Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1291–1303

BIOORGANIC & MEDICINAL CHEMISTRY

Structure–Activity Relationships Among Novel Phenoxybenzamine-Related β-Chloroethylamines

Dario Giardinà,[a,*] Mauro Crucianelli,[a] Piero Angeli,[a] Michela Buccioni,[a] Ugo Gulini,[a] Gabriella Marucci,[a] Gianni Sagratini[a] and Carlo Melchiorre[b]

[a]*Department of Chemical Sciences, University of Camerino, Italy*
[b]*Department of Pharmaceutical Sciences, University of Bologna, Italy*

Received 23 July 2001; accepted 5 November 2001

Abstract—A series of β-chloroethylamines **5**–**18**, structurally related to the irreversible α_1-adrenoceptor antagonist phenoxybenzamine [PB, *N*-benzyl-*N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)amine hydrochloride, **1**] and the competitive antagonist WB4101 [*N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]amine hydrochloride, **2**], were synthesized and evaluated for their activity at α-adrenoceptors of the epididymal and the prostatic portion of young CD rat vas deferens. All compounds displayed irreversible antagonist activity. Most of them showed similar antagonism at both α_1- and α_2-adrenoceptors, whereas compounds **13** and **18**, lacking substituents on both the phenoxy group and the oxyamino carbon chain, displayed a moderate α_1-adrenoceptor selectivity (10–35 times), which was comparable to that of PB. Compounds **14** and **15**, belonging to the benzyl series and bearing, respectively, a 2-ethoxyphenoxy and a 2-*i*-propoxyphenoxy moiety, were the most potent α_1-adrenoceptor antagonists with an affinity value similar to that of PB (pIC_{50} values of 7.17 and 7.06 versus 7.27). Interestingly, several compounds were able to distinguish two α_1-adrenoceptor subtypes in the epididymal tissue, as revealed by the discontinuity of their inhibition curves. A mean ratio of 24:76 for these α_1-adrenoceptors was determined from compounds **8**–**10**, **12**, and **15**–**17**. Furthermore, compounds **9**, **10**, **12**, **16a**, and **16b** showed higher affinity towards the minor population of receptors, whereas compounds **8**, **15**, and **17** preferentially inhibited the major population of α_1-adrenoceptors. In addition, selected pharmacological experiments demonstrated the complementary antagonism of the two series of compounds and their different, preferential affinity for one of the two α_1-adrenoceptor subtypes. In conclusion, we found β-chloroethylamines that demonstrate a multiplicity of α_1-adrenoceptors in the epididymal portion of young CD rat vas deferens and, as a consequence, they are possible useful tools for α_1-adrenoceptor characterization.

Introduction

Phenoxybenzamine [PB, *N*-benzyl-*N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)amine hydrochloride, **1**], the prototype of β-chloroethylamines, has been widely used as an alkylating agent for α_1-adrenoceptor characterization. However, its use is limited due to lack of receptor specificity.[1] It displays selectivity towards α_1-adrenoceptors with respect to α_2-adrenoceptors.[2] However, there is no evidence that PB is able to discriminate between the α_1-adrenoceptor subtypes.[3] PB is the first α_1-adrenoceptor antagonist that has been evaluated in humans for the possible treatment of benign prostatic hypertrophy (BPH).[4]

Currently, α_1-adrenoceptors are classified into three subtypes, namely α_{1A}, α_{1B}, and α_{1D}.[3,5,6] In addition, on the basis of functional studies, the existence of a fourth α_1-adrenoceptor, α_{1L}, has been proposed, which displays a low affinity for prazosin.[7,8] However, a distinct gene encoding for this adrenoceptor remains to be discovered.

The exact nature of the α_1-adrenoceptor subtypes involved in noradrenaline-mediated contractions of the epididymal portion of rat vas deferens has yet to be characterized. Results from some laboratories point to the α_{1A}-adrenoceptor as the only subtype involved in contractions.[9,10] However, it has been reported that this contraction, albeit mainly mediated by only one subtype, named α_{1L} or α_{1A}, is also mediated to a minor extent by an additional α_1-adrenoceptor, that is an α_{1A}-subtype[11] or a non-α_{1A}, non-α_{1B} subtype.[12]

Recently, we reported on a series of novel β-chloroethylamines,[13] structurally related to both PB and WB4101 [*N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-

*Corresponding author at: Department of Chemical Sciences, Via S. Agostino, 1, 62032 Camerino (MC), Italy. Tel.: +39-0737-402265; fax: +39-0737-637345; e-mail: giardina@camserv.unicam.it

0968-0896/02/$ - see front matter
PII: S0968-0896(01)00395-9

N-[2-(2,6-dimethoxyphenoxy)ethyl]amine hydrochloride, **2**], a competitive α_1-adrenoceptor antagonist. These compounds displayed, like PB, an irreversible blocking activity at rat vas deferens α-adrenoceptors, with a slight selectivity for α_1-relative to α_2-adrenoceptors. Interestingly, two compounds of the series, benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]-amine hydrochloride (**3**, CM18) and (2-chloroethyl)-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (**4**, higher melting diastereomer), both bearing a methyl and a methoxy group in their structure, showed a marked discontinuity in their concentration–inhibition curve of α_1-adrenoceptors of the epididymal portion of the rat vas deferens tissue. Of the two enantiomers of **3**, only the (*R*)-(+) stereoisomer showed discontinuity in the α_1-adrenoceptor concentration–inhibition curve.[14]

This finding supports the view that two α_1-adrenoceptor subtypes mediate the noradrenaline-induced contraction of the epididymal portion of young CD rat vas deferens. These subtypes are distinguished by **3** and **4** and are sensitive to stereochemical factors.

These results prompted us to design novel PB- and WB4101-related β-chloroethylamines to establish a structure–biphasicity relationship among these α_1-adrenoceptor alkylating agents.

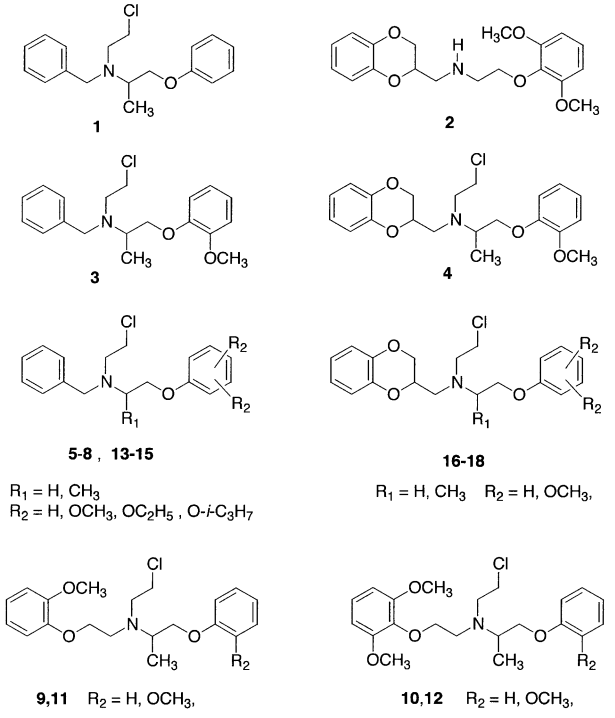


Chart 1.

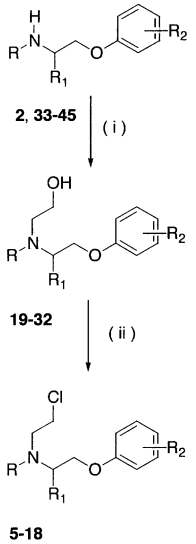
Thus, taking **3** and **4** as chemical starting points, we synthesized compounds **5**–**18** to investigate the effect, if any, on the dual affinity characteristics for α-adrenoceptors of (i) the substituent on the phenoxy moiety (**5**–**7** and **14**–**16**), (ii) the methyl group on the alkane chain (**8**, **13**, **17**, and **18**) and (iii) the opening of the dioxane ring (**9**–**12**).

Chemistry

β-Chloroethylamines **5**–**18**, including the already known **6**[15] and **13**,[16] were synthesized from the corresponding *N*,*N*-disubstituted 2-aminoethanols **19**–**32**, which, in turn, had been obtained from the secondary amines **33**–**45** by alkylation with 2-bromoethanol, upon treatment with thionyl chloride in benzene saturated with HCl (g) (Scheme 1).

Compound **30** afforded, through chromatographic separation, the two diastereomeric alcohols **30a** and **30b**. Intermediates **33**–**45** were obtained following two different procedures (Scheme 2). Amines **33**–**40** and **42**–**44** were synthesized by condensation of the suitable carbonyl compounds and amines, in the presence of sodium cyanoborohydride.[17–19] Amines **41**[20] and **45**[21] were obtained by reduction with BH_3 $(CH_3)_2S$ of amides **46**[22] and **47**, which were synthesized from benzylamine and (2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)amine, respectively, and phenoxyacetic acid. The reference compound WB4101 (**2**) was synthesized as described in literature.[23]

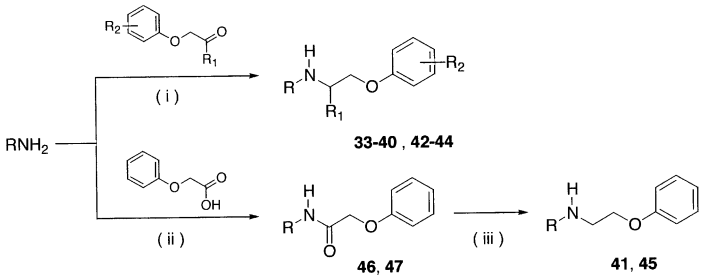
^{1}H NMR spectra of diastereomers **16a** and **16b**, albeit showing some differentiating spectral features, were too complex to permit a configuration assignment. A broad doublet signal relative to the CH_3CH group was present in both diastereomers, whereas, in the range 3.30–4.60 ppm, to a series of overlapping broad signals relative to protons of the non-aromatic carbon framework of **16b**, a more separate succession of complex signals was observed in **16a**. In particular, a narrow multiplet, centred at 4.47 ppm, and allottable, perhaps, to one hydrogen of the methylene vicinal to the chiral centre CH_3CHCH_2, was recognizable in the spectrum.



Compd	R	R_1	R_2
5, 19, 33	Bn	CH_3	3-OCH_3
6, 20, 34	Bn	CH_3	4-OCH_3
7, 21, 35	Bn	CH_3	2,6-di-OCH_3
8, 22, 36	Bn	H	2,6-di-OCH_3
9, 23, 37	MPE	CH_3	2-OCH_3
10, 24, 38	DMPE	CH_3	2-OCH_3
11, 25, 39	MPE	CH_3	H
12, 26, 40	DMPE	CH_3	H
13, 27, 41	Bn	H	H
14, 28, 42	Bn	CH_3	2-OC_2H_5
15, 29, 43	Bn	CH_3	2-*i*-OC_3H_7
16, 30, 44	DBD	CH_3	2,6-di-OCH_3
17, 31, 2	DBD	H	2,6-di-OCH_3
18, 32, 45	DBD	H	H

Bn = Benzyl; MPE = 2-MethoxyPhenoxyEthyl; DMPE = 2,6-DiMethoxyPhenoxyEthyl; DBD = 2,3-DihydroBenzo[1,4]Dioxin-2-ylmethyl

Scheme 1. Reagents: (i) $BrCH_2CH_2OH$, K_2CO_3, ethanol; (ii) $SOCl_2$, HCl (g), benzene.



Scheme 2. Reagents: (i) $NaBH_3CN$, HCl, EtOH, molecular sieves; (ii) *i*-BuOCOCl, Et_3N, CH_2Cl_2; (iii) $BH_3(CH_3)_2S$, DME. Specifications for R, R_1 and R_2 are as reported in Scheme 1.

Kinetic Studies

β-Haloalkylamines inhibit α-adrenoceptors through an irreversible alkylation process, which is mediated by their corresponding aziridinium ions.[24] Since the potency of these antagonists is dependent on their receptor affinity and on the aziridinium ion concentration as well, we determined, at physiological conditions (pH 7.4), the rate of cyclization of β-haloalkylamines **5**–**18** and the hydrolysis rate of the formed aziridinium ions. The aziridinium ion concentration was evaluated applying the Gill and Rang method.[25]

The rate constants (k_1) for the cyclization of **5**–**18** and for the decay (k_2) of the aziridinium ion species were estimated by fitting a kinetic model, based on the consecutive first-order reaction equation $Q=[Q_o k_1/(k_2-k_1)](e^{-k_1 t}-e^{-k_2 t})$ (where Q is the concentration of the 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[26] (Table 1). The new constants were used to calculate correct aziridinium ion concentrations as a time function (Figs 1 and 2).

Table 1. Rates of cyclization (k_1) of β-cloroethylamines **5**–**18** and decay of relative aziridinium ions (k_2) at 37 °C and pH 7.4; phenoxybenzamine (**1**) is reported as reference[a]

Compd	k_1 (s^{-1})×10^2	k_2 (s^{-1})×10^2	Compd	k_1 (s^{-1})×10^2	k_2 (s^{-1})×10^2
5	7.00	2.24	**13**	10.66	1.22
6	11.38	4.35	**14**	12.42	3.24
7	49.91	1.82	**15**	7.57	3.73
8	33.85	1.97	**16a**	4.29	9.04
9	91.75	9.12	**16b**	4.15	10.55
10	147.03	4.49	**17**	6.46	8.27
11	35.18	7.47	**18**	2.38	14.28
12	48.79	7.18	**1**	11.12	1.00

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

Results and Discussion

The biological profile of β-chloroethylamines **5**–**18** at α_1- and α_2-adrenoceptors was assessed on isolated vas deferens[27,28] of young CD rats and the results are reported in Tables 2 and 3, and Figures 3–5. In order to allow comparison of the results, phenoxybenzamine (PB) was used as a reference compound.

α_1-Adrenoceptor blocking activity was assessed by antagonism of (−)-noradrenaline-induced contractions of the epididymal portion of the vas deferens. α_2-Adrenoceptor 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- and α_2-antagonism of tested compounds was determined after a 30-min incubation followed by 30 min of washings. The decrease in maximum response of agonists was evaluated and expressed as a percentage of the control value. Complete concentration–inhibition curves at α_1-adrenoceptors were obtained for all test compounds and are shown in Figures 3–5. The antagonist potency of each compound was expressed as an IC_{50} value, that is the concentration producing 50% inhibition of the agonist maximal response (Tables 2 and 3).

All test compounds showed an irreversible blocking activity at both α_1- and α_2-adrenoceptors since the response was not recovered after extensive washing, following 30 min of incubation.

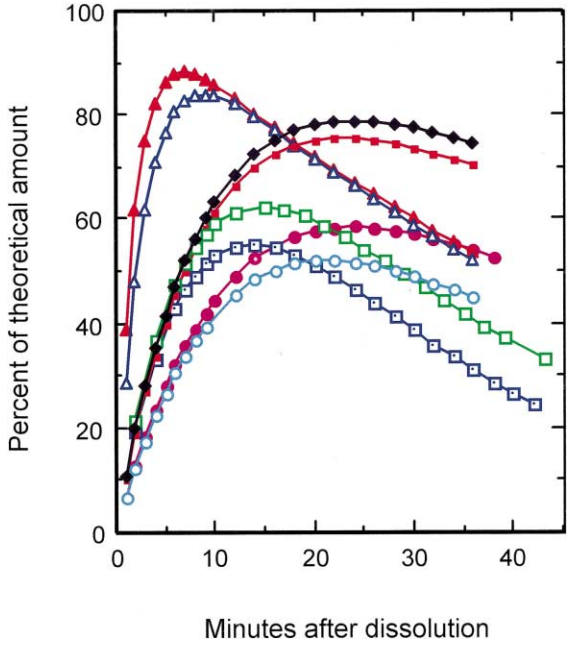


Figure 1. Aziridinium ion formation and decay at pH 7.4 and 37 °C for compounds **5** (●), **6** (□) (**7** (▲), **8** (△), **13** (■), **14** (□), **15** (○) and **1** (◆).

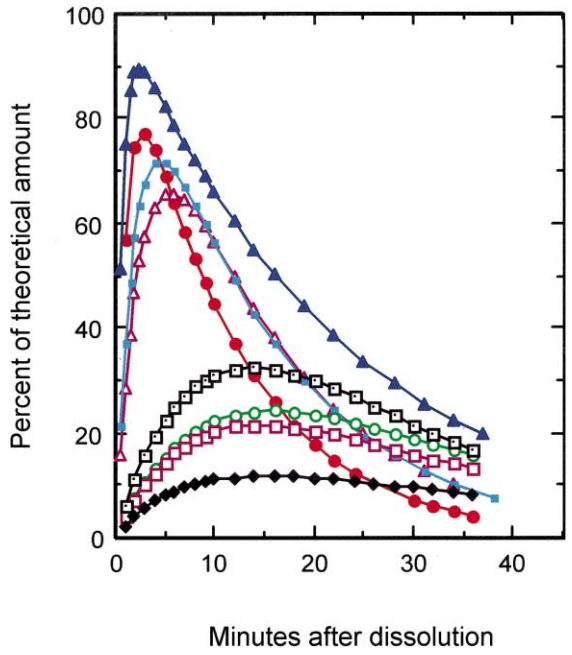
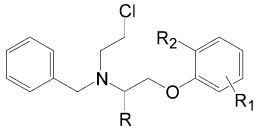


Figure 2. Aziridinium ion formation and decay at pH 7.4 and 37 °C for compounds **9** (●), **10** (▲), **11** (△), **12** (■), **16a** (○), **16b** (□), **17** (⊡) and **18** (◆).

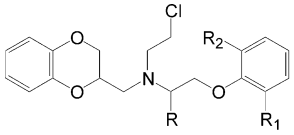
The antagonism ranged from $pIC_{50} = 6.37$ and 7.17 or from 6.62 and 7.93 at α_1-adrenoceptor, whereas it was arranged in a slightly larger gap (1.2–1.9 log units) at α_2-adrenoceptors. Compounds **14** and **15** were the most potent α_1-adrenoceptor antagonists ($pIC_{50} = 7.17$ and 7.06, respectively). These results indicate that the replacement of the methoxy function of **3** with a larger substituent (**14** and **15**) increases potency at both α_1- and α_2-adrenoceptors for compound **14** and only at α_1-adrenoceptor for compound **15**. Introduction of a 3- or 4-methoxy function (**5** and **6**) does not improve the affinity. It should be noted that, among the investigated compounds, only **13** and **18**, both characterized by the absence of substituents on the aliphatic framework and on the phenoxy moiety as well, showed a better α_1-adrenoceptor discriminating ability that was similar to or higher than that of PB, as revealed by their selectivity ratios (**13**, $\alpha_1/\alpha_2 = 10$; **18,** $\alpha_1/\alpha_2 = 35$; PB, $\alpha_1/\alpha_2 = 10$), as a consequence of a very low affinity for α_2-adrenoceptors.

Considering the ability of β-chloroalkylamines to produce aziridinium ions in experimental conditions, it can be observed that all the compounds bearing a 2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl moiety produced a maximal concentration of aziridinium ion lower than that of the corresponding benzyl derivatives (**16a** and **16b** vs **7**; **17** vs **8** and **18** vs **13**), due to both a decreased formation

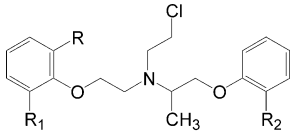
Table 2. α-Adrenoceptor blocking activity of **5**–**18** in the isolated rat vas deferens, in comparison with **1**, **3** and **4**



1, 3, 5-8, 13-15



4, 16-18



9-12

No.	R	R_1	R_2	α_1 pIC_{50}[a]		α_2 pIC_{50}[a]		α_1/α_2[b]
				Exp[c]	Calcd[d]	Exp[c]	Calcd[d]	
1	CH_3	H	H	7.27±0.01	7.38±0.01	6.28±0.01	6.39±0.01	10
3	CH_3	*o*-OCH_3	H	6.85±0.05	7.05±0.05	6.77±0.02	6.87±0.02	1
4	CH_3	OCH_3	H	6.82±0.04	7.93±0.04	6.21±0.03	7.33±0.03	4
5	CH_3	*m*-OCH_3	H	6.80±0.02	7.03±0.02	6.16±0.05	6.39±0.05	4
6	CH_3	*p*-OCH_3	H	6.58±0.06	6.84±0.06	ND[e]		
7	CH_3	*o*-OCH_3	OCH_3	6.57±0.02	6.62±0.02	6.28±0.03	6.33±0.03	2
8	H	*o*-OCH_3	OCH_3	6.66±0.04[f]	6.71±0.04	6.31±0.01	6.39±0.01	2
9	OCH_3	H	OCH_3	6.89±0.01[f]	6.99±0.01	6.42±0.04	6.53±0.04	3
10	OCH_3	OCH_3	OCH_3	6.82±0.05[f]	6.87±0.05	6.89±0.03	6.94±0.03	0.8
11	OCH_3	H	H	6.87±0.01	7.05±0.01	6.25±0.08	6.43±0.08	4
12	OCH_3	OCH_3	H	6.85±0.04[f]	6.99±0.04	6.22±0.02	6.36±0.02	4
13	H	H	H	6.96±0.02	7.08±0.02	5.97±0.05	6.08±0.05	10
14	CH_3	*o*-OC_2H_5	H	7.17±0.01	7.38±0.01	7.10±0.01	7.30±0.01	1
15	CH_3	*o*-O-i-Pr	H	7.06±0.02[f]	7.42±0.02	6.59±0.01	6.88±0.01	3
16a	CH_3	OCH_3	OCH_3	6.37±0.03[f]	6.98±0.03	5.95±0.01	6.57±0.01	3
16b	CH_3	OCH_3	OCH_3	6.56±0.01[f]	7.24±0.01	5.99±0.07	6.67±0.07	3
17	H	OCH_3	OCH_3	6.78±0.02[f]	7.28±0.02	6.62±0.06	7.11±0.06	1
18	H	H	H	6.75±0.02	7.68±0.02	5.21±0.01	6.14±0.01	35

[a]Noradrenaline and clonidine were used as agonists at α_1- and α_2-adrenoceptors, respectively. pIC_{50} values represent the negative logarithm of the concentration that produces 50% inhibition of agonist maximal response.
[b]The α_1/α_2 selectivity ratio is the antilog of the difference between pIC_{50} values at α_1- and α_2-adrenoceptors.
[c]Values deriving from the actual maximal aziridinium ion concentration produced in the experimental conditions by β-chloroethylamines (Figs 1 and 2).
[d]Values calculated considering a complete transformation of β-chloroethylamines into the corresponding aziridinium ions.
[e]ND, not determinable because of inhibition of twitch.
[f]Given the biphasicity of inhibition curves (Fig. 3), these values represent an approximate antagonist potency to block 50% of total number of receptors.

rate and an increased hydrolysis rate (Table 1, Figs 1 and 2). As a consequence, assuming a total transformation of β-chloroalkylamines into the corresponding aziridinium ions, the calculated pIC_{50} values, especially at α_1-adrenoceptors, are higher than the experimental ones for **16a**, **16b**, **17**, and **18**, but not for the benzyl analogues **7**, **8**, and **13**.

This confirms the finding[13] that the 2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl moiety satisfies more favorably than the benzyl group the structural requirements for a better interaction with the α_1- and α_2-adrenoceptors of the rat vas deferens.

The most interesting aspect emerging from the present investigation is the dual mode of inhibition displayed by several β-chloroalkylamines at α_1-adrenoceptors of the epididymal portion of rat vas deferens. Compounds **9**, **10**, **12**, **16a** and **16b**, and **8**, **15** and **17** showed a marked discontinuity in a well defined concentration range, 15–200 and 200–1000 nM, respectively (Fig. 3).

As already hypothesized[13] for **3** and **4**, the two series of compounds may discriminate between two α_1-adrenoceptors, which are functionally active in this tissue and approximately present in a 24:76 ratio. β-Chloroalkylamines **9**, **10**, **12**, **16a** and **16b** displayed higher blocking potency towards the apparent minor fraction of receptors, whereas compounds **8**, **15**, and **17** showed higher affinity at the major fraction (Table 3). Compounds **10**, **12**, and **16b** displayed the best selectivity ratio, ranging from 12 to 16. Thus, treating the tissue with a concentration corresponding to either one plateau of the inhibition curve produced by the compounds of one or the other series, it is possible to block irreversibly and selectively one of the two fractions of α_1-adrenoceptors. As a consequence, the consecutive incubation with a suitable compound of each series, at the above concentrations, should give a complete inhibition of the noradrenaline-induced contractions.

Table 3. Antagonist potency of β-chloroethylamines **8**–**10**, **12**, 15–**17** at high- and low-affinity α_1-adrenoceptor subtypes or subsites of epididymal portion of rat vas deferens

Compd	pIC_{50}[a] versus noradrenaline		$\alpha_{1\ high}/\alpha_{1\ low}$[b]
	$\alpha_{1\ high}$	$\alpha_{1\ low}$	
8	6.69±0.07[c]	5.93±0.02[d]	6
9	7.64±0.02[d]	6.79±0.02[c]	7
10	7.89±0.04[d]	6.70±0.05[c]	15
12	7.92±0.01[d]	6.71±0.04[c]	16
15	7.11±0.01[c]	6.38±0.02[d]	5
16a	7.20±0.01[d]	6.25±0.03[c]	9
16b	7.31±0.03[d]	6.23±0.05[c]	12
17	6.83±0.01[c]	6.03±0.01[d]	6

[a]pIC_{50} values, reported as means±SEM, represent the negative logarithm of concentration producing 50% inhibition of the noradrenaline maximal response. They are calculated from the biphasic inhibition curves considering the plateau as the separation line between the blockade of two different population of α_1-adrenoceptors.
[b]The selectivity ratio represents the antilog of the difference between the pIC_{50} values at the two α_1-adrenoceptor subsites.
[c]Receptor population representing the 76% of the total.
[d]Receptor population representing the 24% of the total.

This aspect has been verified by investigating the antagonism of **8** following pre-treatment of the tissue with 0.1 μM **16a**. As expected, **8** completely blocked, in the range 0.03–1 μM, noradrenaline-induced contractions with a monophasic curve. Complete blockade was achieved with a concentration 3 times lower than that required when using **6** alone (Fig. 4). Similarly, following pre-treatment with 0.6 μM **8**, **16a** caused 100% blockade of noradrenaline-induced contractions with an inhibition curve uniformly increasing with the concentration in the range 0.03–0.2 μM (Fig. 5).

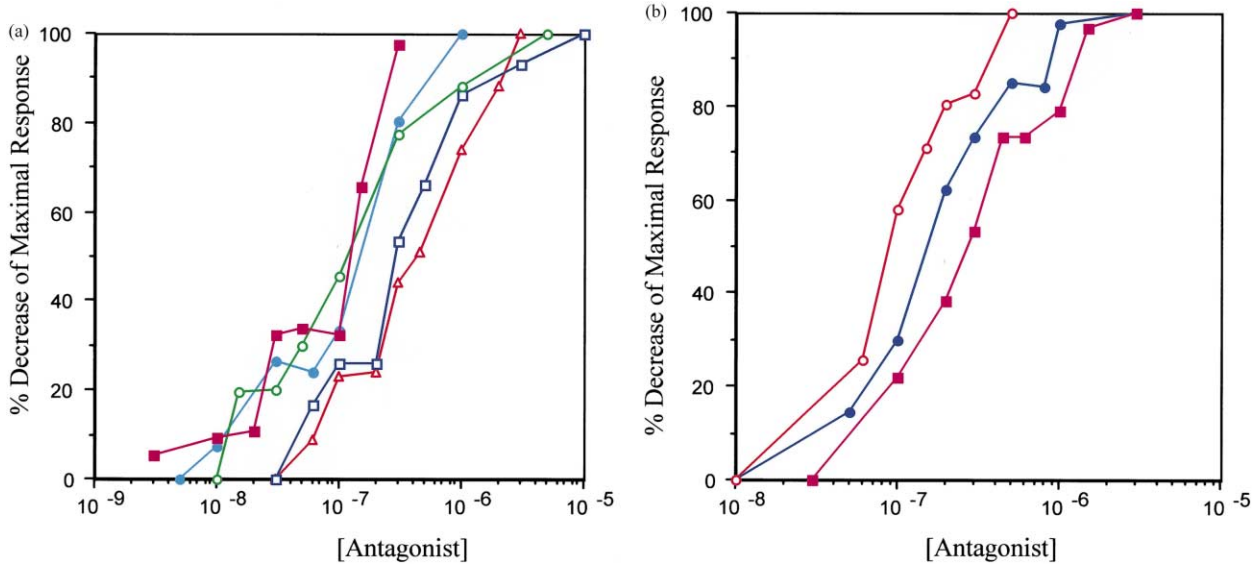


Figure 3. Epididymal portion of rat vas deferens: inhibition course of α_1-adrenoceptors blockade by: (a) compounds **9** (■), **10** (●), **12** (○), **16a** (△), **16b** (□) and (b) compounds, **8** (■), **15** (○), **17** (●). The percent decrease of maximal response to noradrenaline was measured after 30 min of incubation for each concentration followed by 30 min of washing. Results are the mean of three to 10 independent observations. The maximal SEM observed during the experiments did not exceed±8.0.

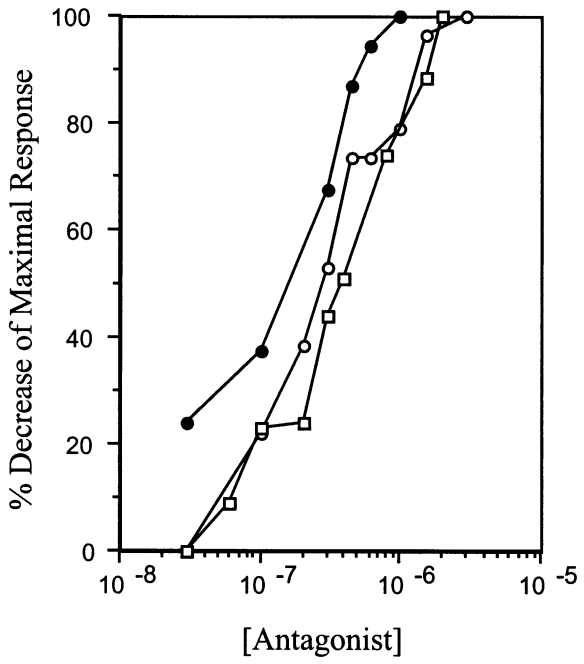


Figure 4. Epididymal portion of rat vas deferens: inhibition course of α_1-adrenoceptors by compounds **8** (○), **16a** (□) and compound **8** after pretreatment with **16a** at plateau concentration, 0.1 μM (●). The percent decrease of maximal response to noradrenaline was measured after 30 min of incubation for each concentration followed by 30 min of washing. In (●) the tissue was first incubated with 0.1 μM solution of **16a** then washed for 30 min before incubation with compound **8**. Results are expressed as mean of two to 10 independent observations. The SEM observed during the experiments did not exceed ± 7.1.

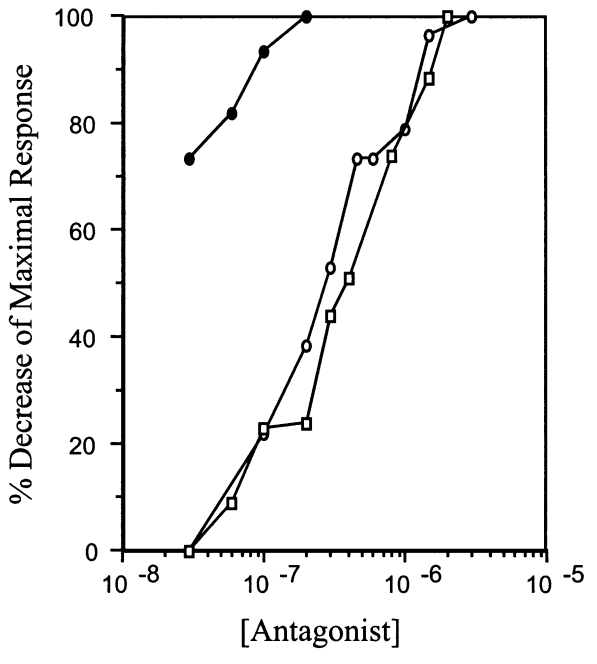


Figure 5. Epididymal portion of rat vas deferens: inhibition course of α_1-adrenoceptors by compounds **8** (○), **16a** (□) and compound **16a** after pretreatment with **8** at plateau concentration, 0.6 μM (●). The percent decrease of maximal response to noradrenaline was measured after 30 min of incubation for each concentration followed by 30 min of washing. In (●) the tissue was first incubated with 0.6 μM solution of **8** then washed for 30 min before incubation with compound **16a**. Results are expressed as mean of two to 10 independent observations. The SEM observed during the experiments did not exceed ± 7.6.

Given the similar shape of the concentration–inhibition curves among the components of the two series of compounds, the results obtained with compounds **8** and **16a** could be extended to the other components of the series. It is suggested that compounds **9**, **10**, **12**, and **16a**,**b** antagonize with high affinity the minor population of α_1-adrenoceptors, which is also blocked by compounds **8**, **15** and **17** but with a lower affinity. Inversely, **8**, **15**, and **17** preferentially inhibit the major population of α_1-adrenoceptors, which is also blocked with a lower affinity by **9**, **10**, **12**, and **16a**,**b**.

Concerning the relationship between structure and biphasicity of the inhibition curve of the studied compounds, the following considerations can be made: (i) A plateau is observed at lower concentrations for those compounds bearing a 2-aminopropoxy chain and, at least, one methoxy substituent at position 2 of the proximal phenoxy moiety (**3**, **4**, **9**, **10**, **16**). However, the dual mechanism of action observed for **12** indicates that the 2-methoxy group can be removed provided that the compound incorporates in some other part of the structure an additional 2,6-dimethoxyphenoxy function (cf., **9**, **11**, and **12**). (ii) A plateau is detected at higher concentrations for those compounds that carry an aminoethoxy rather than an aminopropoxy chain and a 2,6-dimethoxyphenoxy moiety (**8** and **17**) or, alternatively, a 2-aminopropoxy chain and a 2-*i*-propoxyphenoxy function (**15**).

These structural features suggest that steric factors might play an important role in stabilising different specific conformations of the intermediate aziridinium ions, which are able to differentiate two populations of α_1-adrenoceptors in the rat vas deferens tissue.

In conclusion, we discovered two series of novel β-chloroethylamines (**9**, **10**, **12**, **16a**, **16b** and **8**, **15**, **17**, respectively), which demonstrated heterogeneity of α_1-adrenoceptors functionally active in the epididymal portion of young CD rat vas deferens.

Experimental

Chemistry

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin–Elmer 297 and Varian VXR 300 instruments, respectively. The IR spectra, not included, were consistent with all the assigned structures. The elemental analyses of compounds agreed with the calculated values within the range ± 0.4%. 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) ethyl acetate–cyclohexane (0.1:9.9); (B) ethyl acetate–cyclohexane

(2:8); (C) ethyl acetate–cyclohexane (3:7); (D) ethyl acetate–cyclohexane (4:6); (E) ethyl acetate–*n*-hexane (1:4); (F) ethyl acetate–*n*-hexane (5:2); (G) ethyl acetate–*n*-hexane (5:4); (H) ethyl acetate–cyclohexane–methanol (2:5:0.1); (I) ethyl acetate–*n*-hexane–methanol (5:2:0.5); (L) ethyl acetate–*n*-hexane–methanol (5:2:0.1); (M) chloroform–*n*-hexane–methanol (9:1:0.2); (N) methylene chloride–petrol ether–methanol (5:8:1); (O) ethyl acetate–petrol ether–methanol–28% ammonia (10:4:2:0.1); (P) ethyl acetate–petrol ether–methanol–28% ammonia (4:10:1:0.1); (Q) ethyl acetate–petrol ether–methanol–28% ammonia (4:10:0.5:0.05); (R) ethyl acetate–cyclohexane–isopropanol–28% ammonia (6:7:1.5:0.1). 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 ACD/Name, a PC IUPAC name generator, version 1997, Advanced Chemistry Development, Toronto, Canada. Analytical HPLC was performed on a Waters Associates liquid chromatograph, mod. 440, absorbance detector, equipped with a Beckman column, Ultrasphere ODS, 5 μ (250×4.6 mm). Kinetic and pharmacological graphics were drawn by a Cricket Graph computer program, Version 1.3.2., Computer Associates International, Inc.

General procedure for the synthesis of secondary amines 33–40 and 42–44. To a solution of primary amine (7.5 mmol) in ethanol (8 mL) were consecutively added 2.5 mmol of a solution 2.5 M of HCl/EtOH, 1.2 mmol of proper substituted acetone, 1 mmol of sodium cyanoborohydride and an excess of 4 A molecular sieves. The mixture was stirred at room temperature for 72 h, then acidified (pH 1) with 2 N HCl, filtered and evaporated to dryness. The residue was added with H_2O, basified with 2 N KOH and the mixture extracted with Et_2O. The organic layer was extracted with 2 N HCl, the acidic solution basified with 2N KOH and then extracted again with Et_2O. Removal of the dried solvent gave an oil residue that was purified by column chromatography eluting with the proper eluting mixture. The various secondary amines were obtained as oils, and characterised by physical parameters.

***N*-Benzyl-*N*-[2-(3-methoxyphenoxy)-1-methylethyl]amine (33).** Prepared from benzylamine and 1-(3-methoxyphenoxy)acetone. Eluting mixture, I; 60% yield; R_f 0.55 [lit.:[18] $bp_{0.3}$ 150–154 °C]. ^{1}H NMR ($CDCl_3$): δ 1.23 (d, J = 6.52 Hz, 3H, CH_3), 2.52 (s br, 1H, NH, exchangeable with D_2O), 3.10–3.28 (m, 1H, C*H*CH_3), 3.72–4.00 (m, 7H, CH_2Ar, CH_2OAr and OCH_3), 6.44–6.56 (m, 3H, OAr), 7.11–7.42 (m, 6H, OAr e Ar). Hydrochloride salt, mp 99–102 °C (AcOEt). Anal. ($C_{17}H_{22}ClNO_2$) C, H, N.

***N*-Benzyl-*N*-[2-(4-methoxyphenoxy)-1-methylethyl]amine (34).** Prepared from benzylamine and 1-(4-methoxyphenoxy)acetone. Eluting mixture, I; 64% yield; R_f 0.53 [lit.:[18] $bp_{0.4}$ 175–178 °C]. ^{1}H NMR ($CDCl_3$): δ 1.21 (d, J = 6.50 Hz, 3H, CH_3), 2.37 (s br, 1H, NH, exchangeable with D_2O), 3.07–3.25 (m, 1H, C*H*CH_3), 3.72–3.93 (m, 7H, CH_2Ar, CH_2OAr and OCH_3), 6.71–6.95 (m, 4H, OAr), 7.20–7.42 (m, 5H, Ar). Hydrochloride salt, mp 93–95 °C (AcOEt). Anal. ($C_{17}H_{22}ClNO_2$) C, H, N.

***N*-Benzyl-*N*-[2-(2,6-dimethoxyphenoxy)-1-methylethyl]-amine (35).** Prepared from benzylamine and 1-(2,6-dimethoxyphenoxy)acetone. Eluting mixture, N; 62% yield; R_f 0.41. ^{1}H NMR ($CDCl_3$): δ 1.10 (d, J = 6.50 Hz, 3H, CH_3), 2.50 (s, br, 1H, NH, exchangeable with D_2O), 3.10–3.20 (m, 1H, C*H*CH_3), 3.65–3.78 (m, 8H, CH_2Ar, CH_2OAr and OCH_3), 3.95 (d, J = 12.55 Hz, 1H, CH_2Ar), 4.16–4.21 (m, 1H, CH_2OAr), 6.55 (d, J = 7.86 Hz, 2H, OAr), 6.97 (t, J = 7.40 Hz, 1H, OAr), 7.21–7.42 (m, 5H, Ar). Hydrochloride salt, mp 162–164 °C (AcOEt). Anal. ($C_{18}H_{24}ClNO_3 \cdot 0.5H_2O$) C, H, N.

***N*-Benzyl-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]amine (36).** Prepared from benzylamine and 2-(2,6-dimethoxyphenoxy)acetaldehyde. Eluting mixture, O; 51% yield; R_f 0.31. ^{1}H NMR ($CDCl_3$): δ 2.15 (s, br, 1H, NH exchangeable with D_2O), 2.92–2.96 (m, 2H, NCH_2CH_2), 3.79 (s, 6H, OCH_3), 3.87 (s, 2H, CH_2Ar), 4.16–4.20 (m, 2H, CH_2OAr), 6.57 (d, J = 6.63 Hz, 2H, OAr), 6.99 (t, J = 6.63 Hz, 1H, OAr), 7.21–7.40 (m, 5H, Ar). Hydrochloride salt, mp 125–126 °C (*i*-PrOH). Anal. ($C_{17}H_{22}ClNO_3 \cdot 0.5H_2O$) C, H, N.

***N*-[2-(2-Methoxyphenoxy)ethyl]-*N*-[2-(2-methoxyphenoxy)-1-methylethyl]amine (37).** Prepared from 2-(2-methoxyphenoxy)-1-ethanamine and 1-(2-methoxyphenoxy)acetone. Eluting mixture, N; 58% yield; R_f 0.29. ^{1}H NMR ($CDCl_3$): δ 1.35 (d, J = 6.69 Hz, 3H, CH_3), 3.12–3.44 (m, 3H, CH_2NH and C*H*CH_3), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.93–4.32 (m, 5H, CH_2OAr and NH exchangeable with D_2O), 6.83–7.03 (m, 8H, Ar). Anal. ($C_{19}H_{25}NO_4$) C, H, N.

***N*-[2-(2,6-Dimethoxyphenoxy)ethyl]-*N*-[2-(2-methoxyphenoxy)-1-methylethyl]amine (38).** Prepared from 2-(2,6-dimethoxyphenoxy)-1-ethanamine and 1-(2-methoxyphenoxy) acetone. Eluting mixture, M; 55% yield; R_f 0.40. ^{1}H NMR ($CDCl_3$): δ 1.24 (d, J = 6.53 Hz, 3H, CH_3), 2.61 (s br, 1H, NH, exchangeable with D_2O), 2.90–3.13 (m, 2H, CH_2NH), 3.18–3.35 (m, 1H, C*H*CH_3), 3.70–3.96 (m, 10H, OCH_3 and C$H_2OC_6H_4$), 3.98–4.09 (m, 1H, C$H_2OC_6H_4$), 4.17 (t, J = 5.27 Hz, 2H, C$H_2OC_6H_3$), 6.56 (d, J = 8.52 Hz, 2H, C_6H_3), 6.82–7.07 (m, 5H, C_6H_3 and C_6H_4). Anal. ($C_{20}H_{27}NO_5$) C, H, N.

***N*-[2-(2-Methoxyphenoxy)ethyl]-*N*-(1-methyl-2-phenoxyethyl)amine (39).** Prepared from 2-(2-methoxyphenoxy)-1-ethanamine and 1-phenoxyacetone. Eluting mixture, N; 70% yield; R_f 0.47. ^{1}H NMR ($CDCl_3$): δ 1.23 (d, J = 6.67 Hz, 3H, CH_3), 2.36 (s br, 1H, NH, exchangeable with D_2O), 2.98–3.29 (m, 3H, CH_2N and C*H*CH_3), 3.86 (s, 3H, OCH_3), 3.87–3.93 (m, 2H, CHCH_2), 4.07–4.25 (m, 2H, C$H_2OC_6H_4$), 6.83–7.02 (m, 7H, C_6H_4 and C_6H_5), 7.22–7.34 (m, 2H, C_6H_4). Anal. ($C_{18}H_{23}NO_3$) C, H, N.

***N*-[2-(2,6-Dimethoxyphenoxy)ethyl]-*N*-(1-methyl-2-phenoxyethyl)amine (40).** Prepared from 2-(2,6-dimethoxyphenoxy)-1-ethanamine and 1-phenoxyacetone. Eluting mixture, N; 66% yield; R_f 0.30. ^{1}H NMR ($CDCl_3$): δ 1.24 (d, J = 6.27 Hz, 3H, CH_3), 2.58 (s br, 1H, NH, exchangeable with D_2O), 2.88–3.09 (m, 2H, CH_2N), 3.11–3.27 (m, 1H, C*H*CH_3), 3.82 (s, 3H, OCH_3), 3.84 (s,

3H, OCH_3), 3.87–3.96 (m, 2H, $CHCH_2$), 4.04–4.28 (m, 2H, $CH_2OC_6H_3$), 6.62–6.68 (m, 2H, C_6H_3), 6.80–7.04 (m, 4H, C_6H_3 and C_6H_5), 7.22–7.34 (m, 2H, C_6H_5). Anal. ($C_{19}H_{25}NO_4$) C, H, N.

N-Benzyl-N-[2-(2-ethoxyphenoxy)-1-methylethyl]amine (42). Prepared from benzylamine and 1-(2-ethoxyphenoxy)acetone. Eluting mixture, I; 64% yield; R_f 0.54 (lit.:[18] $bp_{0.1}$ 152–155 °C];. ^1H NMR ($CDCl_3$): δ 1.21 (d, $J = 6.47$ Hz, 3H, $CHCH_3$), 1.47 (t, $J = 7.00$ Hz, 3H, OCH_2CH_3), 2.97 (s br, 1H, NH, exchangeable with D_2O), 3.17–3.35 (m, 1H, $CHCH_3$), 3.80–4.11 (m, 6H, CH_2Ar, CH_2OAr and CH_3CH_2), 6.83–6.98 (m, 4H, C_6H_4), 7.22–7.47 (m, 5H, C_6H_5). Hydrochloride salt, mp 115–118 °C (AcOEt). Anal. ($C_{18}H_{24}ClNO_2$) C, H, N.

N-Benzyl-N-[2-(2-isopropoxyphenoxy)-1-methylethyl]-amine (43). Prepared from benzylamine and 1-(2-isopropoxyphenoxy)acetone. Eluting mixture, I; 64% yield; R_f 0.58. ^1H NMR ($CDCl_3$): δ 1.21 (d, $J = 6.52$ Hz, 3H, $CHCH_3$), 1.24–1.40 (m, 6H, $CH(CH_3)_2$), 2.74 (s br, 1H, NH, exchangeable with D_2O), 3.14–3.32 (m, 1H, $CHCH_3$), 3.80–4.05 (m, 4H, CH_2Ar and CH_2OAr), 4.37–4.56 (m, 1H, $CH(CH_3)_2$), 6.85–6.94 (m, 4H, C_6H_4), 7.19–7.42 (m, 5H, C_6H_5). Hydrochloride salt, mp 128–130 °C (AcOEt). Anal. ($C_{19}H_{26}ClNO_2$) C, H, N.

N-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)-N-[2-(2,6-dimethoxyphenoxy)-1-methylethyl]amine (44). Prepared from 2,3-dihydro-1,4-benzodioxin-2-ylmethanamine and 1-(2,6-dimethoxyphenoxy)acetone. Eluting mixture, M; 68% yield; R_f 0.38. ^1H NMR ($CDCl_3$): δ 1.05–1.16 (m, 3H, $CHCH_3$), 2.18 (s, br, 1H, NH exchangeable with D_2O), 2.86–3.12 (m, 3H, CH_2N and $CHCH_3$), 3.58–3.75 (m, 1H, CH_2OAr), 3.84 (s, 6H, OCH_3), 4.01–4.15 (m, 2H, CH_2OAr and H_3 benzodioxin), 4.25–4.39 (m, 2H, H_3 and H_2 benzodioxin), 6.58 (m, 2H, C_6H_3), 6.81–6.93 (m, 4H, C_6H_4), 6.96–7.03 (m, 1H, C_6H_3). Hydrochloride salt, mp 154–156 °C (AcOEt/cyclohexane) [lit.:[20] mp 199–201 °C]. Anal. ($C_{20}H_{26}ClNO_5$) C, H, N.

Synthesis of N-benzyl-N-(2-phenoxyethyl)amine (41) and N-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-N-(2-phenoxyethyl)amine (45). Borane-methyl sulfide 10 M (16.8 g, 221 mmol) in dry ethylene glycol dimethyl ether (20 mL) was added dropwise in 15 min at rt and under nitrogen to a stirred solution of *N*1-benzyl-2-phenoxyacetamide (**46**) (5.3 g, 22.1 mmol) dissolved in dry ethylene glycol dimethyl ether (110 mL). Then the mixture was heated at 80 °C for 16 h. After cooling, the excess of diborane was destroyed at 0 °C by cautiously adding methanol (35 mL), followed by HCl (gas), and successive warming at 80 °C for 2.5 h. The solvents were distilled at reduced pressure, and the residue collected for three consecutive times with methanol (50 mL for time) distilling off every time at reduced pressure. The obtained hydrochloride salt was purified by crystallization with MeOH/*i*-PrOH. The pure free base **41** was obtained as oil from the salt by displacement with 2 N NaOH and extraction with ether: 2.48 g, 50% yield, R_f (eluent mixture Q) 0.53 [lit.:[21] $bp_{0.3}$ 150–154 °C]. ^1H NMR ($CDCl_3$): δ 1.60 (s br, 1H, NH exchangeable with D_2O), 3.13 (t, $J = 6.00$ Hz, 2H, CH_2CH_2OAr), 4.14 (s, 2H, CH_2Ar), 4.29 (t, $J = 6.00$ Hz, 2H, CH_2OAr), 6.90–6.98 (m, 2H, OAr), 7.20–7.28 (m, 3H, OAr), 7.34–7.45 (m, 3H, Ar), 7.55–7.61 (m, 2H, Ar). Hydrochloride salt, mp 190–191 °C (MeOH/*i*-PrOH). Anal. ($C_{15}H_{18}ClNO$) C, H, N.

Similarly, amine **45** was prepared as an oil starting from *N*1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-2-phenoxyacetamide (**47**): 73% yield, R_f (eluent mixture Q) 0.49. Hydrochloride salt, mp 222–224 °C (MeOH/*i*-PrOH) [lit.:[22] 222–223 °C]. ^1H NMR (DMSO-d_6): δ 3.21–3.53 (m, 4H, CH_2NCH_2), 4.02–4.13 (m, 1H, H_3 benzodioxin), 4.26–4.43 (m, 3H, CH_2OAr and H_3 benzodioxin), 4.69–4.77 (m, 1H, H_2 benzodioxin), 6.81–7.06 (m, 7H, C_6H_4 and C_6H_5), 7.27–7.39 (m, 2H, C_6H_5), 9.58 (s br, 2H, NH_2^+ exchangeable with D_2O). Anal. ($C_{17}H_{20}ClNO_3$) C, H, N.

Synthesis of N1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-2-phenoxyacetamide (47). To a solution of phenoxyacetic acid (6 g, 39.4 mmol) in dry dichloromethane (135 mL) was added triethylamine (4.19 g, 41.4 mmol); then the solution was cooled at 0 °C, stirred and added, dropwise, with isobutyl chloroformate (5.65 g, 41.4 mmol). After 20 min, a solution of (2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)amine (6.54 g, 39.4 mmol) in dry dichloromethane (15 mL) was slowly added over 30 min to the cooled and stirred mixture, which was brought to room temperature and stirred for a further 20 h. Then the mixture was washed consecutively with 2 N NaOH, 2 N HCl and H_2O. After drying, the organic solvent was evaporated to give a residue that was purified by column chromatography (eluting mixture D) and crystallized from *i*-PrOH: 6.43 g (55% yield), R_f 0.26, mp 89–90 °C. ^1H NMR ($CDCl_3$): δ 3.52–3.66 (m, 1H, $CHCH_2$), 3.71–3.83 (m, 1H, $CHCH_2$), 3.90–3.98 (m, 1H, H_3 benzodioxin), 4.26–4.36 (m, 2H, H_2 and H_3 benzodioxin), 4.62 (s, 2H, CH_2OAr), 6.82–6.93 (m, 6H, C_6H_5 and C_6H_4), 7.01–7.10 (m, 2H, C_6H_5 and NH), 7.27–7.35 (m, 2H, C_6H_5). Anal. ($C_{17}H_{17}NO_4$) C, H, N.

General procedure for the synthesis of aminoalcohols 19–32. A mixture of the proper amine (1 mmol), bromoethanol (1.1 mmol), and dry K_2CO_3 (2 mmol) in EtOH (10 mL) was heated in a sealed glass tube at 110 °C for 72 h, then filtered and evaporated. The residue was purified by column chromatography with the appropriate eluting mixture to give aminoalcohols **19–32** as oils.

2-Benzyl[2-(3-methoxyphenoxy)-1-methylethyl]amino-1-ethanol (19). Prepared from amine **33**; eluting mixture G; 36% yield; R_f 0.35; MS *m*/*z* 315 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.14 (d, $J = 6.74$ Hz, 3H, CH_3), 2.63–3.15 (m, 3H, CH_2CH_2OH and OH exchangeable with D_2O), 3.21–3.72 (m, 4H, CH_2OH, $CHCH_3$, CH_2Ar), 3.76–4.08 (m, 6H, OCH_3, CH_2Ar and CH_2OAr), 6.42–6.53 (m, 3H, OAr), 7.10–7.42 (m, 6H, OAr and Ar).

2-Benzyl[2-(4-methoxyphenoxy)-1-methylethyl]amino-1-ethanol (20). Prepared from amine **34**; eluting mixture G; 32% yield; R_f 0.34; MS *m*/*z* 315 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.12 (d, $J = 6.74$ Hz, 3H, CH_3), 2.63–2.93 (m, 2H, CH_2CH_2OH), 3.06–3.72 (m, 5H, CH_2OH, $CHCH_3$,

CH_2Ar and OH exchangeable with D_2O), 3.75–4.00 (m, 6H, OCH_3, CH_2Ar and CH_2OAr), 6.78–6.83 (m, 3H, OAr), 7.20–7.37 (m, 6H, OAr and Ar).

2 - Benzyl[2 - (2,6 - dimethoxyphenoxy) - 1 - methylethyl]-amino-1-ethanol (21). Prepared from amine **35**; eluting mixture L; 32% yield; R_f 0.49; MS m/z 345 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.15 (d, $J = 6.50$ Hz, 3H, CH_3), 2.66–2.90 (m, 2H, CH_2CH_2OH), 3.31–3.58 (m, 3H, CH_2OH, $CHCH_3$), 3.59–3.64 (d, $J = 13.6$ Hz, 1H, CH_2Ar), 3.82 (s, 6H, OCH_3), 3.84–3.89 (d, $J = 13.6$ Hz, 1H, CH_2Ar), 3.95–4.10 (m, 2H, CH_2OAr), 6.58 (d, $J = 7.50$ Hz, 2H, OAr), 6.98 (t, $J = 7.50$ Hz, 1H, OAr), 7.20–7.40 (m, 5H, Ar).

2-Benzyl[2-(2,6-dimethoxyphenoxy)ethyl]amino-1-ethanol (22). Prepared from amine **36**; eluting mixture E; 58% yield; R_f 0.48; MS m/z 331 $[M]^+$; ^1H NMR ($CDCl_3$): δ 2.77 (t, $J = 5.62$ Hz, 2H, CH_2CH_2OH), 2.95 (t, $J = 5.74$ Hz, 2H, CH_2CH_2OAr), 3.60 (t, $J = 5.62$ Hz, 2H, CH_2OH), 3.77 (s, 2H, CH_2Ar), 3.84 (s, 6H, OCH_3), 4.06 (t, $J = 5.75$ Hz, 2H, CH_2OAr), 6.58 (d, $J = 8.27$ Hz, 2H, C_6H_3), 6.99 (t, $J = 8.27$ Hz, 1H, C_6H_3), 7.23–7.40 (m, 5H, C_6H_5).

2-[2-(2-Methoxyphenoxy)ethyl][2-(2-methoxyphenoxy)-1-methylethyl]amino-1-ethanol (23). Prepared from amine **37**; eluting mixture I; 50% yield; R_f 0.34; MS m/z 375 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.15 (d, $J = 6.79$ Hz, 3H, CH_3); 1.68 (s br, 1H, OH exchangeable with D_2O), 2.67–3.20 (m, 4H, CH_2NCH_2); 3.22–3.45 (m, 1H, $CHCH_3$), 3.46–3.70 (m, 2H, CH_2OH), 3.83 (s, 6H, OCH_3), 3.86–4.13 (m, 4H, CH_2OAr), 6.78–7.02 (m, 8H, Ar).

2 - [2 - (2,6 - Dimethoxyphenoxy)ethyl][2 - (2 - methoxyphenoxy)-1-methylethyl]amino-1-ethanol (24). Prepared from amine **38**; eluting mixture I; 26% yield; R_f 0.25; MS m/z 405 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.18 (d, $J = 7.08$ Hz, 3H, CH_3), 1.67 (s br, 1H, OH exchangeable with D_2O), 2.70–3.13 (m, 4H, CH_2NCH_2), 3.25–3.45 (m, 1H, $CHCH_3$), 3.50–3.69 (m, 2H, CH_2OH), 3.82 (s, 9H, OCH_3), 3.87–4.16 (m, 4H, CH_2OAr), 6.56 (d, $J = 8.33$ Hz, 2H, C_6H_3), 6.83–7.03 (m, 5H, C_6H_3 and C_6H_4).

2-[2-(2-Methoxyphenoxy)ethyl](1-methyl-2-phenoxyethyl)-amino]-1-ethanol (25). Prepared from amine **39**; eluting mixture I; 50% yield; R_f 0.50; MS m/z 345 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.17 (d, $J = 6.67$ Hz, 3H, CH_3), 1.64 (s br, 1H, OH exchangeable with D_2O), 2.68–2.89 (m, 2H, CH_2CH_2OH), 2.92–3.20 (m, 2H, CH_2CH_2OAr), 3.22–3.47 (m, 1H, $CHCH_3$), 3.50–3.70 (m, 2H, CH_2OH), 3.83 (s, 3H, OCH_3), 3.92–4.13 (m, 4H, CH_2OAr), 6.82–7.03 (m, 7H, C_6H_4 and C_6H_5), 7.22–7.31 (m, 2H, C_6H_5).

2-[2-(2,6-Dimethoxyphenoxy)ethyl](1-methyl-2-phenoxyethyl)amino]-1-ethanol (26). Prepared from amine **40**; eluting mixture I; 49% yield; R_f 0.43; MS m/z 375 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.18 (d, $J = 6.79$ Hz, 3H, CH_3), 1.70 (s br, 1H, OH exchangeable with D_2O), 2.68–3.13 (m, 4H, CH_2NCH_2), 3.18–3.41 (m, 1H, $CHCH_3$), 3.47–3.68 (m, 2H, CH_2OH), 3.82 (s, 6H, OCH_3), 3.90–4.15 (m, 4H, CH_2OAr), 6.56 (d, $J = 8.49$ Hz, 2H, C_6H_3), 6.83–7.03 (m, 4H, C_6H_3 and C_6H_5), 7.22–7.31 (m, 2H, C_6H_5).

2-[Benzyl(2-phenoxyethyl)amino]-1-ethanol (27). Prepared from amine **41**; eluting mixture F; 61% yield; R_f 0.41; MS m/z 271 $[M]^+$ [lit.:[16] bp_6 207–213 °C]; ^1H NMR ($CDCl_3$): δ 1.62 (s br, 1H, OH exchangeable with D_2O), 2.80 (t, $J = 5.34$ Hz, 2H, CH_2CH_2OH), 2.97 (t, $J = 5.85$ Hz, 2H, CH_2CH_2OAr), 3.59 (t, $J = 5.38$ Hz, 2H, CH_2OH), 3.78 (s, 2H, CH_2Ar), 4.01 (t, $J = 5.85$ Hz, 2H, CH_2OAr), 6.85–7.00 (m, 3H, OAr), 7.22–7.31 (m, 7H, Ar and OAr).

2-Benzyl[2-(2-ethoxyphenoxy)-1-methylethyl]amino-1-ethanol (28). Prepared from amine **42**; eluting mixture G; 22% yield; R_f 0.35; MS m/z 329 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.02–1.28 (m, 3H, CH_3CH), 1.47 (t, $J = 7.00$ Hz, 3H, CH_3CH_2), 2.58–2.97 (m, 2H, CH_2CH_2OH), 3.18–3.77 (m, 5H, CH_2OH, $CHCH_3$, CH_2Ar and OH exchangeable with D_2O), 3.79–4.16 (m, 5H, CH_2Ar, CH_3CH_2O and CH_2OAr), 6.77–6.99 (m, 3H, OAr), 7.12–7.50 (m, 6H, OAr and Ar).

2-Benzyl[2-(2-isopropoxyphenoxy)-1-methylethyl]amino-1-ethanol (29). Prepared from amine **43**; eluting mixture G; 40% yield; R_f 0.40; MS m/z 343 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.12 (d, $J = 6.88$ Hz, 3H, $CHCH_3$), 1.24–1.53 (m, 6H, $(CH_3)_2CH$), 2.60–2.98 (m, 2H, CH_2CH_2OH), 3.19–3.77 (m, 5H, CH_2OH, $CHCH_3$, CH_2Ar and OH exchangeable with D_2O), 3.82–4.14 (m, 3H, CH_2Ar and CH_2OAr), 4.44–4.66 (m, 1H, $CH(CH_3)_2$), 6.76–6.98 (m, 3H, OAr), 7.12–7.50 (m, 6H, OAr and Ar).

2-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)[2-(2,6-dimethoxyphenoxy)-1-methylethyl]amino-1-ethanol (diastereomers 30a and 30b). Prepared from amine **44** in a 34% global yield through gravity column chromatography eluting with mixture H.

30a: R_f 0.22; HPLC, R_t 3.95 min (Beckmann Ultrasphere ODS column 5 μm, 250×4.6 mm i.d., eluting mixture *i*-PrOH/*n*-hexane 2:8, flow rate 2 mL/min); MS m/z 403 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.11 (d, $J = 6.38$ Hz, 3H, $CHCH_3$), 1.62 (s br, 1H, OH exchangeable with D_2O), 2.68–3.02 (m, 4H, CH_2NCH_2), 3.21–3.36 (m, 1H, $CHCH_3$), 3.51–3.67 (m, 2H, CH_2OH), 3.82 (s, 6H, OCH_3), 3.85–3.98 (m, 2H, $CH_2OC_6H_3$), 4.08–4.16 (m, 1H, H_3 benzodioxin), 4.20–4.33 (m, 1H, H_2 benzodioxin), 4.38–4.45 (m, 1H, H_3 benzodioxin), 6.56 (d, $J = 7.02$ Hz, 2H, C_6H_3), 6.77–6.90 (m, 4H, C_6H_4), 6.96 (t, $J = 7.02$ Hz, 1H, C_6H_3).

30b: R_f 0.20; HPLC, R_t 7.74 min (Beckmann Ultrasphere ODS column 5 μm, 250×4.6 mm i.d., eluting mixture *i*-PrOH/*n*-hexane 2:8, flow rate 2 mL/min); MS m/z 403 $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.05 (d, $J = 7.08$ Hz, 3H, $CHCH_3$), 1.60 (s br, 1H, OH exchangeable with D_2O), 2.64–2.88 (m, 4H, CH_2NCH_2), 3.25–3.34 (m, 1H, $CHCH_3$), 3.49–3.69 (m, 2H, CH_2OH), 3.78–4.01 (m, 8H, $CH_2OC_6H_3$ and OCH_3), 4.04–4.12 (m, 1H, H_3 benzodioxin), 4.22–4.31 (m, 1H, H_2 benzodioxin), 4.38–4.45 (m, 1H, H_3 benzodioxin), 6.57 (d, $J = 7.46$ Hz, 2H, C_6H_3), 6.78–6.89 (m, 4H, C_6H_4), 6.96 (t, $J = 7.46$ Hz, 1H, C_6H_3).

2-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)[2-(2,6-dimethoxyphenoxy)ethyl]amino-1-ethanol (31). Prepared from amine **2**; eluting mixture M; 43% yield; R_f 0.42; MS *m*/*z* 371 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 2.60–3.13 (m, 6H, CH_2N), 3.39–4.56 (m, 14H, OCH_3, $CH_2OC_6H_3$, CH_2OH, H_2 and H_3 benzodioxin), 6.55 (d, J = 7.80 Hz, 2H, C_6H_3), 6.70–7.16 (m, 5H, C_6H_3 and C_6H_4).

2-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)[2-(2-methoxyphenoxy)ethyl]amino-1-ethanol (32). Prepared from amine **45**; eluting mixture F; 51% yield; R_f 0.49; MS *m*/*z* 329 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 1.62 (s br, 1H, OH exchangeable with D_2O), 2.84–3.13 (m, 6H, NCH_2), 3.50–3.68 (m, 2H, CH_2OH), 3.99–4.11 (m, 3H, H_3 benzodioxin and CH_2OAr), 4.25–4.37 (m, 2H, H_2 and H_3 benzodioxin), 6.80–6.99 (m, 7H, C_6H_3 and C_6H_4), 7.26–7.32 (m, 1H, C_6H_3).

General procedure for the synthesis of β-chloroethylamine hydrochlorides 5–18. HCl (g) was slowly bubbled for 15 min into a stirred and cooled (0 °C) solution of the aminoalcohol (2 mmol) in dry benzene (50 mL); then $SOCl_2$ (2.5 mmol) in dry benzene (5 mL) was added dropwise and the solution refluxed for 8 h. The reaction mixture was distilled to remove both solvent and $SOCl_2$ excess, affording a residue that was purified by column chromatography. Excepting **17** that was obtained as oxalate, all other compounds were transformed into the hydrochloride salt and crystallized.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-[2-(3-methoxyphenoxy)-1-methylethyl]amine hydrochloride (5).** Prepared from aminoalcohol **19**; mp 131–133 °C (EtOAc); R_f 0.35 (eluting mixture A); 24% yield; ^{1}H NMR ($CDCl_3$): δ 1.47–1.77 (m, 3H, CH_3), 3.20–3.64 (m, 2H, CH_2Cl), 3.65–4.03 (m, 6H, $CHCH_3$, CH_2CH_2Cl and OCH_3), 4.05–4.82 (m, 4H, CH_2Ar and CH_2OAr), 6.40–6.55 (m, 4H, OAr), 7.33–7.51 (m, 3H, Ar), 7.71–7.90 (m, 2H, Ar), 13.05 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{19}H_{25}Cl_2NO_2$) C, H, N.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-[2-(4-methoxyphenoxy)-1-methylethyl]amine hydrochloride (6).** Prepared from aminoalcohol **20**; mp 148–150 °C (EtOAc), [lit.:[15] mp 152–153 °C (EtOH/Et_2O)]; R_f 0.30 (eluting mixture A); 15% yield; ^{1}H NMR ($CDCl_3$): δ 1.42–1.80 (m, 3H, CH_3), 3.16–3.62 (m, 2H, CH_2Cl), 3.70–4.04 (m, 6H, $CHCH_3$, CH_2CH_2Cl and OCH_3), 4.05–4.83 (m, 4H, CH_2Ar and CH_2OAr), 6.72–6.97 (m, 4H, OAr), 7.38–7.57 (m, 3H, Ar), 7.72–7.90 (m, 2H, Ar), 13.03 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{19}H_{25}Cl_2NO_2{\cdot}0.25H_2O$) C, H, N.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-[2-(2,6-dimethoxyphenoxy)-ethyl]amine hydrochloride (7).** Prepared from aminoalcohol **21**; mp 167–169 °C (*i*-PrOH); R_f 0.45 (eluting mixture B); 18% yield; ^{1}H NMR ($CDCl_3$): δ 1.58–1.75 (m, 3H, CH_3), 3.80–4.55 (m, 14H, OCH_3, CH_2Ar, CH_2CH_2Cl and CH_2OAr), 4.62–4.80 (m, 1H, $CHCH_3$), 6.58–6.62 (m, 2H, OAr), 7.00–7.10 (m, 1H, OAr), 7.40–7.95 (m, 5H, Ar), 12.45 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{20}H_{27}Cl_2NO_3$) C, H, N.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-[2-(2,6-dimethoxyphenoxy)-ethyl]amine hydrochloride (8).** Prepared from aminoalcohol **22**; mp 156–157 °C (*i*-PrOH); R_f 0.40 (eluting mixture B); 12% yield; ^{1}H NMR ($CDCl_3$): δ 3.40–3.55 (m, 2H, CH_2Cl), 3.57–3.97 (m, 8H, OCH_3 and NCH_2CH_2OAr), 4.08–4.20 (m, 2H, CH_2OAr), 4.33–4.50 (m, 2H, NCH_2CH_2Cl), 4.52–4.70 (m, 2H, NCH_2Ar), 6.60 (d, J = 8.82 Hz, 2H, OAr), 7.07 (t, J = 8.82 Hz, 1H, OAr), 7.42–7.53 (m, 3H, Ar), 7.72–7.83 (m, 2H, Ar), 13.08 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{19}H_{25}Cl_2NO_3$) C, H, N.

***N*-(2-Chloroethyl)-*N*-[2-(2-methoxyphenoxy)ethyl]-*N*-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (9).** Prepared from aminoalcohol **23**; mp 97–99 °C (AcOEt/*n*-hexane); R_f 0.33 (eluting mixture B); 10% yield; ^{1}H NMR ($CDCl_3$): δ 1.56–1.83 (m, 3H, CH_3), 3.56–3.98 (m, 10H, OCH_3 and CH_2CH_2Cl), 4.03–4.39 (m, 4H, CH_2OAr), 4.50–4.78 (m, 3H, CH_2CH_2OAr and $CHCH_3$), 6.80–7.10 (m, 8H, Ar), 13.06 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{21}H_{29}Cl_2NO_4{\cdot}0.25H_2O$) C, H, N.

***N*-(2-Chloroethyl)-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-*N*-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (10).** Prepared from aminoalcohol **24**; mp 114–116 °C (AcOEt/*n*-hexane); R_f 0.33 (eluting mixture B); 12% yield; ^{1}H NMR ($CDCl_3$): δ 1.60–1.84 (m, 3H, CH_3), 3.50–4.10 (m, 13H, OCH_3 and CH_2CH_2Cl), 4.11–4.73 (m, 7H, CH_2CH_2OAr and $CHCH_2OAr$), 6.55 (d, J = 7.60 Hz, 2H, C_6H_3), 6.91–7.12 (m, 5H, C_6H_3 and C_6H_4), 12.82 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{22}H_{31}Cl_2NO_5{\cdot}0.25H_2O$) C, H, N.

***N*-(2-Chloroethyl)-*N*-[2-(2-methoxyphenoxy)ethyl]-*N*-(1-methyl-2-phenoxyethyl)amine hydrochloride (11).** Prepared from aminoalcohol **25**; mp 89–91 °C (AcOEt/*n*-hexane); R_f 0.40 (eluting mixture B); 11% yield; ^{1}H NMR ($CDCl_3$): δ 1.58–1.86 (m, 3H, CH_3), 3.56–3.85 (m, 7H, OCH_3 and CH_2CH_2Cl), 4.05–4.42 (m, 4H, CH_2OAr), 4.55–4.85 (m, 3H, CH_2CH_2OAr and $CHCH_3$), 6.83–7.12 (m, 7H, C_6H_5 and C_6H_4), 7.23–7.38 (m, 2H, C_6H_5), 13.21 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{20}H_{27}Cl_2NO_3{\cdot}0.25H_2O$) C, H, N.

***N*-(2-Chloroethyl)-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-*N*-(1-methyl-2-phenoxyethyl) amine hydrochloride (12).** Prepared from aminoalcohol **26**; mp 114–116 °C (AcOEt/Et_2O); R_f 0.37 (eluting mixture B); 10% yield; ^{1}H NMR ($CDCl_3$): δ 1.52–1.83 (m, 3H, CH_3), 3.50–3.74 (m, 2H, CH_2Cl), 3.83 (s, 6H, OCH_3), 3.90–4.10 (m, 2H, CH_2CH_2Cl), 4.16–4.80 (m, 7H, CH_2CH_2OAr and $CHCH_2OAr$), 6.58 (d, J = 8.42 Hz, 2H, C_6H_3), 6.88–7.10 (m, 4H, C_6H_3 and C_6H_5), 7.23–7.38 (m, 2H, C_6H_5), 13.02 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{21}H_{29}Cl_2NO_4$) C, H, N.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-(2-phenoxyethyl)amine hydrochloride (13).** Prepared from aminoalcohol **27**; mp 99–101 °C (*i*-PrOH), [lit.:[16] mp 106–108 °C (EtOH/Et_2O)]; R_f 0.53 (eluting mixture B); 22% yield; ^{1}H NMR ($CDCl_3$): δ 3.40–3.65 (m, 4H, CH_2CH_2Cl), 4.00–4.20 (m, 2H, CH_2OAr), 4.35–4.48 (m, 2H, CH_2CH_2OAr), 4.50–4.72 (m, 2H, CH_2Ar), 6.90–6.96 (m, 2H, OAr), 6.98–7.05

(m, 1H, OAr), 7.28–7.37 (m, 2H, OAr), 7.40–7.55 (m, 3H, Ar), 7.70–7.80 (m, 2H, Ar), 13.47 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{17}H_{21}Cl_2NO$) C, H, N.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-[2-(2-ethoxyphenoxy)-1-methylethyl]amine hydrochloride (14).** Prepared from aminoalcohol **28**; mp 106–108 °C (AcOEt/Et_2O); R_f 0.35 (eluting mixture A); 16% yield; ^1H NMR ($CDCl_3$): δ 1.30–1.50 (m, 3H, CH_2CH_3), 1.55–1.86 (m, 3H, $CHCH_3$), 3.18–3.79 (m, 2H, CH_2Cl), 3.80–4.88 (m, 9H, $CHCH_3$, CH_2CH_2Cl, CH_3CH_2, CH_2Ar and CH_2OAr), 6.80–7.15 (m, 4H, OAr), 7.40–8.10 (m, 5H, Ar), 12.72 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{20}H_{27}Cl_2NO_2$) C, H, N.

***N*-Benzyl-*N*-(2-chloroethyl)-*N*-[2-(2-isopropoxyphenoxy)-1-methylethyl]amine hydrochloride (15).** Prepared from aminoalcohol **29**; mp 124–126 °C (AcOEt/Et_2O); R_f 0.33 (eluting mixture A); 60% yield; ^1H NMR ($CDCl_3$): δ 1.28–1.43 (m, 6H, $CH(CH_3)_2$), 1.50–1.85 (m, 3H, CH_3CH), 3.20–3.78 (m, 2H, CH_2Cl), 3.81–4.86 (m, 8H, $CHCH_3$, $CH(CH_3)_2$, CH_2CH_2Cl, CH_2Ar and CH_2OAr), 6.80–7.10 (m, 4H, OAr), 7.36–7.57 (m, 3H, Ar), 7.73–8.04 (m, 2H, Ar), 12.72 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{21}H_{29}Cl_2NO_2 \cdot 0.25H_2O$) C, H, N.

***N*-(2-Chloroethyl)-*N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-[2-(2,6-dimethoxy phenoxy)-1-methylethyl]amine hydrochloride (diastereomers 16a and 16b).** Prepared from aminoalcohols **30a** and **30b**, respectively.

16a: mp 162–163 °C (*i*-PrOH); R_f 0.35 (eluting mixture B); 27% yield; ^1H NMR ($CDCl_3$): δ 1.52–1.74 (m, 3H, CH_3), 3.32–3.55 (m, 1H, NCH_2 benzodioxin), 3.60–3.98 (m, 9H, OCH_3, NCH_2 benzodioxin, CH_2Cl), 4.00–4.40 (m, 6H, CH_2CH_2Cl, $CHCH_3$, CH_2OAr, H_3 benzodioxin), 4.47 (m, 1H, CH_2OAr), 5.27–5.62 (m, 1H, H_2 benzodioxin), 6.56 (d, $J = 9.58$ Hz, 2H, C_6H_3), 6.83–6.98 (m, 4H, C_6H_4), 7.03 (t, $J = 9.58$ Hz, 1H, C_6H_3), 13.15 (s, br, 1H, NH exchangeable with D_2O). Anal. ($C_{22}H_{29}Cl_2NO_5 \cdot 0.5C_3H_8O$) C, H, N.

16b: mp 102–104 °C (*i*-PrOH); R_f 0.38 (eluting mixture B); 31% yield; ^1H NMR ($CDCl_3$): δ 1.15–1.92 (m, 3H, CH_3), 3.30–4.60 (m, 17H, OCH_3, CH_2CH_2Cl, $CHCH_3$, CH_2OAr, NCH_2 benzodioxin, H_3 benzodioxin), 5.31–5.65 (m, 1H, H_2 benzodioxin), 6.53 (d, $J = 9.56$ Hz, 2H, C_6H_3), 6.68–7.10 (m, 5H, C_6H_3 and C_6H_4), 13.05 (s, br, 1H, NH exchangeable with D_2O). Anal. ($C_{22}H_{29}Cl_2NO_5 \cdot C_3H_8O$) C, H, N.

***N*-(2-Chloroethyl)-*N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]amine oxalate (17).** Prepared from aminoalcohol **31**; mp 129–132 °C (*i*-PrOH); R_f 0.45 (eluting mixture C); 24% yield; ^1H NMR ($CDCl_3$): δ 3.45–3.96 (m, 14H, OCH_3, CH_2CH_2Cl, CH_2CH_2OAr, NCH_2 benzodioxin), 4.00–4.12 (m, 1H, H_3 benzodioxin), 4.20–4.38 (m, 3H, CH_2OAr and H_3 benzodioxin), 4.81–4.95 (m, 1H, H_2 benzodioxin), 6.57 (d, $J = 8.78$ Hz, 2H, C_6H_3), 6.80–6.94 (m, 4H, C_6H_4), 7.04 (t, $J = 8.78$ Hz, 1H, C_6H_3), 13.03 (s, br, 2H, NH and COOH exchangeable with D_2O). Anal. ($C_{23}H_{28}ClNO_9$) C, H, N.

***N*-(2-Chloroethyl)-*N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-(2-phenoxyethyl)amine hydrochloride (18).** Prepared from aminoalcohol **32**; mp 103–105 °C (AcOEt/Et_2O); R_f 0.43 (eluting mixture B); 76% yield; ^1H NMR ($CDCl_3$): δ 3.38–3.52 (m, 1H, NCH_2 benzodioxin), 3.55–3.92 (m, 5H, CH_2CH_2Cl and NCH_2 benzodioxin), 3.98–4.09 (m, 3H, CH_2CH_2OAr and H_3 benzodioxin), 4.22–4.35 (m, 1H, H_3 benzodioxin), 4.42–4.68 (m, 2H, CH_2OAr), 5.20–5.33 (m, 1H, H_2 benzodioxin), 6.80–6.95 (m, 6H, C_6H_4 and C_6H_5), 6.98–7.10 (m, 1H, C_6H_5), 7.26–7.39 (m, 2H, C_6H_5), 13.90 (s br, 1H, NH exchangeable with D_2O). Anal. ($C_{19}H_{23}Cl_2NO_3$) C, H, N.

Measurement of aziridinium ion concentrations

Aziridinium ion concentration of β-chloroethylamines **5**–**18** was calculated by applying the Gill and Rang method.[25] The adopted procedure for **9** is reported as an example.

A mixture of sodium–potassium phosphate buffer (50 mM, pH 7.4, 96 mL) and MeOH (14 mL) was added to a stirred solution (37 °C) of **7** (0.021 g, 0.048 mmol) in methanol (10 mL). After 1 min stirring, 22 aliquots (5 mL) of solution were removed during 34 min, the first 10 every 60 s while the other 12 every 120 s. Each aliquot was poured into glacial acetic acid (1 mL) to stop cyclization and the resulting solution was treated with 0.01 N sodium thiosulfate (1 mL). In the range of 10–20 min, the mixtures were titrated with a 3.30 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.[26]

The obtained new rate constants k_1 and k_2 were used to calculate correct aziridinium ion concentrations that were graphed, as a time function, by a Cricket Graph program.

Functional antagonism in isolated rat vas deferens

Male albino rats (CD, BR 125–150 g, Charles River, Como, Italy) 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 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.[29]

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 a voltage of 10–35 V. The stimulation voltage was fixed throughout the experiments. In the case of α_1-adrenoceptor assays, propranolol hydrochloride (1 μM) and cocaine hydrochloride (10 μM) were present in the Krebs solution throughout the experiments outlined below to block β-adrenoceptors and neuronal uptake mechanisms, respectively.

The α_1-adrenoceptor 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 **5**, **6**, **11**, **13**, and **14** were tested at 5–6 different concentrations and each concentration was investigated 3–7 times, while compounds **7**–**10**, **12**, **15**–**18** were investigated at 7–9 concentrations, each of these being tested 3–10 times. The antagonist potency of compounds at α_1-adrenoceptors was expressed by the negative logarithm of concentration that causes 50% inhibition of agonist action (pIC_{50}).

When assessing the complementary antagonism of the two series of novel β-chloroethylamines, tissues were pre-incubated for 30 min with **8** or **16a** at the concentration producing the plateau in their inhibition curves (0.6 μM and 0.1 μM, respectively). Following 30 min of washings, a third dose–response curve to NA was obtained. Then, after 30 min of equilibration, tissues were incubated for 30 min with increasing concentrations of **16a** (0.03–0.2 μM) or **8** (0.03–1.0 μM). In both cases, following 30 min of washings, a fourth NA dose–response curve was constructed and the total inhibition produced by the two antagonists, given consecutively, that is **8** followed by **16a** in one case and **16a** followed by **8** in the other one, was calculated from the fourth NA dose–response curve relative to the second one, taken as control. Each concentration of antagonist was tested independently 4–9 times. Control experiments were performed with **8** or **16a** alone in order to correct for time-dependent changes in antagonist inhibition during the whole experiment. β-Chloroethylamine **8**, unlike **16a**, showed a $31.6 \pm 4.2\%$ ($n = 8$) loss of inhibition of the maximum response to NA, which was taken into account when calculating the actual decrease in the fourth NA dose–response curve.

The α_2-adrenoceptor blocking activity was assessed on the prostatic portion of the vas deferens by antagonism to clonidine, which inhibits the twitch responses of the field-stimulated vas deferens by acting on the α_2-adrenoceptor.[30,31] 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 a 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-adrenoceptors was expressed by the negative logarithm of concentration that causes 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.

Analytical data of unknown secondary amines **35**–**37**, **39**, **40**, **43**, and amide **47**

Compd	Formula	Calcd (%)			Found (%)		
		C	H	N	C	H	N
35	$C_{18}H_{24}ClNO_3 \cdot 0.5H_2O$	62.32	7.27	4.03	62.39	7.27	3.80
36	$C_{17}H_{22}ClNO_3 \cdot 0.5H_2O$	61.35	6.97	4.20	61.19	7.22	4.04
37	$C_{19}H_{25}NO_4$	68.86	7.60	4.23	68.75	7.29	4.17
39	$C_{18}H_{23}NO_3$	71.73	7.69	4.65	71.50	7.78	4.81
40	$C_{19}H_{25}NO_4$	68.86	7.60	4.23	68.91	7.48	4.39
43	$C_{19}H_{26}ClNO_2$	67.94	7.80	4.17	67.60	8.12	4.11
47	$C_{17}H_{17}NO_4$	68.22	5.72	4.68	68.18	5.83	4.40

Analytical data of β-chloroethylamines salts **5**–**18**

Compd	Formula	Calcd (%)			Found (%)		
		C	H	N	C	H	N
5	$C_{19}H_{25}Cl_2NO_2$	61.63	6.80	3.78	61.46	7.08	3.65
6	$C_{19}H_{25}Cl_2NO_2 \cdot 0.25H_2O$	60.88	6.80	3.78	60.95	6.93	3.60
7	$C_{20}H_{27}Cl_2NO_3$	59.99	6.80	3.50	60.04	7.01	3.51
8	$C_{19}H_{25}Cl_2NO_3$	59.07	6.52	3.63	59.37	6.60	3.63
9	$C_{21}H_{29}Cl_2NO_4 \cdot 0.25H_2O$	58.00	6.84	3.22	58.17	7.17	3.18
10	$C_{22}H_{31}Cl_2NO_5 \cdot 0.25H_2O$	56.84	6.83	3.01	56.80	7.14	2.96
11	$C_{20}H_{27}Cl_2NO_3 \cdot 0.25H_2O$	59.34	6.85	3.34	59.49	7.14	3.34
12	$C_{21}H_{29}Cl_2NO_4$	58.61	7.26	3.25	58.23	7.22	3.14
13	$C_{17}H_{21}Cl_2NO$	62.58	6.49	4.29	62.97	6.60	4.15
14	$C_{20}H_{27}Cl_2NO_2$	62.50	7.08	3.64	62.44	7.27	3.63
15	$C_{21}H_{29}Cl_2NO_2 \cdot 0.25H_2O$	62.61	7.38	3.48	62.79	7.68	3.39
16a	$C_{22}H_{29}Cl_2NO_5 \cdot 0.5C_3H_8O$	57.79	6.81	2.87	58.11	6.60	2.54
16b	$C_{22}H_{29}Cl_2NO_5 \cdot C_3H_8O$	57.91	7.19	2.70	58.27	6.97	2.52
17	$C_{23}H_{28}ClNO_9$	55.48	5.67	2.81	55.26	5.70	2.64
18	$C_{19}H_{23}Cl_2NO_3$	59.38	6.03	3.64	59.57	6.18	3.42

Acknowledgements

This work was supported by a grant from Cofinanziamento Murst 1999.

References

1. Browning, C.; Giles, H.; Martin, G. R. *Br. J. Pharmacol.* **1995**, *116*, 262 P.
2. Ruffolo, R. R., Jr.; Nichols, A. J.; Stadel, J. M.; Hieble, J. P. *Pharmacol. Rev.* **1991**, *43*, 475.
3. Hieble, J. P.; Bondinell, W. E.; Ruffolo, R. R., Jr. *J. Med. Chem.* **1995**, *38*, 3415.
4. Caine, M.; Perlberg, S.; Meretyk, S. *Br. J. Urol.* **1978**, *50*, 551.
5. (a) Hanft, G.; Gross, G.; Beckeringh, J. J.; Korstanje, C. *J. Pharm. Pharmacol.* **1989**, *41*, 714. (b) Oshita, M.; Kigoshi, S.; Muramatsu, I. *Br. J. Pharmacol.* **1991**, *104*, 961.
6. (a) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R., Jr.; Trendelenburg, U. *Pharmacol. Rev.* **1994**, *46*, 121. (b) Ford, A. P. D. W.; Williams, T. J.; Blue, D. R.; Clarke, D. E. *Trends Pharmacol. Sci.* **1994**, *15*, 167. (c) Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R., Jr. *Pharmacol. Rev.* **1995**, *47*, 267.
7. Muramatsu, I.; Ohmura, T.; Kigoshi, S.; Hashimoto, S.; Oshita, M. *Br. J. Pharmacol.* **1990**, *99*, 197.
8. Oshita, M.; Kigoshi, S.; Muramatsu, I. *Br. J. Pharmacol.* **1993**, *108*, 1071.
9. Burt, R. P.; Chapple, C. R.; Marshall, I. *Br. J. Pharmacol.* **1995**, *115*, 467.
10. Marshall, I.; Burt, R. P.; Green, G. M.; Hussain, M. B.; Chapple, C. R. *Br. J. Pharmacol.* **1996**, *119*, 407.
11. Ohmura, T.; Oshita, M.; Kigoshi, S.; Muramatsu, I. *Br. J. Pharmacol.* **1992**, *107*, 697.
12. Aboud, R.; Shafii, M.; Docherty, J. R. *Br. J. Pharmacol.* **1993**, *109*, 80.
13. Giardinà, D.; Crucianelli, M.; Marucci, G.; Paparelli, F.; Melchiorre, C. *Bioorg. Med. Chem.* **1995**, *3*, 85.
14. Giardinà, D.; Crucianelli, M.; Marucci, G.; Angeli, P.; Melchiorre, C.; Antolini, L. *Bioorg. Med. Chem.* **1997**, *5*, 1775.
15. SmithKline & French International Co. Brit. 673,509, June 4, 1952; *Chem. Abst.* **1955**, *49*, 1794.
16. Gump, W. S.; Nikawitz, E. J. *J. Am. Chem. Soc.* **1950**, *72*, 3846.
17. Fujikura, T.; Niigata, K.; Hashimoto, S.; Imai, K.; Takenaka, T. *Chem. Pharm. Bull.* **1982**, *30*, 4092.
18. Ferri, V.; Pallavicini, M.; Piccini, D.; Valoti, E.; Villa, L.; Brunello, N.; Racagni, G. *Il Farmaco* **1988**, *43* (1 Suppl.), 1153.
19. Villa, L.; Valoti, E.; Villa, A. M.; Pallavicini, M.; Ferri, V.; Iuliano, E.; Brunello, N. *Il Farmaco* **1994**, *49*, 587.
20. Copp, F. C. (Wellcome Foundation Ltd.) Ger. 1,117,600, Nov 23, 1961; *Chem. Abst.* **1962**, *56*, 14166a.
21. Ward Blenkinsop & Co. Ltd. Neth. Appl. 6,407,012, Dec 21, 1964; *Chem. Abst.* **1965**, *62*, 16260e.
22. Kushner, S.; Cassell, R. I.; Morton, J., II; Williams, J. H. *J. Org. Chem.* **1951**, *16*, 1283.
23. Green, P. N.; Shapero, M.; Wilson, C. *J. Med. Chem.* **1969**, *12*, 326.
24. Henkel, J. G.; Portoghese, P. S.; Miller, J. W.; Lewis, P. *J. Med. Chem.* **1976**, *19*, 6.
25. Gill, E. W.; Rang, H. P. *Mol. Pharmacol.* **1966**, *2*, 284.
26. Ebert, K.; Ederer, H.; Isenhour, J. L. In *Computer Applications in Chemistry*; VCH: Weinheim, 1989; Vol. 11, p 343–400.
27. McGrath, J. C. *J. Physiol. (London)* **1978**, *283*, 23.
28. Brown, C. M.; McGrath, J. C.; Summers, R. J. *Br. J. Pharmacol.* **1979**, *66*, 553.
29. Furchgott, R. F. In *Cathecolamines (Handbook of Experimental Pharmacology)*; Blanschko, H., Muscholl, E., Eds; Springer: Berlin, 1972; Vol. 33, p 283–335.
30. Drew, G. M. *Eur. J. Pharmacol.* **1977**, *42*, 123.
31. Doxey, J. C.; Smith, C. F. C.; Walker, J. M. *Br. J. Pharmacol.* **1977**, *60*, 91.