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Original article

Synthesis and α_1-adrenoceptor antagonist activity of tamsulosin analogues

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ABSTRACT

Tamsulosin (−)-**1** is the most utilized α_1-adrenoceptor antagonist in the benign prostatic hyperplasia therapy owing to its uroselective antagonism and capability in relieving both obstructive and irritative lower urinary tract symptoms. Here we report the synthesis and pharmacological study of the homochiral (−)-**1** analogues (−)-**2**–(−)-**5**, bearing definite modifications in the 2-substituted phenoxyethylamino group in order to evaluate their influence on the affinity profile for α_1-adrenoceptor subtypes. The benzyl analogue (−)-**3**, displaying a preferential antagonist profile for α_{1A}-than α_{1D}-and α_{1B}-adrenoceptors, and a 12-fold higher potency at α_{1A}-adrenoceptors with respect to the α_{1B} subtype, may have improved uroselectivity compared to (−)-**1**.

1. Introduction

Three native adrenoceptors, α_{1A}, α_{1B} and α_{1D}, have been pharmacologically detected in animal and human tissues, corresponding to the cloned α_{1a}, α_{1b}, and α_{1d} subtypes expressed in various cell lines [1]. A fourth α_1-adrenoceptor subtype, α_{1L}, displaying low affinity for prazosin [2] and conformationally related to the α_{1A} subtype [3] has also been reported.

Multiple α_1-adrenoceptor subtypes are present in a variety of organs, as brain, heart, blood vessels, liver, spleen, kidney, prostate, where mediate, on activation by endogenous noradrenaline or adrenaline, a range of physiological functions including neuronal transmission, contraction of blood vessels and low urinary tract smooth muscles, myocardial growth and inotropy [4].

Pharmacological evidences indicate that the α_{1A}-and the α_{1B}-adrenoceptors are the subtypes predominantly expressed in human blood vessels and involved in contraction of vasculatures responsible of blood pressure in young men and in the elderly, respectively [5–8]. As a consequence, their specific antagonists display therapeutic indications for the control of hypertension. Furthermore, the α_{1A}-adrenoceptor is predominantly expressed, with respect to the α_{1D}, in human prostate gland where it mediates contractions producing obstructive symptoms to the urine flow [9,10]. At the same time, the α_{1D}-adrenoceptor is the most abundant subtype in human bladder detrusor [11] whose stimulation causes bladder instability and irritability [10]. For these reasons, beside the non-selective antagonists, some subtype selective antagonists are currently used as first line medical treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia (BPH) [12,13].

(*R*)-(−)-5-[2-(2-(2-Ethoxyphenoxy)ethylamino)propyl]-2-methoxybenzenesulfonamide ((−)-**1**, tamsulosin), a chiral sulfamoylphenethylamine derivative, is a potent α_1-adrenoceptor antagonist [14]. Rather different results, with regard selectivity, were obtained for (−)-**1** in binding and functional assays. In binding experiments, it showed high and similar affinity at α_{1A}-and α_{1D}-adrenoceptors and a slightly lower affinity for the α_{1B} subtype [15–17]. In functional studies on animal tissues, (−)-**1** resulted almost equipotent at α_{1D}-and α_{1A}-adrenoceptors while being a weaker antagonist at the α_{1B} subtype [18]. However, in other studies, (−)-**1** was significantly more potent at α_{1D}-adrenoceptors than at α_{1A}- or α_{1B}-adrenoceptors [16,17,19].

Tamsulosin is the most utilized α_1-adrenoceptor antagonist in the BPH therapy [20] owing to its uroselective α_1-adrenoceptor antagonism, with a superior profile in relieving both obstructive and irritative lower urinary tract symptoms [21,22]. In this regard, most of the pharmacological evidences indicate that α_1-adrenoceptor antagonists with higher affinity for the α_{1A}-adrenoceptor

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over the α_{1B} subtype and, at the same time, with an important affinity for the α_{1D}-adrenoceptor, that is with a balanced $\alpha_{1A/D}$ selectivity, should find an optimal use in the BPH therapy [8,23,24].

Although endowed with minor side effects, such as orthostatic hypotension and dizziness, in comparison to non-selective quinazoline antagonists, (−)-**1** gave more frequently ejaculatory disorders [8,25,26].

Here we report the design and synthesis of the homochiral (−)-**1** analogues (−)-**2**, (−**3**), and (−)-**4** [27], bearing definite modifications in the 2-substituted phenoxyethylamino group in order to evaluate their influence on the affinity profile for α_1-adrenoceptors, which could, hopefully, lead to an improvement of the therapeutic utilization. Specifically, the 2-ethoxy group of (−)-**1** was replaced with an *i*-propoxy ((−)-**2**), a benzyloxy ((−)-**3**) or a 2,2,2-trifluoroethoxy moiety ((−)-**4**), to increase at different extent the hindrance, the hydrophobic properties, or the polar character of the progenitor. In addition, the *i*-propoxy [28] and the 2,2,2-trifluoroethoxy [29] groups are structural component of some known potent α_1-adrenoceptor antagonists. In particular, some 2,2,2-trifluoroethoxy-bearing compounds [13,30,31] displayed high affinity and selectivity for the human cloned α_{1a}-adrenoceptor together with a marked uroselectivity. This finding suggested that the 2,2,2-trifluoroethoxy moiety could be a key structural element for prostate selectivity [32].

In addition, to evaluate the effect of a specific conformational constraint on affinity, the 2-(2-ethoxyphenoxy)ethanamine moiety of (−)-**1** was replaced with the 1-*cis*-(3-methyl-2,3-dihydro-1,4-benzodioxin-2-yl)methanamino group affording the diastereomers (−)-**5a** and (−)-**5b**. In fact, a similar structural modification on a WB4101-related compound resulted in a α_1-adrenoceptor antagonist potency and α_1/α_2 selectivity higher than those observed for the corresponding *trans* isomer [33] (Fig. 1).

2. Chemistry

Compounds (−)-**2**–(−)-**4** were synthesized by reaction of (−)-5-[(2*R*)-2-aminopropyl]-2-methoxybenzenesulfonamide ((−)-**6**) [34] with the appropriate alkylating agent 1-(2-bromoethoxy)-2-isopropoxybenzene (**7**), 1-(benzyloxy)-2-(2-bromoethoxy)benzene (**8**) [35], or 2-[2-(2,2,2-trifluoroethoxy)-phenoxy]ethyl 4-methylbenzenesulfonate (**9**) [36] in refluxing *i*-AmOH. (−)-**5a** and (−)-**5b** were synthesized as diastereomers by reductive alkylation of amine (−)-**6** with (±)-*cis*-3-methyl-2,3-dihydro-1,4-benzodioxine-2-carbaldehyde (**10**) and sodium cyanoborohydride in MeOH (Scheme 1).

The intermediate amine (−)-**6**, which was included in a patent [34], was obtained by resolution with (*R*)-(−)-mandelic acid of the corresponding racemic amine (±)-**6** [37], synthesized by reductive amination of 2-methoxy-5-(2-oxopropyl)-1-benzenesulfonamide [38] with ammonium acetate and sodium cyanoborohydride in MeOH. After repeated crystallizations from ethanol a pure (−)-mandelate was obtained. The treatment of an aqueous solution of this salt with a K_2CO_3 solution gave (−)-**6** with the same physical characteristics of the reported compound [34]. Its optical purity (99.3%) was determined by reversed phase HPLC analysis of the relative carbamate, obtained by reaction with (1*R*)-(−)-menthyl chloroformate, in comparison with the mixture of menthylcarbamates of (±)-**6**. In the same conditions, the carbamates from (±)-**6** showed two peaks with retention time of 36.19 and 36.53 min, whereas that deriving from (−)-**6** displayed, beside the principal peak at 36.50 min, a significant and detectable small peak relative to the less abundant diastereomer.

The alkylating agent **7** was synthesized by reaction of 1,2-dibromoethane with 2-isopropoxyphenol in presence of KOH in EtOH. The Swern oxidation [39] was used for the synthesis of (±)-*cis*-3-methyl-2,3-dihydro-1,4-benzodioxine-2-carbaldehyde (**10**) starting from (±)-[*cis*-3-methyl-2,3-dihydro-1,4-benzodioxin-2-yl]methanol [33] and a mixture of oxalyl chloride and dimethylsulfoxide in dichloromethane at low temperature.

Compounds (−)-**2**–(−)-**4**, (−)-**5a** and (−)-**5b** were purified by chromatography and characterized by elemental analysis, ^{1}H NMR, specific rotation and chromatographic parameters.

3. Pharmacology

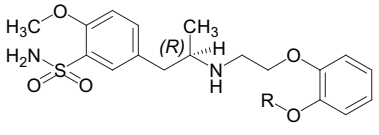
The affinity profile of compounds (−)-**2**–(−)-**4**, (−)-**5a**, (−)-**5b**, and (−)-**1** as reference, was evaluated in radioreceptor binding assays on human cloned α_1-adrenoceptors. Competition experiments were performed using [^{3}H]prazosin to label α_1-adrenoceptor binding sites on membranes of Chinese hamster ovary (CHO) cells expressing human α_{1a}, α_{1b}, and α_{1d}-adrenoceptor subtypes [40]. Binding affinities were expressed as pK_i values derived using the Cheng–Prusoff equation [41].

All compounds were also studied on α_1-adrenoceptor subtypes of rat isolated tissues, using (−)-**1** as reference compound. α_{1A}-and α_{1D}-adrenoceptor blocking activities were evaluated by antagonism of (−)-noradrenaline-induced contractions of rat prostatic vas deferens [42] and aorta [43], respectively, whereas the α_{1B}-adrenoceptor antagonism was determined on the rat spleen tissue by using phenylephrine as agonist [44]. The potency of (−)-**4** and (−)-**5a** at all α_1 subtypes, of (−)-**5b** at α_{1A} and α_{1D}-adrenoceptors, and of (−)-**2** at the α_{1D} subtype was expressed as the pA_2 value calculated by Schild plots at three different concentrations according to Arunlakshana and Schild [45]. The potency of (−)-**1** and (−)-**3** at all α_1 subtypes, of (−)-**2** at α_{1A} and α_{1B}-adrenoceptors, and of (−)-**5b** at the α_{1B} subtype was expressed by the pK_B value according to van Rossum [46] because the slope of the Schild plot was significantly different from unity. In this latter case, the pK_B value was calculated at the lowest antagonist concentration giving a significant rightward shift of the agonist concentration-response curve [log (concentration ratio −1) ≥ 0.5].

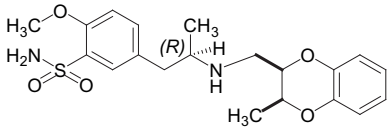
The experimental data were subjected to statistical analysis by means of Student's *t*-test. A p value < 0.05 was taken to indicate a statistically significant difference.

4. Results and discussion

The results of radioligand binding assays and functional tests are presented in Tables 1 and 2, respectively. In binding experiments,

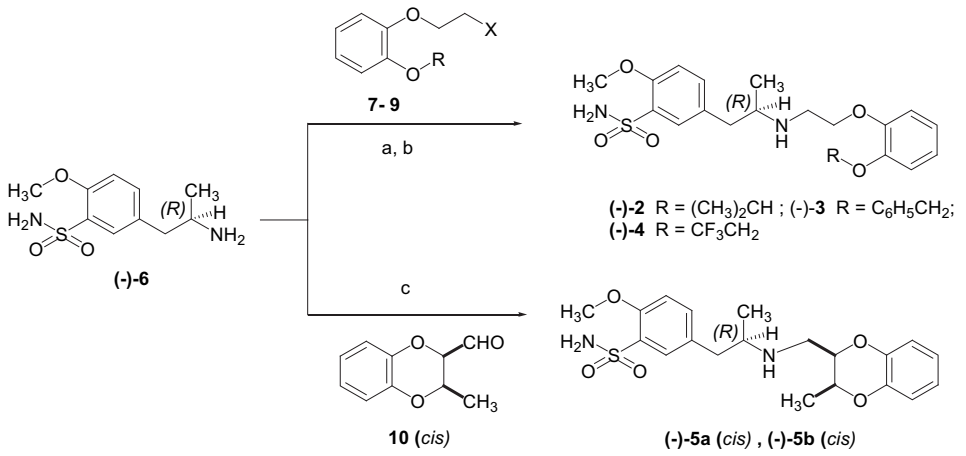


(-)-1 R = CH_3CH_2; **(-)-2** R = $(CH_3)_2CH$;
(-)-3 R = $C_6H_5CH_2$; **(-)-4** R = CF_3CH_2



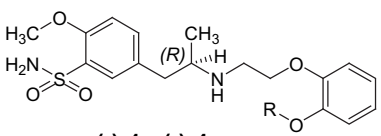
(-)-5a (*cis*) , **(-)-5b** (*cis*)

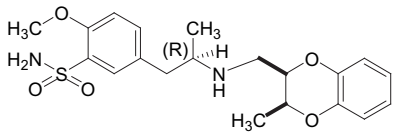
Fig. 1. Structure of compounds (−)-**1** (tamsulosin) and analogues (−)-**2**–(−)-**5**.



Scheme 1.. Reagents and conditions: (a) X = Br with R = $(CH_3)_2CH$ (**7**) and $C_6H_5CH_2$ (**8**); X = TsO with R = CF_3CH_2 (**9**); (b) *i*-AmOH, reflux; (c) $NaBH_3CN$, MeOH, H^+, r.t.

Table 1
Binding affinity constants, expressed as pK_i, of compounds (−)-**2**–(−)-**4**, (−)-**5a**, (−)-**5b**, and (−)-**1** as reference, at cloned human α_1-adrenoceptor subtypes expressed in CHO cells.





Compound	pK_i[a]			Selectivity ratio[b]		
	α_{1a}	α_{1b}	α_{1d}	α_{1a}/α_{1b}	α_{1d}/α_{1b}	α_{1a}/α_{1d}
(−)-**1**	10.30 ± 0.04	9.20 ± 0.08	10.00 ± 0.04	13	6	2
(−)-**2**	9.47 ± 0.02	8.72 ± 0.04	9.61 ± 0.01	5	8	0.7
(−)-**3**	9.46 ± 0.06	8.68 ± 0.01	9.49 ± 0.04	6	6	0.9
(−)-**4**	9.22 ± 0.02	8.26 ± 0.03	9.02 ± 0.07	9	6	2
(−)-**5a**	7.72 ± 0.03	7.17 ± 0.07	7.76 ± 0.01	4	4	0.9
(−)-**5b**	7.94 ± 0.01	7.50 ± 0.09	7.81 ± 0.02	3	2	1

[a] Equilibrium dissociation constants (K_i) were calculated from IC_{50} values using the Cheng–Prusoff equation. The affinity estimates, derived from displacement of [^{3}H] prazosin binding from α_1-adrenoceptors and expressed as mean values of pK_i ± SEM, were from two to three experiments performed in triplicate, which agreed within ±20%.
[b] Calculated by the antilog of the difference between pK_i values at different α_1-adrenoceptor subtypes.

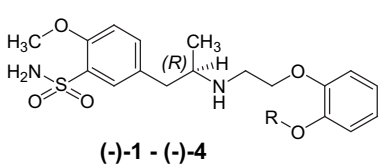
compounds (−)-**2**–(−)-**4** displayed a slightly lower affinity than (−)-**1** at all the three α_1-adrenoceptor subtypes, which was more pronounced (9- to 12-fold) for the fluoro derivative (−)-**4**. In addition, all compounds, like (−)-**1**, showed similar and low $\alpha_{1a,d}$ over α_{1b} selectivity. In contrast, the cyclic analogues (−)-**5a** and (−)-**5b** were markedly less potent than non-cyclic analogues and (−)-**1** at all three α_1-adrenoceptor subtypes.

In rat tissues, (−)-**1** displayed a slightly higher potency for the α_{1D}-adrenoceptor with respect to α_{1A} and α_{1B} subtypes (3- and 5-fold, respectively) as previously reported [17,18]. Compounds (−)-**2**–(−)-**4** were almost equipotent with (−)-**1** at α_{1A} and α_{1B} subtypes, and slightly less potent (5–10-fold) at the α_{1D} subtype, whereas (−)-**5a** and (−)-**5b**, in agreement with the binding results, were much weaker antagonists at all α_1-adrenoceptors (from 35 to 600 times less potent than (−)-**1**). Compound (−)-**3** displayed the best antagonist profile, being as potent as (−)-**1** at the α_{1A}-adrenoceptor (pK_B, 9.66 vs. 9.46) and 5–6-fold less potent at the α_{1B} and α_{1D} subtypes (pK_B, 8.57 and 9.20 vs. 9.30 and 10.00, respectively). Thus, it was 12-fold more selective for the α_{1A}-adrenoceptor over the α_{1B} subtype while maintaining a relevant blocking activity at the α_{1D}-adrenoceptor.

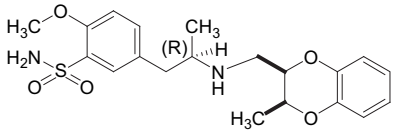
The slightly reduced potency of (−)-**2** at α_{1D}-, and (−)-**3** at α_{1B}- and α_{1D}-adrenoceptors, relatively to (−)-**1**, could be related to an increased steric hindrance of the *i*-propoxy and benzyloxy groups in comparison with the ethoxy moiety of (−)-**1**. Differently, the electronic properties of the 2,2,2-trifluoroethoxy group might be responsible for the reduced potency of (−)-**4** at α_{1D}-adrenoceptor.

On 2002, a molecular modeling study [47] has shown that (−)-**1** binds at the α_1-adrenoceptor subtypes through the interaction of its secondary amine function and methoxy group with three aspartate residues in the third transmembrane (TM3) domain and with three serine residues in TM5 domain, respectively. An additional interaction would take place between the nitrogen atom of the sulphonamide moiety of (−)-**1** and a glutamate residue located into the TM4 domain of α_{1D}-adrenoceptor. Furthermore, previous mutagenesis studies [48] had identified two phenylalanine residues, which are conserved in TM7 domains of all α_1-adrenoceptor subtypes, as binding sites for a non-selective interaction with many α_1-antagonists. As consequence, we hypothesize that the phenyl ring of the 2-ethoxyphenoxy moiety of (−)-**1** could interact with these phenylalanine residues, as advanced for the related 2,6-dimethoxyphenoxy fragment of the α_{1A}-adrenoceptor antagonist

Table 2
Functional antagonist affinities, expressed as pA_2 or pK_B, of compounds (−)-**2** − (−)-**4**, (−)-**5a**, (−)-**5b**, and (−)-**1** as reference, at α_1-adrenoceptor subtypes of isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D}).



(-)-1 - (-)-4



(-)-5a (*cis*) , **(-)-5b** (*cis*)

Compound	R	pA_2[a] or pK_B[b]			Selectivity ratio[c]		
		α_{1A}	α_{1B}	α_{1D}	α_{1A}/α_{1B}	α_{1A}/α_{1D}	α_{1D}/α_{1B}
(−)-**1**	CH_3CH_2	9.46 ± 0.13[b]	9.30 ± 0.08[b]	10.00 ± 0.10[b]	1.5	0.3	5
(−)-**2**	$(CH_3)_2CH$	9.66 ± 0.08[b]	8.81 ± 0.10[b]	9.30 ± 0.04[a]	7	2	3
(−)-**3**	$C_6H_5CH_2$	9.66 ± 0.01[b]	8.57 ± 0.02[b]	9.20 ± 0.04[b]	12	3	4
(−)-**4**	CF_3CH_2	9.58 ± 0.08[a]	8.96 ± 0.02[a]	9.02 ± 0.01[a]	4	4	1
(−)-**5a**		7.52 ± 0.06[a]	7.51 ± 0.04[a]	7.22 ± 0.03[a]	1	2	0.5
(−)-**5b**		7.79 ± 0.04[a]	7.76 ± 0.08[b]	7.67 ± 0.03[a]	1	1	0.8

[a] pA_2 values, expressed as means ± SEM of three different concentrations, each tested at least four times.
[b] pK_B values (±SEM) calculated according to van Rossum.
[c] Calculated by the antilog of the difference between pA_2 or pK_B values at different α_1-adrenoceptor subtypes.

WB4101 [49], whereas the 2-ethoxy function could bind the OH serine residue present into the TM6 domain of all three α_1-adrenoceptors [47]. Thus, it is possible to speculate that specific alkoxy or arylalkoxy groups, in place of the ethoxy moiety of (−)-1, could disrupt these interactions by steric or electronic effects established with some aminoacidic residues into the α_1-adrenoceptor TM domains (Fig. 2). This may explain the small reduction of antagonist activity at α_{1B}-and α_{1D}-adrenoceptors, with respect to (−)-**1**, of compounds (−)-**2**–(−)-**4**, bearing an *i*-propoxy, benzyloxy, and 2,2,2-trifluoroethoxy groups, respectively.

On the other hand, the marked drop in affinity observed for (−)-**5a** and (−)-**5b** at all the α_1-adrenoceptor subtypes could be due to the reduced flexibility and the conformational constraint induced by cyclization of the 2-ethoxyphenoxyethylamino fragment of (−)-**1** into the *cis*-3-methyl-[2,3-dihydro-1,4-benzodioxin-2-yl]methylamino moiety. This structural modification could negatively affect one or both of above supposed interactions with TM6 and TM7 domains with a possible and important reduction of the overall binding (Fig. 2).

5. Conclusion

The benzyl analogue (−)-**3** of (−)-**1** is the most interesting compound of the present study, owing to its antagonist profile at α_{1A}-and α_{1D}-adrenoceptors ($\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$) in functional tests, with a 12-fold higher potency at α_{1A}-adrenoceptor with respect to the α_{1B} subtype. These properties could have relevance in BPH therapy because compounds like (−)-**3** may have improved

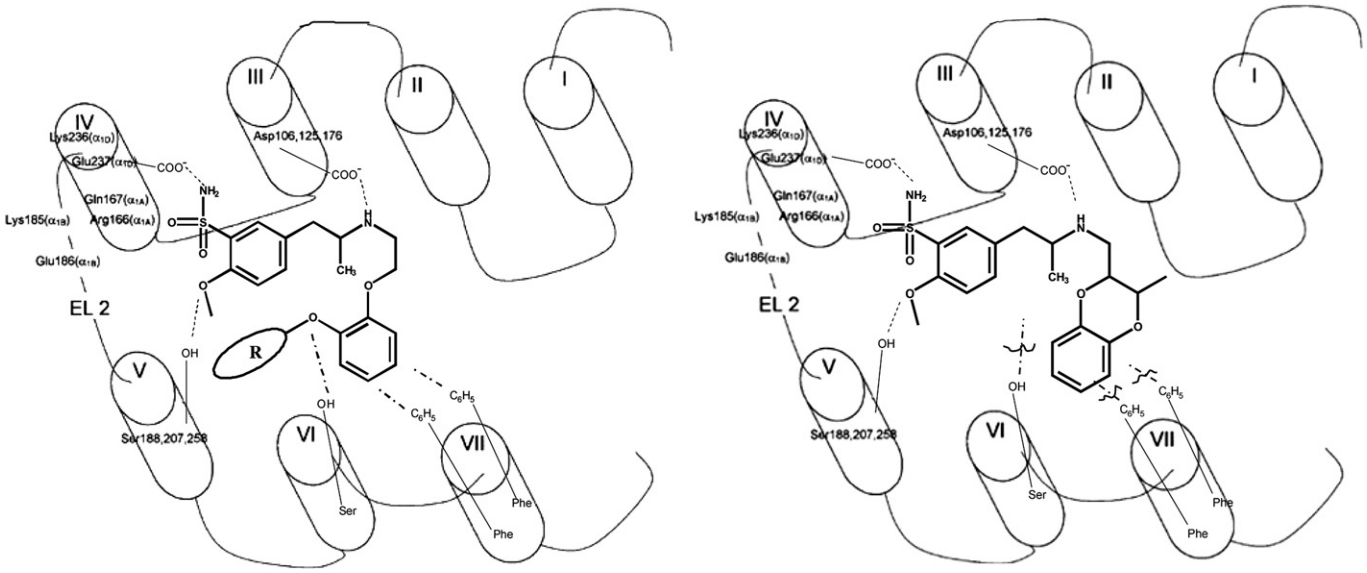


Fig. 2. Hypothetical interaction model of tamsulosin analogue compounds (−)-**2** − (−)-**4** and (−)-**5a** and (−)-**5b** with the aminoacidic residues of seven transmembrane domains of α_1-adrenoceptors. The model derives from that suggested by Ishiguro for tamsulosin [47], involving the binding of some chemical functions with the TM3, TM4, and TM5 domains, which has been here adapted with new supposed interactions. It is conceivable that compounds (−)-**2**–(−)-**4**, with R = $(CH_3)_2CH$, $C_6H_5CH_2$, and CF_3CH_2, would bind also to the serine and phenylalanine residues of TM6 and TM7 domains, respectively (*left*); on the contrary, the same interactions would be missing for compounds (−)-**5a** and (−)-**5b** (*right*).

uroselectivity compared to (−)-**1**. This is because of the potential major beneficial clinical effects deriving from the preferential antagonism of the α_{1A}-adrenoceptor, which relieves the voiding symptoms due to the bladder outlet obstruction mediated by prostate smooth muscle contraction, and from an effective antagonism towards the α_{1D} subtype, which alleviates the symptoms of bladder filling. At the same time, the reduced blocking activity of the α_{1B}-adrenoceptor subserves the uroselectivity character of (−)-**3** because of minimization of the hypotensive side effect, especially in the elderly.

6. Experimental protocols

6.1. 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, performed on a Fisons instrument mod. EA1108CHNS-O, agreed with the calculated values within the range ±0.4%. Electron impact ionization (EI) mass spectra were obtained with a Hewlett–Packard instrument, consisting of model 5890 A for the separation section and model 5971 A for the mass section. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. Analytical HPLC was performed on a Hewlett–Packard 1090 apparatus, series II, with UV detector, equipped with a Luna C_{18} column (250 mm × 4.6 mm i.d., 5 μm) from Phenomenex (Chesire, UK). Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash (when non specified) or gravity 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, chloroform–ethyl acetate–methanol–28% ammonia (4:4:2:0.1); B, ethyl acetate–cyclohexane (5:5); C, chloroform–ethyl acetate–methanol–28% ammonia (4:4:1:0.1); D, chloroform–ethyl acetate–methanol–28% ammonia (4:4:0.5:0.05); E, ethyl acetate–petroleum ether (2:8); F, chloroform–ethyl acetate–methanol–28% ammonia (4:4:0.2:0.05); G, petroleum ether–methanol (9:1). 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 software, version 7.0 (Advanced Chemistry Development, Inc., Toronto, Canada). Yields of purified products were not optimized. Chemicals and reagents were purchased from Sigma–Aldrich Srl (Milano, Italy) or Lancaster research chemicals (Chiminord, Srl, Cusano Milanino, Milano, Italy).

6.1.1. (±)-5-(2-Aminopropyl)-2-methoxybenzenesulfonamide [(±)-6]

Amonium acetate (8.41 g, 109 mmol) and sodium cyanoborohydride (0.69 g, 10.9 mmol) were added consecutively to a suspension of 2-methoxy-5-(2-oxopropyl)-1-benzenesulfonamide (2.5 g, 10.9 mmol) in MeOH (75 mL), then the mixture was stirred at room temperature for 15 h. After removal of inorganic salts by filtration through a short silica column and quick elution with mixture A, evaporation of the solvent left an oily residue that was transformed into the hydrochloride salt, which was crystallized from MeOH. The corresponding free base was obtained treating an aqueous solution (11 mL) with K_2CO_3 (7.46 g). After 2 h stirring at room temperature, the crude precipitate was collected and purified by column chromatography eluting with mixture A to give the racemic amine (±)-**6** as amorphous solid: 0.9 g (35%); mp 166–168 °C (ref. 30, 166–167 °C); R_f = 0.30 (mixture A); ^{1}H NMR (DMSO-d_6): δ 0.93 (d, J = 6.2 Hz, 3H, CH_3CH), 2.42–2.66 (m, 2H, CH_2Ar, partly overlapped to solvent), 2.98 (sextet, J = 6.2 Hz, 1H, CHCH$_3$), 3.85 (s, 3H, OCH$_3$), 7.10 (m, 1H, Ar–H^3), 7.32–7.45 (m, 1H, Ar–H^4), 7.53 (m, 1H, Ar–H^6). The NH_2 and SO_2NH_2 protons are unapparent on the spectrum; they are expected under the waters' low and flattened hump observed in the range 3.20–3.80 δ. Anal. Calc. for $C_{10}H_{16}N_2O_3S\cdot0.25H_2O$: C, 48.28; H, 6.68; N, 11.25. Found: C, 48.27; H, 6.98; N, 11.16%.

*6.1.1.1. Resolution of (±)-**6**.* A solution of (±)-**6** (20 g, 81.86 mmol) and (*R*)-(−)-mandelic acid (12.46 g, 81.86 mmol) in MeOH (180 mL) was evaporated to dryness to give a residue that was crystallized seven times from EtOH, affording the less soluble diastereomeric mandelate: 1.1 g, mp 205–206 °C, $[\alpha]_D^{20} = -44.6$ (c = 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.03 (d, J = 6.2 Hz, 3H, CH_3CH), 2.46–2.68 (m, 1H, CH_2CH, partly overlapped to solvent), 2.80–2.96 (m, 1H, CH_2CH), 3.41 (sextet, J = 6.2 Hz, 1H, CHCH$_3$), 3.84 (s, 3H, OCH$_3$), 4.50 (s, 1H, CHOH), 6.80–7.42 (m, 13H, Ar–H, Ar–H^3, Ar–H^4, SO_2NH_2, NH_3^+, OH, partly exchangeable with D_2O), 7.55 (m, 1H, Ar–H^6). Anal. Calc. for $C_{18}H_{24}N_2O_6S\cdot1.5H_2O\cdot0.1C_2H_5$-OH: C, 51.06; H, 6.49; N, 6.47. Found: C, 50.77; H, 6.21; N, 6.17%.

Potassium carbonate (3.5 g) was added to a solution of the above salt (0.7 g) dissolved in H_2O (11.2 mL). Following 3 h stirring at room temperature, the precipitate was collected by filtration to give enantiomer (−)-**6** as amorphous white solid: 0.45 g; mp 165–166 °C; $[\alpha]_D^{20} = -16.8$ (c = 1, MeOH) (ref. 27, mp 166–167 °C, $[\alpha]_D^{23} = -17.3$ (c = 1.07, MeOH)). ^{1}H NMR (DMSO-d_6): δ 0.92 (d, J = 6.2 Hz, 3H, CH_3CH), 2.40–2.70 (m, 2H, CH_2CH, partly overlapped to solvent), 2.95 (sextet, J = 6.2 Hz, 1H, CHCH$_3$), 3.85 (s, 3H, OCH$_3$), 7.10 (m, 1H, Ar–H^3), 7.30–7.40 (m, 1H, Ar–H^4), 7.51 (m, 1H, Ar–H^6). The NH_2 and SO_2NH_2 protons are unapparent on the spectrum; they are expected under the waters' low and flattened hump observed in the range 3.20–3.80 δ. Anal. Calc. for $C_{10}H_{16}N_2O_3S\cdot0.25H_2O$: C, 48.28; H, 6.68; N, 11.25. Found: C, 48.01; H, 6.77; N, 11.44%.

*6.1.1.2. Determination of the optical purity of (−)-**6**.* (1*R*)-(−)-Menthyl chloroformate (0.13 g, 0.61 mmol) and 2 N NaOH (0.15 mL, 0.31 mmol) were simultaneously added dropwise to a cooled (0 °C) and stirred solution of (±)-**6** (0.15 g, 0.61 mmol) in 2 N NaOH (0.15 mL, 0.31 mmol). After stirring 2 h at 0 °C and 2 h at room temperature, the reaction mixture was acidified with 2 N HCl and extracted with ethyl acetate. Removal of the dried solvent gave a crude residue that was purified by column chromatography (mixture B) to give 0.1 g of a mixture of the two diastereomers as waxy solid: MS (EI) m/z = 426 [M$^+$]. ^{1}H NMR (DMSO-d_6), δ: 0.75–2.08 (m, 21H, CH_3CHN and menthyl), 2.60–2.92 (m, 2H, CH_2CHN), 4.02 (s, 3H, OCH$_3$), 4.32–4.60 (m, 2H, CH$_3$CHN and OCH menthyl), 5.06 (br s, 3H, CONH and SO_2NH_2, exchangeable with D_2O), 6.98 (m, 1H, Ar–H^3), 7.32–7.44 (m, 1H, Ar–H^4), 7.73 (m, 1H, Ar–H^6).

Similarly, the diastereomer of (−)-**6** with (1*R*)-(−)-menthyl chloroformate was prepared following the above procedure: MS (EI) m/z = 426 [M$^+$]. ^{1}H NMR (DMSO-d_6), δ: 0.68–2.07 (m, 21H, CH_3CHN and menthyl), 2.60–2.93 (m, 2H, CH_2CHN), 4.00 (s, 3H, OCH$_3$), 4.30–4.60 (m, 2H, CH$_3$CHN and OCH menthyl), 5.12 (br s, 3H, CONH and SO_2NH_2, exchangeable with D_2O), 6.99 (m, 1H, Ar–H^3), 7.30–7.44 (m, 1H, Ar–H^4), 7.53 (m, 1H, Ar–H^6).

The HPLC analysis of the diastereomer obtained from (−)-**6** and of the mixture of the two diastereomers mixture obtained from (±)-**6** was performed with a reversed phase Luna C_{18} column (250 mm × 4.6 mm i.d., 5 μm) fluxed (1 mL/min) 20 min with a mixture 70:30 (v/v) of H_2O/CH_3CN, then changed to reach in 15 min a 30:70 ratio. The injection of a sample solution (5 μL, c 2.5 mg/mL) of the mixture obtained from (±)-**6**, detectable at 275 nm, displayed two peaks with retention time of 36.19 and

36.53 min, whereas the diastereomer obtained from (−)-**6** gave a principal peak at a retention time of 36.50 min with a 99.3% of enantiomeric purity.

*6.1.2. General procedure for the synthesis of (−)-2–(−)-**4***

A mixture of (−)-**6** (0.25 g, 1.23 mmol) and the appropriate alkylating agent **7**, **8**, or **9** (0.51 mmol) in *i*-AmOH (10 mL) was refluxed 3 h. After filtration, the solvent was distilled at reduced pressure and the residue was purified by gravity column chromatography.

*6.1.2.1. (−)-5-((2R)-2-{[2-(2-Isopropoxyphenoxy)ethyl]amino} propyl)-2-methoxybenzenesulfonamide (−)-**2**.* 0.08 g (37%); mp 108–109 °C; $R_f = 0.32$ (mixture C); $[\alpha]_D^{20} = -15$ ($c = 0.5$, MeOH). ^{1}H NMR (DMSO-d_6): δ 0.91 (d, $J = 6.0$ Hz, 3H, CH_3CH), 1.20 (d, $J = 6.1$ Hz, 6H, $(CH_3)_2CH$), 1.62 (br s, 1H, NH, exchangeable with D_2O), 2.37–2.51 (m, 1H, CH_2CH, partly overlapped to solvent), 2.66–2.98 (m, 4H, CH_2CH, CH_3CH, and NCH_2), 3.84 (s, 3H, OCH_3), 3.97 (t, $J = 5.6$ Hz, 2H, CH_2CH_2O), 4.43 (septet, $J = 6.1$ Hz, 1H, $(CH_3)_2CH$), 6.82–7.15 (m, 7H, Ar–H, Ar–H^3, SO_2NH_2, partly exchangeable with D_2O), 7.33–7.40 (m, 1H, Ar–H^4), 7.54 (m, 1H, Ar–H^6). Anal. Calc. for $C_{21}H_{30}N_2O_5S \cdot 0.25H_2O$: C, 59.06; H, 7.20; N, 6.56. Found: C, 59.24; H, 7.59; N, 6.67%.

*6.1.2.2. (−)-5-[(2R)-2-({2-[2-(Benzyloxy)phenoxy]ethyl}amino) propyl]-2-methoxybenzenesulfonamide (−)-**3**.* 0.06 g (25%); mp 126–127 °C; $R_f = 0.44$ (mixture D); $[\alpha]_D^{20} = -11.2$ ($c = 0.5$, MeOH). ^{1}H NMR (DMSO-d_6): δ 0.87 (d, $J = 5.8$ Hz, 3H, CH_3CH), 1.75 (br s, 1H, NH, exchangeable with D_2O), 2.33–2.52 (m, 1H, CH_2CH, partly overlapped to solvent), 2.63–3.00 (m, 4H, CH_2CH, CH_3CH, and NCH_2), 3.83 (s, 3H, OCH_3), 4.01 (t, $J = 5.6$ Hz, 2H, CH_2CH_2O), 5.08 (s, 2H, $C_6H_5CH_2$), 6.82–7.10 (m, 7H, Ar–H, Ar–H^3, SO_2NH_2, partly exchangeable with D_2O), 7.28–7.46 (m, 6H, $C_6H_5CH_2$, Ar–H^4), 7.53 (m, 1H, Ar–H^6). Anal. Calc. for $C_{25}H_{30}N_2O_5S \cdot 0.5H_2O$: C, 62.61; H, 6.51; N, 5.84. Found: C, 62.88; H, 6.86; N, 5.51%.

*6.1.2.3. (−)-2-Methoxy-5-[(2R)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino)propyl]benzenesulfonamide (−)-**4**.* 0.01 g (4%); mp 117–118 °C; $R_f = 0.42$ (mixture C); $[\alpha]_D^{20} = -12$ ($c = 0.5$, MeOH). ^{1}H NMR (DMSO-d_6): δ 0.92 (d, $J = 6.2$ Hz, 3H, CH_3CH), 1.70 (br s, 1H, NH, exchangeable with D_2O), 2.36–2.52 (m, 1H, CH_2CH, partly overlapped to solvent), 2.68–3.06 (m, 4H, CH_2CH, CH_3CH, and NCH_2), 3.84 (s, 3H, OCH_3), 4.04 (t, $J = 5.6$ Hz, 2H, CH_2CH_2O), 4.68 (quartet, $J = 9.0$, 2H, CH_2CF_3), 6.88–7.16 (m, 7H, Ar–H, Ar–H^3, SO_2NH_2, partly exchangeable with D_2O), 7.32–7.43 (m, 1H, Ar–H^4), 7.55 (m, 1H, Ar–H^6). Anal. Calc. for $C_{20}H_{25}F_3N_2O_5S \cdot 0.5H_2O$: C, 50.94; H, 5.55; N, 5.94. Found: C, 50.65; H, 5.36; N, 6.30%.

*6.1.3. (±)-cis-3-Methyl-2,3-dihydro-1,4-benzodioxine-2-carbaldehyde (**10**)*

A solution of DMSO (0.4 g, 5.28 mmol) in dry CH_2Cl_2 (0.5 mL) was added dropwise to a stirred and cooled at − 60 °C solution of $(COCl)_2$ (0.31 g, 2.4 mmol) in dry CH_2Cl_2 (12 mL). After 2 min, the temperature was allowed to raise until − 30 °C, then a solution of [*cis*-3-methyl-2,3-dihydro-1,4-benzodioxin-2-yl]methanol (0.2 g, 1.10 mmol) in dry CH_2Cl_2 (2 mL) was added to reaction mixture. After stirring for additional 15 min, triethylamine (0.56 g, 5.5 mmol) was dropped inwards at the same temperature, which was left to increase to room temperature. The resulting mixture was stirred for further 2 h, treated with H_2O/CH_2Cl_2 and then extracted several time with $CHCl_3$. After washing with 1% HCl, 5% Na_2CO_3 and H_2O, the organic solvents were dried and then evaporated. The residue was purified by gravity column chromatography (mixture E) to give the aldheyde **10** as an oil: 0.11 g (61%); $R_f = 0.45$ (mixture E); MS (EI) $m/z = 178$ [M$^+$]; ^{1}H NMR (DMSO-d_6): δ 1.30 (d, $J = 6.6$ Hz, 3H, CH_3), 4.19 (dq, $J = 2.8$, 6.6 Hz, 1H, H^3), 4.30 (d, $J = 2.8$ Hz, 1H, H^2), 6.80–7.00 (m, 4H, Ar–H), 9.63 (s, 1H, CHO).

*6.1.4. (−)-2-Methoxy-5-((2R)-2-cis-{[(3-methyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]-amino} propyl)benzenesulfonamide [(−)-**5a** and (−)-**5b**]*

A solution of **10** (0.09 g, 0.51 mmol) in MeOH (5 mL), $NaBH_3CN$ (0.032 g, 0.51 mmol), and an excess of molecular sieves 3 Å were consecutively added to a solution of (−)-**6** (0.25 g, 1.23 mmol) in MeOH (10 mL) acidified to pH 6 with 2 N HCl in MeOH. After 72 h stirring at room temperature, the molecular sieves were removed by filtration and the solvent evaporated. The residue was purified by gravity column chromatography (mixture F) to give diastereomers (−)-**5**a and (−)-**5b** as white spongy solids.

*6.1.4.1. (−)-**5a**.* 0.011 g; mp 106–108 °C; $R_f = 0.48$ (mixture F); $[\alpha]_D^{20} = -23.6$ ($c = 0.5$, MeOH). ^{1}H NMR (DMSO-d_6): δ 0.93 (d, $J = 5.8$ Hz, 3H, C*H*$_3$CHN), 1.27 (d, 3H, C*H*$_3$CHO), 1.68 (br s, 1H, NH, exchangeable with D_2O), 2.35–2.60 (m, 1H, C*H*$_2$CH, partly overlapped to solvent), 2.64–3.05 (m, 4H, C*H*$_2$CH, CH$_3$C*H*N, NCH_2), 3.75–3.95 (m, 4H OCH_3, H^2 benzodioxin), 4.00–4.20 (m, 1H, H^3 benzodioxin), 6.75–6.90 (m, 4H, Ar–H benzodioxin), 6.95–7.15 (m, 3H, H^3, SO_2NH_2, partly exchangeable with D_2O), 7.35–7.43 (m, 1H, H^4), 7.55 (m, 1H, H^6). Anal. Calc. for $C_{20}H_{26}N_2O_5S \cdot 0.75H_2O$: C, 57.19; H, 6.59; N, 6.66. Found: C, 57.46; H, 6.39; N, 6.59%.

*6.1.4.2. (−)-**5b**.* 0.018 g; mp 106–108 °C; $R_f = 0.40$ (mixture F); $[\alpha]_D^{20} = -19.6$ ($c = 0.5$, MeOH). ^{1}H NMR (DMSO-d_6): δ 0.91 (d, $J = 5.8$ Hz, 3H, C*H*$_3$CHN), 1.18 (d, 3H, C*H*$_3$CHO), 1.72 (br s, 1H, NH, exchangeable with D_2O), 2.35–2.55 (m, 1H, C*H*$_2$CHN, partly overlapped to solvent), 2.62–2.98 (m, 4H, C*H*$_2$CHN, CH$_3$C*H*N, NCH_2), 3.85 (s, 3H, OCH_3), 4.05–4.20 (m, 1H, H^2 benzodioxin), 4.35–4.48 (m, 1H, H^3 benzodioxin), 6.75–6.87 (m, 4H, Ar–H benzodioxin), 6.95–7.16 (m, 3H, H^3, SO_2NH_2, partly exchangeable with D_2O), 7.30–7.42 (m, 1H, H^4), 7.54 (m, 1H, H^6). Anal. Calc. for $C_{20}H_{26}N_2O_5S \cdot 0.25H_2O$: C, 58.45; H, 6.50; N, 6.81. Found: C, 58.30; H, 6.82; N, 6.58%.

*6.1.5. 1-(2-Bromoethoxy)-2-isopropoxybenzene (**7**)*

A mixture of 2-isopropoxyphenol (3 g, 19 mmol), 1,2-dibromoethane (29.62 g, 150 mmol), and KOH (1.06 g, 19 mmol) in EtOH (50 mL) was refluxed for 75 h. After distillation of solvent and 1,2-dibromoethane excess at reduced pressure, the residue was washed with 2 N NaOH and extracted with $CHCl_3$. Removal of the dried solvent gave a residue that was purified by column chromatography (mixture G), affording **7** as an oil: 2.2 g (45%); $R_f = 0.56$ (mixture G); MS (EI) $m/z = 258$ [M$^+$]. ^{1}H NMR ($CDCl_3$): δ 1.37 (d, $J = 6.2$ Hz, 6H, $(CH_3)_2CH$), 3.66 (t, $J = 6.6$ Hz, 2H, CH_2Br), 4.33 (t, $J = 6.6$ Hz, 2H, OCH_2), 4.56 (septet, $J = 6.2$ Hz, 1H, $(CH_3)_2CH$), 6.82–7.12 (m, 4H, Ar–H).

6.2. Pharmacology

6.2.1. Binding assays

Competition binding assays to cloned human α_{1a}, α_{1b}, and α_{1d}-adrenoceptor subtypes were performed in membrane preparations from CHO (Chinese Hamster Ovary) cell lines transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoceptor. Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described [40]. Briefly, CHO cells membranes (30 μg proteins) were incubated in 50 mM Tris–HCl buffer, pH 7.4, with 0.1–0.4 nM [^{3}H]prazosin, in a final volume of 1.02 mL for 30 min at 25 °C, in the absence or presence of competing drugs (1 pM-10 μM). Non-specific binding was determined in the presence of 10 μM phentolamine. The

incubation was stopped by addition of ice-cold Tris–HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

6.2.2. Functional experiments

Tissues for experiments were taken from male Wistar rats (275–300 g; Charles River, Como, Italy). All animal testing was carried out according to the European Community Council Directive of 24 November 1986 (86/609/EEC). Animals were sacrificed by cervical dislocation and the required organs were isolated. Vas deferens prostatic portion, spleen and aorta were freed from adhering connective tissue and set up rapidly, under a suitable tension, in 20-mL organ baths. The bath medium, containing physiological salt solution (pH 7.4), was kept at 37 °C and aerated with 5% CO_2: 95% O_2. Concentration-response curves were constructed by cumulative addition of agonist. The agonist concentration in the bath was increased approximately 3-fold at each step, with each addition being made only after the response of the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force displacement transducer connected to the MacLab System PowerLab/800.

In all experiments a control agonist concentration-response curve (vehicle) was constructed in the presence of the maximum DMSO concentration (0.5%) contained in the bathing solutions being the solvent used for dissolution of tested antagonists on preparing the initial stock solution. These curves were not different from the previous one indicating no interference of solvent in the agonist effect. The agonist-elicited concentration-response curves obtained in the presence of the tested concentrations of antagonist were related to the vehicle control curve, taking the maximal response as 100%. Parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity. The experimental conditions used for the investigation at α_1-adrenoceptor subtypes are procedures taken from quoted literatures.

All pharmacological graphics were drawn by a Prism 4.0 computer program (GraphPad Software, Inc., San Diego, CA, USA). Chemicals, (−)-noradrenaline bitartrate, (−)-phenylephrine hydrochloride, cocaine hydrochloride, normetanephrine hydrochloride and (±)-propranolol hydrochloride were purchased from Sigma–Aldrich Srl (Milano, Italy).

6.2.2.1. Prostatic rat vas deferens. Affinity at α_{1A}-adrenoceptor was evaluated on prostatic rat vas deferens according to a reported procedure [42]. Prostatic portions of 2 cm length were mounted under 0.35 g tension at 37 °C in Tyrode solution of the following composition (mM): NaCl, 130; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 0.89; NaH_2PO_4, 0.42; $NaHCO_3$, 25; glucose, 5.6. To prevent the neuronal uptake of the agonist noradrenaline, cocaine hydrochloride (10 μM) was added to the Tyrode solution 20 min before the agonist cumulative concentration-response curve. Vasa deferentia were equilibrated for 45 min, with washing every 15 min. After the equilibration period, tissues were primed twice by addition of 10 μM noradrenaline in order to obtain a constant response. After another washing and equilibration period of 45 min, a cumulative isotonic noradrenaline concentration-response curve was constructed to determine the relationship between agonist concentrations and contractile response. When measuring the effect of the antagonist, it was allowed to equilibrate with the tissue for 30 min before constructing a new concentration-response curve to the agonist. The noradrenaline solution contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

6.2.2.2. Aorta. Affinity at rat aorta α_{1D}-adrenoceptor was evaluated using a procedure adapted from that already reported [43]. Two strips (15 mm × 3 mm) were cut helically from rat thoracic aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of 100 μM acetylcholine-induced relaxation to preparations contracted with 1 μM noradrenaline was taken as an indicator that the vessel was denuded successfully. The strips were then tied with surgical thread and suspended in an organ bath containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.7. Cocaine hydrochloride (10 μM), normetanephrine hydrochloride (1 μM), and propranolol hydrochloride (1 μM) were added to prevent the neuronal and extraneuronal uptake of the agonist noradrenaline and to block the β-adrenoceptors, respectively. In the absence of these inhibitors the noradrenaline concentration-response curve was significantly displaced to the right (data not shown).

After an equilibration period of at least 2 h under an optimal tension of 1 g, cumulative noradrenaline concentration-response curves were recorded isometrically at 1 h intervals, the first being discarded and the second one taken as control. After inspection of vehicle activity, the antagonist was allowed to equilibrate with the tissue for 30 min before generation of the third cumulative concentration-response curve to the agonist. Noradrenaline solutions contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

6.2.2.3. Spleen. Affinity at rat spleen α_{1B}-adrenoceptor was evaluated according to a reported procedure [44]. The spleen was removed and bisected longitudinally in two strips, which were suspended in tissue baths containing Krebs solution of the following composition (mM): NaCl, 120; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.5; KH_2PO_4, 1.2; $NaHCO_3$, 20; glucose, 11; EDTA, 0.01. Propranolol hydrochloride (4 μM) was added to block β-adrenoceptors. Following the reported procedure the spleen strips were placed under 1 g resting tension and equilibrated for 2 h. A first cumulative concentration-response curve to the agonist phenylephrine was fast taken isometrically, followed by 30 min washing. Subsequently, a second cumulative curve was constructed followed by 30 min washing. Each tissue was then incubated for 30 min either with vehicle or different antagonist concentrations before constructing the new phenylephrine concentration-response curve (third curve).

6.2.3. Data analysis

Data from binding assays were analysed using a non-linear curve-fitting program Allfit [50]. Scatchard plots were linear in all preparations and the pseudo-Hill coefficients non significantly different from the unity ($p > 0.05$). The inhibition of the radioligand specific binding by tested compounds allowed the estimation of IC_{50} values that were converted to affinity constants (K_i) by the Cheng–Prusoff equation [41]: $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. Results were expressed as pK_i values.

In functional studies, responses were expressed as percentage of the maximal contraction observed in the agonist concentration-response curve taken as control. Each response was plotted graphically as a mean from at least four separate experiments. Curves were fitted to all the data by a non-linear regression using the Prism 3.0 program to calculate pEC_{50} values. In all cases, 50% of the maximum for each concentration-response curve was used to evaluate the EC_{50}. This value, calculated in presence and in absence of antagonist in a single tissue, was used to determine the concentration ratio.

Schild plots were constructed to estimate the pA_2 values and the slope of the regression line using experimental series obtained from at least three different concentrations [45]. The Schild diagrams were constructed by plotting the log (concentration ratio −1) against the log [antagonist] and deriving it from a linear regression using the Prism 3.0 program. When the Schild plot slope

was not significantly different from unity ($p > 0.05$), the regression was recalculated with a constrained slope of 1 and the result given as a pA_2 value. In a number of cases, Schild analysis could not be performed due to the nonparallel slopes of concentration-response curves. As consequence, pK_B values were calculated, according to van Rossum [46], at the lowest antagonist concentration giving a significant rightward shift of the agonist concentration-response curve [log (concentration ratio -1) ≥ 0.5]. Thus, the potency of (−)-**4** and (−)-**5a** at all α_1 subtypes, of (−)-**5b** at α_{1A} and α_{1D}-adrenoceptors, and of (−)-**2** at the α_{1D} subtype was expressed as the pA_2 value, whereas the potency of (−)-**1** and (−)-**3** at all α_1 subtypes, of (−)-**2** at α_{1A} and α_{1B}-adrenoceptors, and of (−)-**5b** at the α_{1B} subtype was expressed by the pK_B value.

All data were compared by Student's *t*-test and presented as means ± SEM of 2–3 experiments, performed in triplicate, in binging assays, and of 4–6 experiments in functional tests. A p value < 0.05 was taken to indicate a statistically significant difference.

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