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Synthesis and α-Adrenoceptor Blocking Activity of the Enantiomers of Benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine Hydrochloride

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Abstract—The enantiomers of benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (**1**, CM18) were synthesized and studied pharmacologically for their irreversible antagonism at rat vas deferens α-adrenoceptors. In addition, assignment of the absolute configuration of the two enantiomers of **1** was made by X-ray crystallographic analysis performed on the intermediate amine (+)-**2** hydrochloride. The enantiomer (*R*)-(+)-**1** [(R)-(+)-CM18] (a) had a 10-fold preferential blocking activity for α_1- versus α_2-adrenoceptors, (b) discriminated, like racemic **1**, between two possible α_1-adrenoceptor subsites/subtypes, with a selectivity ratio of 6.5 and (c) was 10–23 times as potent as the (*S*)-(−)-enantiomer at α_2- and α_1-adrenoceptors. Thus, it may be a valuable tool for the characterization of rat vas deferens α_1-adrenoceptor subtypes. © 1997 Elsevier Science Ltd.

Introduction

Adrenergic receptors have been subdivided into three major types, designated as α_1, α_2 and β, distinguished by their relative affinities for selective agonists and antagonists. They are members of a receptor superfamily coupled with different G-proteins and mediating the action of endogenous catecholamines.[1] Many components of this family have been cloned during recent years and a number of subtypes have been pharmacologically characterized in all classes.

Pharmacological and binding studies have shown that α_1-adrenoceptors can be classified into at least three subtypes, namely α_{1A}, α_{1B} and α_{1D}.[2,3] The α_{1A} subtype has a high affinity for antagonists such as WB 4101, 5-methylurapidil, and (*S*)-(+)-niguldipine, and is insensitive to inactivation by chloroethylclonidine (CEC).[4] The α_{1B} subtype displays lower affinity for the above antagonists, but is preferentially inactivated by the alkylating agent CEC,[4] whereas the α_{1D} subtype has a high affinity for the antagonist BMY7378.[5] Current evidence indicates that rat submaxillary gland,[6] human liver[7] and various tissues such as rat vas deferens,[8–10] rabbit prostate and prostatic urethra[11] contain predominantly the α_{1A}-adrenoceptor, whereas rat liver and spleen[12,13] are considered α_{1B}-adrenoceptor preparations and the α_{1D}-adrenoceptor mediates the contraction in rat aorta.[13–15] Cloning studies have confirmed the existence of three distinct α_1-adrenoceptors, which are now designated as α_{1a}, α_{1b} and α_{1d} subtypes.[16–19] The recombinant α_{1a}-adrenoceptor (formerly designated as α_{1c}),[17] corresponds to the native α_{1A}-adrenoceptor,[20] the recombinant α_{1b} to the native α_{1B} and the α_{1d} (formerly designated as $\alpha_{1a/d}$ in some publications) to the native α_{1D}-adrenoceptor recently characterized in rat aorta. Thus, α_1-adrenoceptors are now classified as α_{1A} (α_{1a}), α_{1B} (α_{1b}) and α_{1D} (α_{1d}), with upper- and lower-case subscripts being used to designate native or recombinant receptor, respectively.[3]

However, an alternative classification scheme of α_1-adrenoceptors, based on high and low affinity for prazosin, α_{1H} and α_{1L}, respectively, has been adopted.[21] In this scheme α_{1H}-adrenoceptors encompass all α_{1A}, α_{1B} and α_{1D} subtypes, whereas for the α_{1L}-adrenoceptor only functional pharmacological evidence is available.[22,23]

The nature of the α_1-adrenoceptor subtype involved in contractions due to noradrenaline of the epididymal portion of rat vas deferens is still matter of debate. Besides various evidence indicating in the pharmacologically defined α_{1A}-adrenoceptor the only subtype involved in contractions,[9,10] other investigations have shown that the contribution of a second α_1-adrenoceptor has to be considered. In fact, it has been reported that, in addition to mediation by the α_{1A}-subtype, the contraction of this tissue is mainly mediated by an α_{1L}-adrenoceptor,[24] or else by a minor contribution of a non-α_{1A}, non-α_{1B} adrenoceptor subtype.[13]

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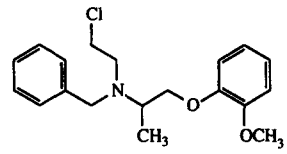
Recently, we studied some β-chloroethylamines which are structurally related to WB4101, a potent and competitive α_1-adrenoceptor antagonist, and to phenoxybenzamine, an irreversible α-adrenoceptor antagonist.[25] These compounds, like the prototype phenoxybenzamine, displayed an irreversible blocking activity at rat vas deferens α-adrenoceptors, with a slight selectivity for α_1- relative to α_2-adrenoceptors. Interestingly, two compounds, benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (**1**, CM18) and (2-chloroethyl)-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-[2-(2-methoxyphenoxy)-1-methylethyl]amine hydrochloride (the 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, suggesting a possible discriminating ability between two α_1-adrenoceptor subtypes mediating the noradrenaline-induced contraction of rat vas deferens tissue.[25]

It is well known that stereochemistry plays a relevant role in drug–receptor interaction processes. For example, among α_1-adrenoceptor ligands, the (*S*)-(+)-enantiomer of niguldipine, a potent calcium channel antagonist displaying also high affinity for α_1-adrenoceptors,[4] exhibited 340- and 630-fold selectivity in binding to the cloned human α_{1a}-adrenoceptor relative to the α_{1b}- and α_{1d}-subtypes, respectively. The (*R*)-(−)-niguldipine, instead, was 29-fold less potent at the α_{1a}-adrenoceptor than its enantiomer and also less subtype-selective.[26] In addition, the (*R*)-(−)-tamsulosin displayed a 20, 80 and 50 times higher affinity than the (*S*)-(+)-enantiomer, respectively, at cloned human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors, whereas both enantiomers showed a higher affinity for the α_{1a} over the α_{1b}-adrenoceptor (fivefold to 25-fold), failing to discriminate between α_{1a}- and α_{1d}-subtypes.[27] Recently, mephendioxan, another competitive antagonist, displayed significant enantioselectivity.[28] In fact, in binding to native and cloned α_1-adrenoceptors (2*S*,3*S*)-(−)-mephendioxan was 10–30 times more potent than its enantiomer. Furthermore, whereas (2*R*,3*R*)-(+)-mephendioxan was 20-fold selective for α_{1a}- versus α_{1b}- and α_{1d}-adrenoceptors, (2*S*,3*S*)-(−)-mephendioxan, in comparison, displayed a higher selectivity for α_{1a}-adrenoceptor with respect to the α_{1b} (36- to 60-fold), and a 20-fold selectivity with respect to the α_{1d}-subtype.

Among the irreversible antagonists stereochemistry was also seen to play a crucial role, as indicated by the activity of the (*R*)-(+)- and (*S*)-(−)-enantiomers of the alkylating agent phenoxybenzamine. They displayed, in fact, a different α-adrenergic blocking potency, with the (*R*)-(+) enantiomer being 14.5-fold more potent than the (*S*)-(−) one.[29]

Since the already studied β-chloroethylamines incorporate chiral centres in their structures, we thought it of interest to investigate the effect of their stereochemistry on the α-adrenoceptor blocking activity. We report here



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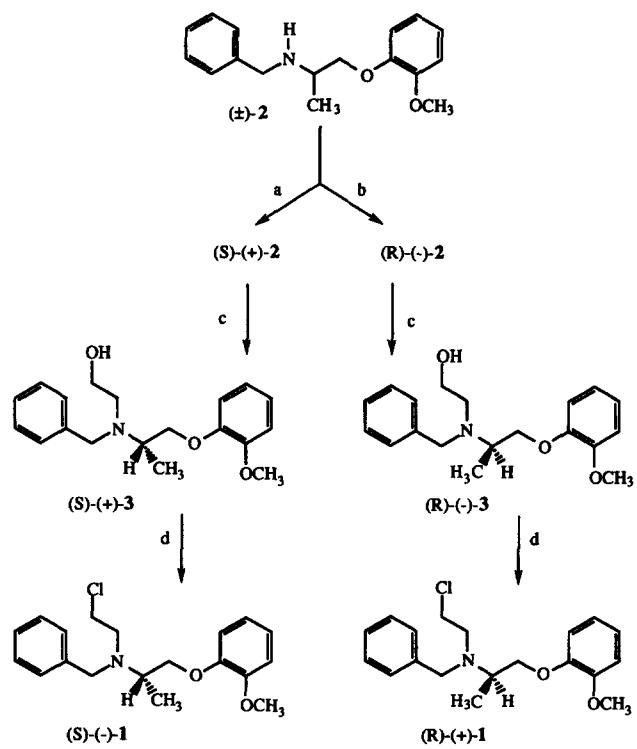
the synthesis, absolute configuration, and biological evaluation of compound **1** enantiomers.

Chemistry

Compounds (+)-**1** and (−)-**1** were synthesized as hydrochloride salts following a procedure similar to that used for the synthesis of racemic **1** (CM18) (Scheme 1) and were characterized by ^{1}H NMR and elemental analysis. The intermediate racemic amine **2**[25] was resolved in the respective enantiomers (+)-**2** and (−)-**2** with (*D*)-(+)- and (*L*)-(−)-*O*,*O*′-dibenzoyl tartaric acids. Enantiomeric purity was assessed, in comparison with racemic **2**, by HPLC and ^{1}H NMR analysis upon their transformation with (*R*)-(−)-1-(1-naphthyl)-ethyl isocyanate into the corresponding diastereomeric ureas 1-benzyl-1-[2-(2-methoxyphenoxy)-1-methylethyl]-3-(1-naphthalen-1-ylethyl)urea. MS spectra of all three ureas revealed the absence of the molecular ion and the cleavage of the molecules mainly into the starting materials and related fragments. HPLC analysis (normal phase, hypersil column, elution solvent *n*-hex-



Scheme 1. Reagents and conditions: (a) (−)-*O*,*O*′-dibenzoyl-L-tartaric acid, MeOH, rt; (b) (+)-*O*,*O*′-dibenzoyl-D-tartaric acid, MeOH, rt; (c) $Br(CH_2)_2OH$, K_2CO_3, EtOH; (d) $SOCl_2$, HCl (gas), C_6H_6.

ane:ethyl acetate 80:20% v/v) showed a single peak with a retention time of 6.06 and 6.95 min for the ureas obtained from (−)-**2** and (+)-**2**, respectively. In the same conditions, the urea from racemic **2** showed two peaks with a retention time of 6.17 and 7.06 min, respectively (Fig. 1). Since a purposely prepared mixture of ureas obtained from (+)-**2** and (−)-**2**, respectively in a 98:2 ratio, showed a detectable appearance of the less abundant diastereomer, we attributed to the amine (+)-**2** a stereochemical purity higher than 98%. Similarly, analysis of a mixture of the same ureas in a 3.5:96.5 ratio allowed us to attribute to amine (−)-**2** a stereochemical purity higher than 96.5 %. These assignments were confirmed by ^{1}H NMR, focusing on the methoxy resonance which allows comparison between the ureas obtained from the enantiomers and racemic **2**. In the spectrum of racemic amine diastereomeric urea, the methoxy group gives two singlets at 3.72 and 3.75 ppm, whereas in the ureas from the two enantiomers a single peak is observed at the corresponding chemical shift. However, whereas in the urea from (+)-**2** the signal attributed to the (−)-enantiomer derivative is not significant, in that from (−)-**2** about 3–3.5 % of the other diastereomer is detectable.

Suitable crystals of (+)-**2** hydrochloride were obtained for X-ray crystallographic analysis which allowed us to know its absolute configuration and, as a consequence, that of related compounds. Alkylation of (+)-**2** and (−)-**2** with 2-bromoethanol afforded the corresponding *N,N*-disubstituted 2-aminoethanols (+)-**3** and (−)-**3**. Following a reported procedure,[29] these were, in turn, converted into the corresponding β-chloroethylamines (−)-**1** and (+)-**1**, respectively, with thionyl chloride. Given the experimental conditions, conversion of the enantiomers of **2** into those of **1** presumably proceeded without racemization. Thus, we assumed that (+)-**1** and (−)-**1** have the same optical purity as the corresponding enantiomers of **2**.

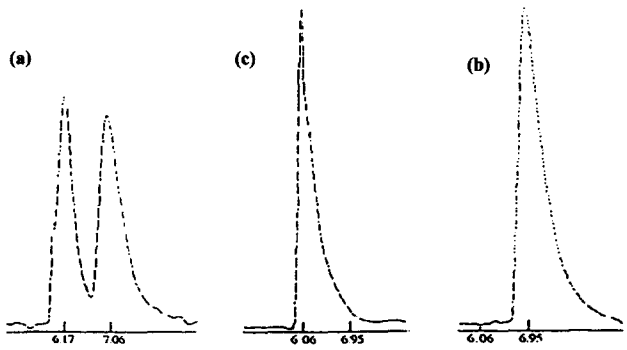


Figure 1. Analytical HPLC chromatograms of the diastereomeric naphthylethyl ureas of racemic **2** (a) and its enantiomers (*S*)-(+)-**2** (b) and (*R*)-(−)-**2** (c). Chromatographic conditions: normal phase, hypersil column, 5 μm (200 × 2.1 ID), elution with *n*-hexane–ethyl acetate 80:20 v/v at a flow rate of 0.5 mL/min, UV detection at 254 nm, chart speed 7.8 mm/min. Retention times (min) are indicated at each peak.

Results and Discussion

The absolute configuration of (+)-**2** hydrochloride, determined by X-ray crystallographic analysis (Fig. 2), proved to be *S*, and, as a consequence, that of the aminoalcohol (+)-**3** and β-chloroethylamine (−)-**1** did as well. From this, it follows that amine (−)-**2**, aminoalcohol (−)-**3** and β-chloroethylamine (+)-**1** have an *R* configuration.

The biological profiles of (*R*)-(+)-**1** and (*S*)-(−)-**1** were evaluated at α_1- and α_2-adrenoceptors on isolated rat vas deferens[30,31] and the results are reported in Table 1 and Figure 3.

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- 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-adrenoceptors were obtained for both enantiomers (*R*)-(+)-**1** and (*S*)-(−)-**1** and are shown in Figure 3 together with that of racemic **1**. The potency of each compound was expressed as IC_{50} value, the concentration that produces 50% inhibition of the agonist maximal response (Table 1).

The antagonist potency at both α_1- and α_2-adrenoceptors is dependent on the concentration of aziridinium ion, the active species of β-chloroethylamines in α-adrenergic blockade.[32] Since the rate of cyclization of (*R*)-(+)-**1** and (*S*)-(−)-**1** into the aziridinium ion and the rate of hydrolysis are identical by virtue of their enantiomeric relationships, the concentration of the aziridinium ion formed from the two stereoisomers is the same as and equal to that obtained from racemic **1**. Thus, a 30-min incubation time of the tissues with investigated drugs is justified by kinetic data.[25]

As expected, the two enantiomers showed an irreversible blocking activity at both α_1- and α_2-adrenoceptors

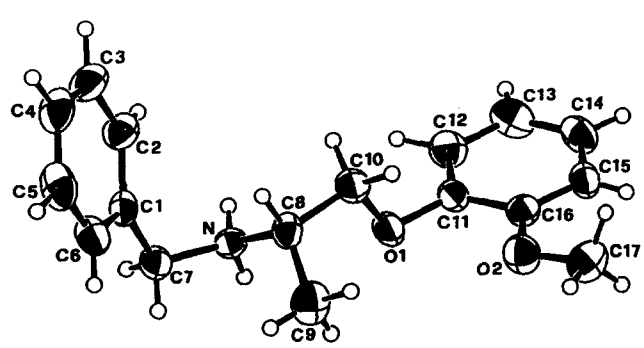


Figure 2. ORTEP view of (+)-**2** as hydrochloride salt showing the atom numbering scheme and thermal motion ellipsoids (40%).

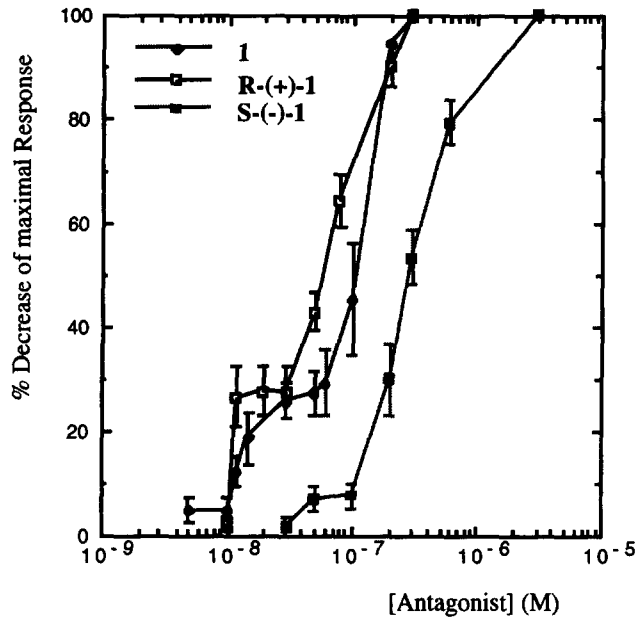


Figure 3. Covalent occupancy of α_1-adrenoceptors of the epidydimal portion of rat vas deferens by **1** and its enantiomers *R*-(+)-**1** and *S*-(−)-**1**. The percent decrease of Maximal response to noradrenaline was measured after a 30-min incubation for each concentration of antagonist followed by washing with the bath solution for 30 min. Results are expressed as the mean±SEM of four to 10 independent observations.

since the response was not recovered after extensive washing following incubation. Both enantiomers, like racemic **1**, were slightly more potent at α_1- than at α_2-adrenoceptors. (*R*)-(+)-**1** was the most potent also in comparision with phenoxybenzamine (pIC_{50} = 7.94±0.02 and 7.27±0.01,[25] respectively). However, the most interesting finding was the inhibition curve of noradrenaline-induced contractions at α_1-adrenoceptors displayed by (*R*)-(+)-**1**. In fact, (*R*)-(+)-**1** showed, as already observed with racemic **1**, a marked discontinuity in the concentration–inhibition curve with a plateau in the 10–30 nM range. This behavior confirms and strengthens the hypothesis of a possible nonhomogeneous population of α_1-adrenoceptors in the epididymal portion of rat vas deferens tissue.[25] On the other hand, the other enantiomer (*S*)-(−)-**1**, was not able to discriminate among possible α_1-adrenoceptor subtypes, as revealed by its monophasic inhibition of noradrenaline-induced responses (Fig. 3).

In agreement with previous hypotheses made by other authors,[13,24] the biphasic inhibition of α_1-adrenoceptors observed for (*R*)-(+)-**1** might indicate the involvement of two subtypes which control the rat vas deferens contractile mechanism in a proportion of about 30% and 70% of the effect.

The (*R*)-(+)-**1** enantiomer, like racemic **1**, discriminated between a high and a low affinity site with a $IC_{50(high)}/IC_{50(low)}$ selectivity ratio of about one order of magnitude (Table 1). Furthermore, (*R*)-(+)-**1** was 10- to 23-fold more potent than the (−)-enantiomer toward α_2-adrenoceptors and the α_1-adrenoceptor high affinity site, revealing that stereochemistry has a similar effect at both α-adrenoceptors.

As a consequence of its observed selectivity, the irreversible antagonist (*R*)-(+)-**1** may be a useful tool for the characterization of α_1-adrenoceptor subtypes of the epididymal portion of rat vas deferens.

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 model 5890A for the separation section and model 5971A for the mass section. Analytical HPLC analysis was performed

Table 1. α-Adrenoceptor blocking activity of (±)-**1**, (*R*)-(+)-**1** and (*S*)-(−)-**1** in the isolated rat vas deferens

	pIC_{50}[a]			
Compd	α_{1high}	α_{1low}	$\alpha_{1high}/\alpha_{1low}$[b]	α_2
R-(+)-**1**	7.94±0.02[c]	7.13±0.02[c]	6.5	7.01±0.03
S-(−)-**1**	6.58±0.02			6.03±0.00
(±)-**1**	7.88±0.03[c]	6.85±0.04[c]	10	6.77±0.02

[a] pIC_{50} values represent the negative logarithm of the concentration that produces 50% inhibition of the agonist maximal response and are expressed as means±SEM.
[b] The $\alpha_{1high}/\alpha_{1low}$ selectivity ratio is the antilog of the difference between the pIC_{50} values at α_1-adrenoceptor subsites.
[c] These pIC_{50} values are calculated assuming that the plateau in the inhibition curves (Fig. 3) for (±)-**1** and (*R*)-(+)-**1** identify the line of separation in the blockade of two α_1-adrenoceptor subtypes or subsites.

on a Hewlett Packard 1090 apparatus series II, with a UV 254 detector, using a Hypersil column, 5 μm (200 × 2.1 ID). The optical rotation was measured on a Perkin–Elmer 241 MC polarimeter. Chromatographic separations were made 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, petroleum ether–ethyl acetate–methanol–28% ammonia (10:4:1:0.05); B, ethyl acetate–*n*-hexane (2:5); C, chloroform–ethyl acetate (8:2); D, ethyl acetate–cyclohexane (2:8). Crystallographic analysis was performed on a CAD4 automatic four-circle diffractometer. 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 naming in organic chemistry (Beilstein-Institut and Springer-Verlag). (Analytical results are given in Table 2.)

Resolution of (±)-benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]amine (2). A solution of racemic **2** (7.76 g, 28.6 mmol) in MeOH (80 mL) was treated with a solution of (−)-*O*,*O*′-dibenzoyl-L-tartaric acid monohydrate (10.76 g, 28.6 mmol) in MeOH (100 mL). The solution was evaporated to dryness to give a residue that was crystallized by dissolving the solid in 400 mL of hot *i*-PrOH. The precipitate was recrystallized two further times with the same solvent to give 6.91 g of (−)-*O*,*O*′-dibenzoyl tartrate salt; mp 93–95 °C; $[\alpha]_D^{20}$ −62.14 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.37 (d, *J* = 6.59 Hz, 3H, CH_3), 3.43–3.60 (m, 1H, CH_3CH), 3.68 (s, 3H, OCH_3), 3.98–4.25 (m, 3H, $ArCH_2$, CH_2O), 4.34–4.48 (m, 1H, $ArCH_2$), 5.80 (s, 2H, CHCOOH), 6.77–6.85 (m, 3H, Ar), 6.90–7.02 (m, 1H, Ar), 7.18–7.38 (m, 7H, Ar), 7.42–7.53 (m, 4H, Ar), 7.98–8.08 (*m*, 4H, Ar). Anal. ($C_{35}H_{35}NO_{10}\cdot1.5H_2O\cdot0.75C_3H_8O$) C, H, N.

The salt was dissolved in water, the ice-cooled solution made alkalinic with 5% Na_2CO_3 and the resulting mixture extracted with chloroform (4 × 100 mL). Removal of dried solvent gave (R)-(−)-benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]amine [(*R*)-(−)-2] as oily liquid: 1.94 g; R_f 0.43 (mixture A). This liquid was transformed into the hydrochloride salt and recrystallized with ethyl acetate: mp 146–147 °C; $[\alpha]_D^{20}$ −119.75 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$, free base) δ 1.20 (d, *J* = 7.37 Hz, 3H, CH_3), 2.33 (br s, 1H, NH), 3.14–3.32 (m, 1H, CH_3CH), 3.78–4.03 (m, 7H, OCH_3, $ArCH_2$, CH_2O), 6.80–7.02 (m, 4H, OAr), 7.19–7.42 (m, 5H, Ar). Anal. ($C_{17}H_{22}ClNO_2$) C, H, N.

The amine recovered by alkaline treatment with cold 2 N NaOH of combined mother liquors of the above dibenzoyl tartrate (5.75 g, 21.2 mmol) was dissolved in MeOH (50 mL) and treated with a solution of (+)-*O*,*O*′-dibenzoyl-D-tartaric acid monohydrate (7.97 g, 21.2 mmol) in MeOH (80 mL). The resulting mixture was evaporated to dryness and the residue was crystallized three times with *i*-PrOH to give 5.90 g of (+)-*O*,*O*′-dibenzoyl tartrate salt; mp 93–95 °C; $[\alpha]_D^{20}$ +62.64 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.39 (d, *J* = 6.67 Hz, 3H, CH_3), 3.41–3.60 (m, 1H, CH_3CH), 3.67 (s, 3H, OCH_3), 3.95–4.25 (m, 3H, $ArCH_2$, CH_2O), 4.37–4.50 (m, 1H, $ArCH_2$), 5.80 (s, 2H, CHCOOH), 6.72–6.87 (m, 3H, Ar), 6.91–7.02 (m, 1H, Ar), 7.17–7.38 (m, 7H, Ar), 7.43–7.55 (m, 4H, Ar), 7.98–8.05 (m, 4H, Ar). Anal. ($C_{35}H_{35}NO_{10}\cdot1.5H_2O\cdot0.75C_3H_8O$) C, H, N.

This salt was treated as described for the other enantiomer to give (*S*)-(+)-benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]amine [(*S*)-(+)-2] as oily liquid: 1.38 g; R_f 0.43 (mixture A). This liquid was transformed into the hydrochloride salt and recrystallized with ethyl acetate: mp 146–147 °C; $[\alpha]_D^{20}$ +119.60 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$, free base) δ 1.20 (d, *J* = 7.37 Hz, 3H, CH_3), 2.24 (br s, 1H, NH), 3.14–3.32 (m, 1H, CH_3CH), 3.78–4.03 (m, 7H, OCH_3, $ArCH_2$, CH_2O), 6.80–7.02 (m, 4H, OAr), 7.19–7.42 (m, 5H, Ar). Anal. ($C_{17}H_{22}ClNO_2$) C, H, N.

Determination of optical purity of (*S*)-(+)-2 and (*R*)-(−)-2. A mixture of racemic **2** (0.05 g, 0.19 mmol) and (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (0.04 g, 0.19 mmol) in dry dichloromethane (10 mL) was stirred 4 h at room temperature. Removal of the solvent gave a residue that was purified by column chromatography. Eluting with mixture B afforded the 1-benzyl-1-[2-(2-methoxyphenoxy)-1-methylethyl]-3-(1-naphthalen-1-yl-ethyl)urea as diastereomeric mixture: 0.07 g (oil); R_f 0.18; MS (EI) *m/z* (rel int), 104 (2), 134 (100), 238 (1), 271 (2) (M^+ of **2**), 127 (4), 155 (4), 182 (12), 197 (6) (M^+ of naphthylethyl isocyanate) and 253 (2), 302 (2), 329 (100), 345 (31); ^{1}H NMR ($CDCl_3$) δ 1.34 (d, *J* = 7.08 Hz, 3H, CH_3CHCH_2), 1.36 (d, *J* = 7.08 Hz, 3H, CH_3CHCH_2), 1.51 (d, *J* = 6.68 Hz, 6H, CH_3CHNH),

Table 2. Analytical data

Compound	Formula	Calcd (%)			Found (%)		
		C	H	N	C	H	N
(+)-**1**	$C_{19}H_{25}Cl_2NO_2$	61.63	6.80	3.78	61.53	7.07	4.02
(−)-**1**	$C_{19}H_{25}Cl_2NO_2$	61.63	6.80	3.78	61.56	7.07	3.90
(+)-**2** DBT salt	$C_{35}H_{53}NO_{10}$ $1.5H_2O\cdot0.75C_3H_8O$	63.76	6.32	2.00	64.02	6.58	1.78
(−)-**2** DBT salt	$C_{35}H_{53}NO_{10}$ $1.5H_2O\cdot0.75C_3H_8O$	63.76	6.32	2.00	63.75	6.57	1.80

3.72 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.90–4.18 (m, 4H, CH_2O), 4.43 (d, J = 17.30, 1H, CH_2Ar), 4.44 (d, J = 17.50 Hz, 1H, CH_2Ar), 4.71 (d, J = 17.21 Hz, 1H, CH_2Ar), 4.72 (d, J = 17.39 Hz, 1H, CH_2Ar), 5.09–5.27 (m, 2H, $CHCH_2O$), 5.70–5.92 (m, 2H, CHNH), 6.76–7.00 (m, 8H, Ar), 7.12–7.55 (m, 20H, Ar), 7.65–7.89 (m, 4H, NH and Ar), 8.08–8.22 (m, 2H, Ar). HPLC analysis was performed with *n*-hexane–ethyl acetate (4:1) as the eluting mixture at a flow rate of 0.5 mL/min. Under these conditions, injected samples (1.5 μL, c = 5 mg/mL), which were detected by absorbance at 254 nm, gave two peaks with retention times of 6.17 and 7.06 min (Fig. 1).

The urea derivative of (−)-**2** was prepared as described for racemic **2**. Oil; R_f 0.18 (mixture B); MS (EI) *m/z* (rel int), 104 (3), 134 (100), 271 (1) (M^+ of **2**), 127 (34), 155 (40), 182 (100), 197 (73) (M^+ of naphthylethyl isocyanate) and 253 (1), 302 (1), 329 (100), 345 (18); ^{1}H NMR ($CDCl_3$) δ 1.35 (d, J = 7.04 Hz, 3H, CH_3CHCH_2), 1.50 (d, J = 6.78 Hz, 3H, CH_3CHNH), 3.72 (s, 3H, OCH_3), 3.94–4.09 (m, 2H, CH_2O), 4.42 (d, J = 17.22 Hz, 1H, CH_2Ar), 4.69 (d, J = 17.22 Hz, 1H, CH_2Ar), 5.08–5.20 (m, 1H, $CHCH_2O$), 5.70–5.87 (m, 1H, CHNH), 6.75–6.98 (m, 4H, Ar), 7.11–7.54 (m, 10H, Ar), 7.68 (d, J = 7.64 Hz, 1H, NH), 7.76–7.85 (m, 1H, Ar), 8.06–8.16 (m, 1H, Ar). HPLC analysis revealed a peak with retention time of 6.06 min (Fig. 1).

The urea derivative of (+)-**2** was prepared with a similar procedure: oil; R_f 0.17 (mixture B); MS (EI) *m/z* (rel int), 104 (2), 134 (100), 271 (1) (M^+ of **2**), 127 (29), 155 (39), 182 (100), 197 (56) (M^+ of naphthylethyl isocyanate) and 329 (100), 345 (21); ^{1}H NMR ($CDCl_3$) δ 1.33 (d, J = 7.12 Hz, 3H, CH_3CHCH_2), 1.52 (d, J = 6.78 Hz, 3H, CH_3CHNH), 3.75 (s, 3H, OCH_3), 3.90–4.11 (m, 2H, CH_2O), 4.43 (d, J = 17.16 Hz, 1H, CH_2Ar), 4.72 (d, J = 17.20 Hz, 1H, CH_2Ar), 5.10–5.26 (m, 1H, $CHCH_2O$), 5.70–5.92 (m, 1H, CHNH), 6.75–6.98 (m, 4H, Ar), 7.15–7.56 (m, 10H, Ar), 7.73 (d, J = 7.64 Hz, 1H, NH), 7.79–7.88 (m, 1H, Ar), 8.08–8.21 (m, 1H, Ar). HPLC analysis revealed a single peak with retention time of 6.95 min (Fig. 1).

(*R*)-(−)-2-[Benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]-amino]ethanol [(*R*)-(−)-3]. A mixture of the amine (−)-**2** (1.8 g, 6.63 mmol), bromoethanol (0.91 g, 7.30 mmol), and dry K_2CO_3 (1.8 g, 13.27 mmol) in ethanol (50 mL) 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 2N HCl. The acidic solution was basified with 2N NaOH and extracted with Et_2O. Removal of dried solvent gave a residue that was purified by chromatography. Elution with mixture C gave 0.98 g of (−)-**3** as viscous oil; R_f 0.43; $[\alpha]_D^{20}$ −40.16 (*c* 1, MeOH); MS (EI) *m/z* (rel int), 91 (97), 178 (100), 284 (19), 315 (1) $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 1.12 (d, J = 6.84 Hz, 3H, CH_3), 2.60–2.97 (m, 2H, CH_2CH_2OH), 3.32–3.73 (m, 4H, CH_2OH, CH_2Ar and CH_2OAr), 3.78–4.05 (m, 6H, OCH_3, CH_2Ar, CH_2OAr and CH_3CH), 6.76–6.96 (m, 4H, OAr), 7.12–7.46 (m, 5H, Ar).

(*S*)-(+)-2-[Benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]-amino]ethanol [(*S*)-(+)-3]. This was obtained from the amine (+)-**2** following the above procedure: viscous oil; 42% yield; R_f 0.43 (mixture C); $[\alpha]_D^{20}$ +39.61 (*c* 1, MeOH); MS (EI) *m/z* (rel int), 91 (100), 178 (99), 284 (16), 315 (1) $(M)^+$; ^{1}H NMR ($CDCl_3$) δ 1.11 (d, J = 6.51 Hz, 3H, CH_3), 2.55–2.98 (m, 2H, CH_2CH_2OH), 3.21–3.73 (m, 4H, CH_2OH, CH_2Ar and CH_2OAr), 3.80–4.05 (m, 6H, OCH_3, CH_2Ar, CH_2OAr and CH_3CH), 6.76–7.05 (m, 4H, OAr), 7.12–7.50 (m, 5H, Ar).

(*R*)-(+)-Benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl] amine hydrochloride [(*R*)-(+)-1]. HCl (g) was slowly bubbled for 15 min into a stirred and cooled (0 °C) solution of 0.83 g (2.63 mmol) of aminoalcohol (−)-**3** in dry benzene (70 mL), then $SOCl_2$ (0.39 g, 3.32 mmol) in dry benzene (12 mL) was added dropwise and the mixture refluxed for 8 h. Removal of the solvent and $SOCl_2$ excess gave a residue that was purified by chromatography eluting with mixture D. A total of 0.23 g of (+)-**1** was obtained; mp 112–113 °C (from *i*PrOH); R_f 0.49; $[\alpha]_D^{20}$ +152.13 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.56–1.80 (m, 3H, CH_3), 3.30–3.80 (m, 2H, CH_2Cl), 3.82–4.50 (m, 8H, OCH_3, CH_3CH, NCH_2CH_2, CH_2Ar, CH_2OAr), 4.55–4.84 (m, 2H, CH_2Ar, CH_2OAr), 6.86–7.12 (m, 4H, OAr), 7.38–7.60 (m, 3H, Ar), 7.74–8.01 (m, 2H, Ar), 12.85 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{19}H_{25}Cl_2NO_2$) C, H, N.

(*S*)-(−)-Benzyl-(2-chloroethyl)-[2-(2-methoxyphenoxy)-1-methylethyl] amine hydrochloride [(*S*)-(−)-1]. This was obtained from the aminoalcohol (+)-**3** following the above procedure: 30% yield; mp 113–114 °C (from ethyl acetate–*n*-hexane); R_f 0.49 (mixture D); $[\alpha]_D^{20}$ −151.46 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.56–1.80 (m, 3H, CH_3), 3.30–3.78 (m, 2H, CH_2Cl), 3.82–4.50 (m, 8H, OCH_3, CH_3CH, NCH_2CH_2, CH_2Ar, CH_2OAr), 4.55–4.85 (m, 2H, CH_2Ar, CH_2OAr), 6.85–7.18 (m, 4H, OAr), 7.38–7.60 (m, 3H, Ar), 7.72–8.00 (m, 2H, Ar), 12.85 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{19}H_{25}Cl_2NO_2$) C, H, N.

X-ray crystallography

Crystals of (+)-benzyl-[2-(2-methoxyphenoxy)-1-methylethyl]amine [(+)-**2**] hydrochloride for X-ray diffraction were grown from ethyl acetate. A crystal of approximate dimensions 0.35 × 0.30 × 0.25 mm^3 was used for data collection.

Crystal data. $C_{17}H_{22}ClNO_2$, M = 307.81, monoclinic, a = 10.293 (2), b = 7.601 (1), c = 10.533 (2) Å, β = 90.90 (2)°, V = 823.9 (3) Å^3, space group $P2_1$, Z = 2, D_c = 1.241 mg m^{-3}, μ (M_0–K_α) = 0.204 mm^{-1}, F(000) = 330. Diffractometric data were collected at room temperature in the range 2° ≤ φ ≤ 28 °, including Friedel pairs.

Intensities were corrected for Lorentz and polarization effects, and an empirical absorption correction, based on the ψ-scan, was applied. The structure was solved by

direct methods using the SHELX-86 program, and refined on F^2 with SHELX-93. All non-H atoms were refined anisotropically, whereas H-atoms, located in ΔF maps, were refined isotropically. The absolute configuration was determined by refinement on a Flack χ-parameter, whose final value was 0.06 (4). The final agreement factors were $R = 0.031$ and $wR_2 = 0.078$ for 271 parameters and 3139 observed reflections. The largest difference peak and hole were 0.173 and -0.154 e·Å^{-3}, respectively.

The results of the X-ray structure analysis unambiguously show the absolute configuration at the C_8 chiral centre to be S. All bond distances and angles are in the expected ranges. The crystal packing is mainly determined by two strong hydrogen bond interactions which involve the Cl^- ion and both the N-bonded hydrogen atoms. A drawing of the organic cation is shown in Figure 2, along with the labelling scheme used.

Functional antagonism in isolated rat vas deferens

Male albino rats [rat outbred, Charles River line: CD (SD) BR], 125–150 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 10–20 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-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 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 (*S*)-(−)-**1** and (*R*)-(+)-**1** were investigated at seven and eight different concentrations, respectively, and each concentration was tested four to 10 times. The antagonist potency of compounds at α_1-adrenoceptors was expressed by the negative logarithm of concentration that caused 50% inhibition of agonist action (pIC_{50}).

The α_2-adrenoceptor 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-adrenoceptor.[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-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.

Supplementary material available

Final fractional coordinates and equivalent isotropic temperature factors, bond distances and angles, anisotropic thermal parameters, torsion angles, and selected least-squares planes for compound (+)-**2** hydrochloride salt (eight tables).

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