (*m*, 7H, H-3 of benzodioxan, CH_2CH_2Cl, CH_3CH and CH_2OAr), 5.42 (*br s*, 1H, H-2 of benzodioxan), 6.78–7.05 (*m*, 8H, Ar), 13.32 (*br s*, 1H, NH, exchangeable with D_2O). Anal. ($C_{21}H_{27}Cl_2NO_4$) C, H, N.

Measurements of formation and decay of aziridinium ions

The method used to quantify aziridinium ions was based on that of Gill and Rang.[24] Here we describe the procedure followed for the standard compound **1** and used also for **2–8**.

A mixture of sodium–potassium phosphate buffer (50 mM, pH 7.4) and methanol (130 mL; 8:2, v/v) was added to a stirred solution (37 °C) of **1** (17.7 mg) in methanol (2 mL). After 1 min stirring, aliquots (3.5 mL) of solution were removed every 30 s, during 30–35 min, and poured into glacial acetic acid (1 mL) to stop cyclization. The resulting solution was treated with 0.01 N sodium thiosulfate solution (1 mL) and, over 10–20 min, titrated with a 3.84 mM iodine solution by an automatic microburet using an amperometric method to reveal the end point. Calculated aziridinium ion concentrations were fitted to a first order kinetic model by an unweighted Gauss–Newton non-linear regression routine.[25]

Functional antagonism in isolated rat vas deferens

Male albino rats (175–200 g) were killed by a sharp blow on the head and both vasa deferentia were isolated, freed from adhering connective tissue and transversely bisected. Prostatic, 12 mm in length, and epididymal portions, 14 mm in length, were prepared and mounted individually in baths of 20 mL working volume containing Krebs solution, pH 7.4, of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. $MgSO_4$ concentration was reduced to 0.6 mM when twitch response to field stimulation was studied. The medium was maintained at 37 °C and gassed with 95% O_2–5% CO_2. The loading tension used to assess α_1- or α_2-blocking activities was 0.4 g or 0.5–0.8 g, respectively, and contractions were recorded by means of force transducers connected to a two channel Gemini 7070 polygraph.

The tissues were allowed to equilibrate for at least 1 h before addition of any drug. Parallel experiments, in which tissues did not receive any antagonist, were run in order to correct for time-dependent changes in agonist sensitivity.[33]

Field stimulation of the tissue was carried out by means of two platinum electrodes, placed near the top and bottom of the vas deferens, at 0.1 Hz using square pulses of 3 ms duration at voltage of 10–35 V. The stimulation voltage was fixed throughout the experiments. Propranolol hydrochloride (1 μM) and cocaine hydrochloride (10 μM) were present in the Krebs solution throughout the experiments outlined below to block β-adrenoreceptors and neuronal uptake mechanisms, respectively.

The α_1-adrenoreceptor blocking activity was determined on the epididymal portion of the vas deferens. Noradrenaline dose–response curves were obtained cumulatively, the first one being discarded and the second one taken as control. After incubation with the antagonist for 30 min and washing with physiological solution for 30 min, a third dose–response curve was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. Compounds **1**, **2** and **4–8** were tested at four to seven different concentrations and each concentration was investigated four to six times, while compound **3** was investigated at 10 concentrations, each of these being tested six to 10 times. The antagonist potency of compounds at α_1-adrenoreceptors was expressed by the negative logarithm of concentration that cause 50% inhibition of agonist action (pIC_{50}).

The α_2-adrenoreceptor blocking activity was assessed on the prostatic portion of the vas deferens by antagonism to clonidine which inhibits twitch responses of the field-stimulated vas deferens by acting on the α_2-adrenoreceptor.[34,35] A first clonidine dose–response curve, taken as control, was obtained cumulatively avoiding the inhibition of more than 90% of twitch responses, while the concentration of clonidine causing 100% inhibition was deduced from the second dose–response curve obtained from parallel experiments. Under these conditions it was possible to obtain a second dose–response curve which was not significantly different from the first one. Thus, after incubation with antagonist for 30 min and washing with physiological solution for 30 min, a dose–response curve was obtained and results were expressed as percentage of the maximal response obtained in the control curve. Each antagonist was tested at three different concentrations and each concentration was investigated at least four times. The antagonist potency of compounds at α_2-adrenoreceptors was expressed by the negative logarithm of concentration that cause 50% inhibition of agonist action (pIC_{50}).

All data are presented as the mean ± SE of *n* experiments. Differences between mean values were tested for significance by Student's *t*-test.

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Analytical Data

		Calcd %			Found%		
no.	Formula	C	H	N	C	H	N
2	$C_{18}H_{23}Cl_2NO_2$	60.68	6.51	3.93	61.03	6.64	3.56
3	$C_{19}H_{25}Cl_2NO_2$	61.63	6.80	3.78	61.61	6.87	3.58
4	$C_{20}H_{25}Cl_2NO_3$	60.31	6.33	3.52	60.57	6.56	3.31
5	$C_{20}H_{25}Cl_2NO_3$	60.31	6.33	3.52	60.61	6.60	3.14
6	$C_{20}H_{25}Cl_2NO_4$	57.98	6.08	3.38	57.81	6.18	3.13
7	$C_{21}H_{27}Cl_2NO_4 \cdot 0.25C_4H_8O_2$	58.67	6.49	3.11	58.99	6.43	2.80
8	$C_{21}H_{27}Cl_2NO_4$	58.88	6.35	3.27	59.10	6.56	3.34
9	$C_{17}H_{22}ClNO_2$	66.30	7.20	4.55	66.58	7.32	4.35
10	$C_{18}H_{22}ClNO_3 \cdot 0.5H_20$	62.70	6.72	4.06	62.33	6.48	3.91

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