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6-Methoxy-7-benzofuranoxy and 6-Methoxy-7-indolyloxy Analogues of 2-[2-(2,6-Dimethoxyphenoxy)ethyl]aminomethyl-1,4-benzodioxane (WB4101):[1] Discovery of a Potent and Selective α_{1D}-Adrenoceptor Antagonist

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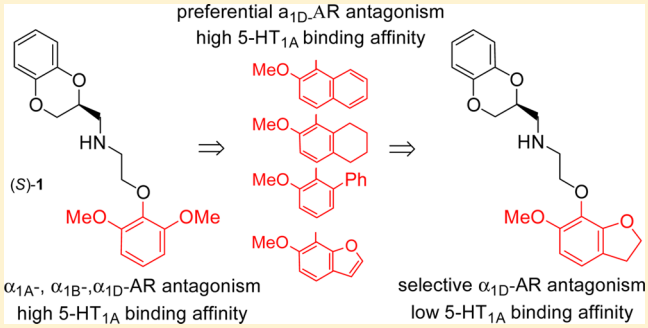
Supporting Information

ABSTRACT: Previous results have shown that replacement of one of the two *o*-methoxy groups at the phenoxy residue of the potent, but not subtype-selective, α_1-AR antagonist (*S*)-WB4101 [(*S*)-**1**] by phenyl, or by ortho,meta-fused cyclohexane, or especially by ortho,meta-fused benzene preferentially elicits α_{1D}-AR antagonist affinity. Such observations inspired the design of four new analogues of **1** bearing, in lieu of the 2,6-dimethoxyphenoxy residue, a 6-methoxy-substituted 7-benzofuranoxy or 7-indolyloxy group or, alternatively, their corresponding 2,3-dihydro form. Of these new compounds, which maintain, rigidified, the characteristic ortho heterodisubstituted phenoxy substructure of **1**, the *S* enantiomer of the dihydrobenzofuranoxy derivative exhibited the highest α_{1D}-AR antagonist affinity (pA_2 9.58) with significant α_{1D}/α_{1A} and α_{1D}/α_{1B} selectivity. In addition, compared both to α_{1D}-AR antagonists structurally related to **1** and to the well-known α_{1D}-AR antagonist BMY7378, this derivative had modest 5-HT_{1A} affinity and neutral α_1-AR antagonist behavior.



INTRODUCTION

The availability of potent and selective antagonists for each of the three α_1 adrenoceptor (α_1-AR) subtypes remains a demanding challenge. A recent review on the functional characterization of α_1-AR subtypes laments the lack of reliable α_{1B}- and α_{1D}-AR antagonists without questionable selectivity in functional tests and with no affinity at receptors other than α_1-ARs, such as 5-HT_{1A} and α_2-AR.[2] The unavailability of truly selective α_{1B}-AR agonists and antagonists hampers studies on the function of α_{1B}-AR, thus making necessary the use of knockout technology, while to investigate the responses mediated by α_{1D}-AR, the only pharmacological tool seems to be **2** (BMY 7378) (Chart 1), a molecule which dates back to 1995 and is an antagonist also at the 5-HT_{1A} and α_{2C} receptors.[3] On the other hand, an attractive candidate for structural modifications aimed at maintaining one of the three antagonist activities while depressing the two others remains (*S*)-**1** [(*S*)-WB4101] (Chart 1), a molecule exhibiting subnanomolar antagonist affinities at all the three subtypes and consisting of two distinct rigid substructures connected by a relatively flexible five-atom linear chain.[3]

In the course of several years, we have studied more than 50 enatiomeric pairs of analogues of **1** modified at the aminoalkyloxy chain,[5] at the benzodioxane,[6−8] at the phenoxy residue,[7,9−11] or at two of these three fragments,[4,9] finding the potent and very selective α_{1A}-AR antagonist (*S*)-**3**[3] and, recently, the very potent and remarkably selective α_{1B}-AR antagonist (*S*)-**4**[8] (Chart 1,Table 1). The latter was obtained by introducing a methoxyl substituent at the 8 position of benzodioxane of (*S*)-**1**, while the former was obtained by replacing the benzodioxane and the 2,6-dimethoxyphenoxy moiety of (*S*)-**1** with tetrahydronaphthodioxane and 2-methoxy-1-naphthoxyl, respectively. Furthermore, the only replacement of 2,6-dimethoxyphenoxy by 2-methoxy-1-naphthoxy without modifying the benzodioxane nucleus resulted in the impressively potent α_{1D}-AR antagonist (*S*)-**5**, whose α_{1B} and α_{1A} antagonist affinities are very high but significantly lower than that at the α_{1D}-AR (Chart 1, Table 1).[4]

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Chart 1. Structures of 2, 30, (*S*)-1, and α_1-AR Subtype-Selective Antagonists Related to (*S*)-1

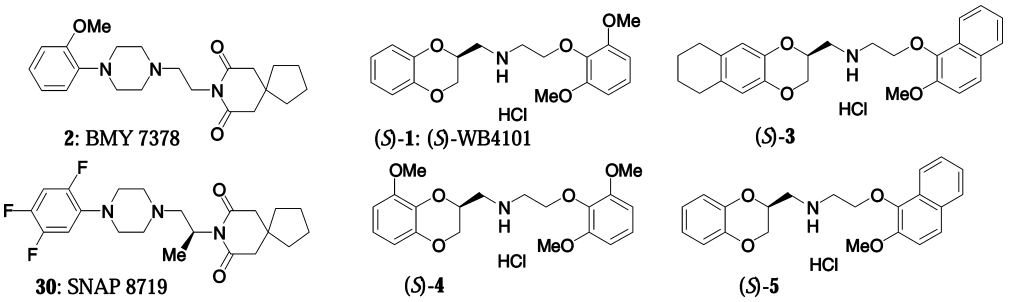


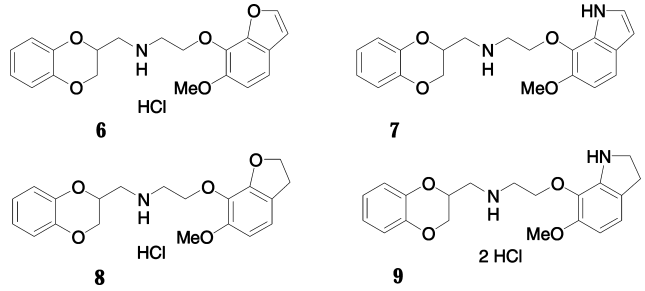
Table 1. Antagonist Affinities, Expressed as pA_2, of (*S*)-1, (*S*)-3, (*S*)-4, and (*S*)-5 at α_1- and α_2-Adrenoceptors on Isolated Rat Tissues, Namely Prostate (α_{1A}), Spleen (α_{1B}), Thoracic Aorta (α_{1D}), and Prostatic Vas Deferens (α_2)

	pA_2[a]					
	α_{1A}	α_{1B}	α_{1D}	α_2		
compd	prostate	spleen	thoracic aorta	prostatic vas deferens	affinity ratios	
(*S*)-**1**	9.49	9.17	9.20	6.92	2.1 (α_{1A}/α_{1B})	1.9 (α_{1A}/α_{1D})
(*S*)-**3**	7.98	<5	5.59	<5	954 (α_{1A}/α_{1B})	245 (α_{1A}/α_{1D})
(*S*)-**4**	8.55	9.58	7.93	6.23	11 (α_{1B}/α_{1A})	45 (α_{1B}/α_{1D})
(*S*)-**5**	8.96	9.69	10.68	<5	52 (α_{1D}/α_{1A})	10 (α_{1D}/α_{1B})

[a]Data are taken from refs 4 and 8. pA_2 values (n = 5–7) were calculated from Schild plots. The ratio of the potency (EC_{50}) of (−)-noradrenaline (α_{1A} and α_{1D}), phenylephrine (α_{1B}), and clonidine (α_2), in the presence of the antagonist and in its absence, was measured.

The antagonist activity profile of (*S*)-**5**, discriminating α_{1D} relative to α_{1B}- and α_{1A}-ARs, focused our interest on the 2-methoxy-1-naphthoxy group as a 2,6-dimethoxyphenoxy substitute orienting to α_{1D} antagonist affinity and prompted us to optimize its interaction potential. Therefore, it seemed proper to replace naphthalene with benzofuran or indole, which are naphthalene isosteres and offer the additional chance of disubstitution with heteroatoms at the benzene ring. In particular, the substitution of the 2,6-dimethoxyphenoxy portion of **1** with 6-methoxy-7-benzofuranoxy, compared to that with 2-methoxy-1-naphthoxy, maintains the salient feature of the 2-methoxy-1-naphthoxy derivative **5**, that is the presence of the *o*-methoxy-substituted aromatic bicycle but also the phenoxy substructure ortho-disubstituted with two nonhydroxylic HBA oxygens, which characterizes **1**. This means that the methoxybenzofuranoxy derivative **6** (Chart 2) is, at the same time, an isostere of **5** and a rigidified analogue of **1**. Alternatively, the 6-methoxy-7-indolyloxy residue of compound **7** (Figure 2) is also isosteric to 2-methoxy-1-naphthoxy but the two ortho heteroatoms of its phenoxy substructure, O and N, have quite different character and interaction potential with the binding site. To complete the investigation, it seemed worthwhile to also consider the 2,3-dihydro-6-methoxy-7-benzofuranoxy and 2,3-dihydro-6-methoxy-7-indolyloxy analogues, **8** and **9**, respectively (Chart 2), as we had contemplated the 2-methoxytetrahydronaphthoxy derivative besides **5** in the previous series of analogues of **1**[4] and, moreover, because 2,3-dihydro-6-methoxy-7-benzofuranoxyl is a conformationally constrained mimic of the 2,6-dimethoxyphenoxy residue of **1**.

Chart 2. Target Benzofuranoxy and Indolyloxy Analogues of 1



CHEMISTRY

(*S*)- and (*R*)-**1**, necessary in the binding and functional tests as reference compounds, are accessible, as previously reported, from different unichiral synthons, such as (*S*)-isopropylidene glycerol,[5] (*R*)- and (*S*)-1,4-benzodioxane-2-carboxylic acid,[12−14] and the respective methyl esters.[12,15] The enantiomers of **4** were synthesized by N-alkylation of 6-methoxy-7-(2-aminoethoxy)benzofuran (**18**) with (*R*)- and (*S*)-2-mesyloxymethyl-1,4-benzodioxane,[7,16] while the enantiomers of **7**, **8**, and **9** were synthesized by N-alkylation of (*S*)- and (*R*)-2-aminomethyl-1,4-benzodioxane[7,17] with 6-methoxy-7-(2-mesyloxyethoxy)indole (**26**), 6-methoxy-7-(2-mesyloxyethoxy)-2,3-dihydrobenzofuran (**20**), and *N*-Cbz-6-methoxy-7-(2-bromoethoxy)-2,3-dihydroindole (**29**), respectively (Scheme 1). The enantiomers of **6** and **8** were isolated as hydrochlorides, those of **9** as dihydrochlorides, and those of **7** as free amines.

The synthesis of the benzofuran and dihydrobenzofuran building blocks, namely **18** and **20**, is illustrated in Scheme 2. *o*,*m*-Dimethoxyphenol was reacted with ethyl chloroacetate to give the phenoxyacetate **10**, which was hydrolyzed to the corresponding carboxylic acid **11** and then converted into the phenoxyacetyl chloride **12**. The subsequent intramolecular Friedel−Crafts yielded **13**, whose OH was etherified with ethyl chloroacetate to obtain the acetate **14**, which was reduced to the corresponding alcohol **15** and then mesylated. The mesyl ester **16** was converted into the azide **17** and reduced to **18** with hydrazine. Hydrogenation of the intermediate **15** provided the 2,3-dihydrobenzofuran **19**, which was transformed into the mesylate **20**.

The synthesis of the indole and dihydroindole building blocks, namely **26** and **29**, is shown in Scheme 3. *o*,*m*-Dimethoxybenzoic acid was converted into 6-methoxy-7-hydroxyindole (**24**) in four steps according to slightly modified literature procedures.[18,19] The successive reaction with ethylene

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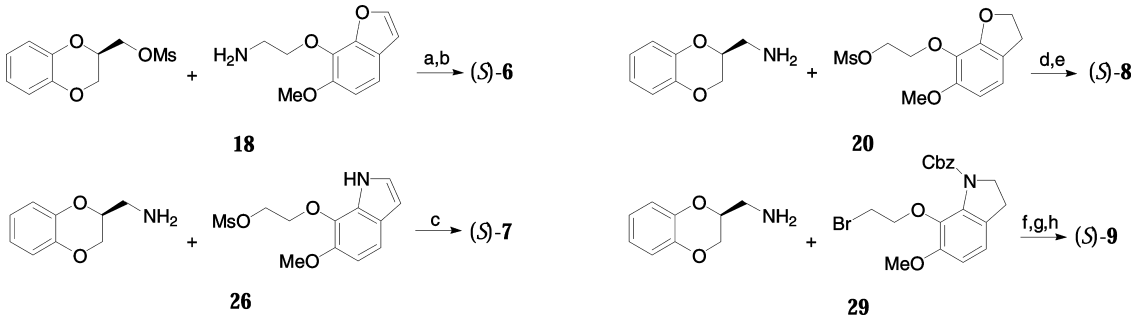
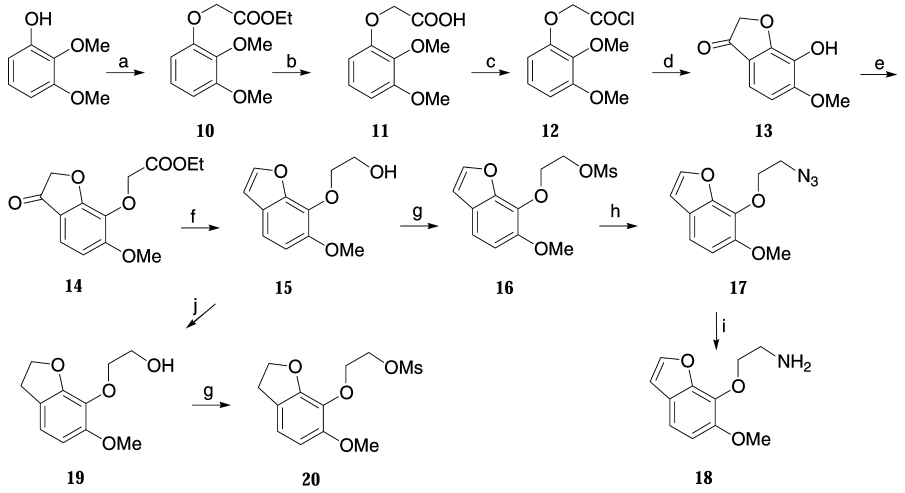
Scheme 1. Synthesis of the *S* Stereoisomers of Compounds 6–9[a]



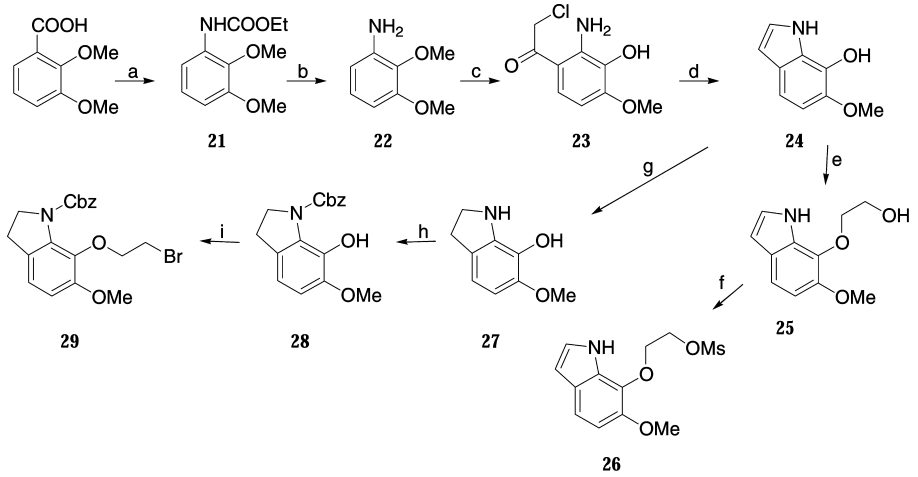
[a]Reagents and conditions: (a) 2-Methylpropanol, 120 °C, 45 min, mw; (b) 3 N HCl/EtOH; (c) 2-methypropanol, 120 °C, 1 h, mw; (d) 2-methylpropanol, 90 °C, 2 h, mw; (e) 3 N HCl/EtOH; (f) 2-methylpropanol, reflux, 18 h; (g) H_2, Pd/C, MeOH, rt, 16 h; (h) 1.5 N HCl/EtOH.

Scheme 2. Synthesis of the Intermediates 18 and 19[a]



[a]Reagents and conditions: (a) Ethyl chloroacetate, NaH, THF/DMSO, rt, 15 h; (b) NaOH, MeOH, rt, 1 h; (c) oxalyl chloride, 60 °C, 1 h; (d) $AlCl_3$, dichloroethane, rt, 18 h, then 10% aqueous HCl; (e) potassium *tert*-butoxide, ethyl chloroacetate, THF/DMSO, rt, 4 h; (f) $LiAlH_4$, THF, reflux, 5 h; (g) MsCl, TEA, DCM, rt, 1 h; (h) NaN_3, DMF/water, 90 °C, 1 h; (i) hydrazine hydrate, PdO, MeOH, reflux, 2 h; (j) H_2, Pd/C, EtOH, rt, 24 h.

Scheme 3. Synthesis of the Intermediates 26 and 29[a]



[a]Reagents and conditions: (a) DPPA, TEA, THF, EtOH, reflux, 4 h; (b) KOH, EtOH, water, reflux, 3 h; (c) BCl_3, DCM, 0 °C then $ClCH_2CN$, dichloroethane, reflux, 3 h; (d) $NaBH_4$, dioxane, 90 °C, 3 h; (e) ethylene carbonate, K_2CO_3, dioxane, 150 °C, 60 min mw; (f) MsCl, TEA, DCM, rt, 30 min; (g) H_2, Pd/C, CH_3COOH, rt, 16 h; (h) benzyl chloroformate, DCM, Py, 10 °C, 3 h; (i) 1,2-dibromoethane, NaH, THF/DMSO, reflux, 16 h.

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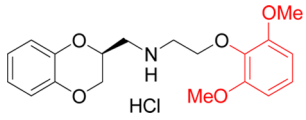
Table 2. Affinity Constants, Expressed as pK_i (−log K_i, M) of (*S*)-1, (*S*)-5, and the Enantiomers of Compounds 6−9 for Cloned Human α_1-Adrenoceptor Subtypes and the 5-HT_{1A} Receptor



(*S*)-**1**

		pK_i (±SE)[a]				affinity ratios[b]		
compd	group replacing 2,6-dimethoxyphenyl of **1**	α_{1a}	α_{1b}	α_{1d}	5-HT_{1A}	α_{1a}/α_{1b}	α_{1a}/α_{1d}	α_{1a}/5-HT_{1A}
(*S*)-**1**		9.39 (±0.06)	8.24 (±0.04)	9.29 (±0.11)	8.61 (±0.04)	14.1	1.3	6.0
(*S*)-**5**	2-methoxy-1-naphthyl	8.80 (±0.09)	7.80 (±0.07)	8.18 (±0.13)	7.95 (±0.06)	10.0	4.2	7.1
(*S*)-**6**	6-methoxy-7-benzofuranyl	9.31 (±0.02)	7.54 (±0.12)	8.72 (±0.14)	8.59 (±0.08)	58.9	3.9	5.2
(*R*)-**6**		8.04 (±0.07)	6.90 (±0.06)	7.74 (±0.09)	7.96 (±0.15)	13.8	2.0	1.2
(*S*)-**8**	2,3-dihydro-6-methoxy-7-benzofuranyl	8.70 (±0.03)	7.66 (±0.01)	8.65 (±0.10)	7.33 (±0.07)	11.0	1.1	23.4
(*R*)-**8**		7.77 (±0.05)	6.79 (±0.05)	7.74 (±0.08)	6.79 (±0.08)	9.5	1.1	9.5
(*S*)-**7**	6-methoxy-7-indolyl	6.75 (±0.04)	6.31 (±0.11)	6.20 (±0.10)	6.47 (±0.09)	2.8	3.5	1.9
(*R*)-**7**		6.36 (±0.05)	5.72 (±0.13)	5.87 (±0.10)	5.77 (±0.18)	4.4	3.1	3.9
(*S*)-**9**	2,3-dihydro-6-methoxy-7-indolyl	8.42 (±0.09)	7.45 (±0.8)	7.89 (±0.10)	6.96 (±0.07)	9.3	3.4	28.8
(*R*)-**9**		7.50 (±0.05)	6.43 (±0.09)	7.08 (±0.04)	6.66 (±0.11)	11.7	26.3	6.9

[a]Data of (*S*)-**1** and (*S*)-**3** are reported in ref 3. [b]Antilog of ΔpK_i.

Table 3. *S* Enantiomers of Compounds 6−9: Antagonist Affinities, Expressed as pA_2, at α_{1A}-, α_{1B}-, α_{1D}-AR on Isolated Rat Tissues, and Inverse Agonism Expressed as Magnitude of Inhibition of Calcium-Induced Increase in the Resting Tension (IRT) of Calcium-Depleted Guinea Pig Thoracic Aorta

	pA_2[a]					
	α_{1A}		α_{1B}	α_{1D}	α_2	
compd	prostate	prostatic vas deferens	spleen	thoracic aorta	prostatic vas deferens	inhibition of Ca^{2+}-induced IRT (%)[b]
(*S*)-**6**	8.71 (±0.09)	8.26 (±0.07)	8.68 (±0.02)	9.01 (±0.09)	6.55 (±0.05)	0
(*S*)-**8**	7.88 (±0.09)	7.47 (±0.03)	8.49 (±0.03)	9.58 (±0.06)	6.63 (±0.01)	0
(*S*)-**7**	7.88 (±0.01)	8.31 (±0.05)	7.77 (±0.08)	8.52 (±0.03)	6.09 (±0.01)	25
(*S*)-**9**	8.44 (±0.01)	8.03 (±0.06)	9.08 (±0.01)	8.62 (±0.09)	6.05 (±0.02)	100

[a]pA_2 values ± SE (n = 5−7) were calculated from Schild plots,[23] constrained to a slope of −1.0, unless otherwise specified.[24] pA_2 is the positive value of the intercept of the line derived by plotting log (DR-1) vs log[antagonist]. The log(DR-1) was calculated for least at three different antagonist concentrations, and each was tested from three to five times. Dose-ratio (DR) values represent the ratio of the potency (EC_{50}) of the agonist (α_{1A} and α_{1D}: (−)-noradrenaline; α_{1B}: phenylephrine; α_2: clonidine) in the presence of the antagonist and in its absence. Parallelism of dose−response curves was checked by linear regression, and the slopes were tested for significance ($p < 0.05$). [b]Data are the percent decreases of the Ca^{2+} (1.8 mM)-induced IRT in the presence of the antagonist at 10 nM concentration, calculated assuming as a reference IRT the Ca^{2+} (1.8 mM)-induced IRT in the absence of any agent. Data represent the mean ± SE from four to seven experiments.

carbonate gave the hydroxyethyl ether **25**, which was mesylated to **26**. To prepare **29**, the intermediate **24** was hydrogenated, Cbz N-protected, and O-2-bromoethylated with dibromoethane.

RESULTS AND DISCUSSION

Table 2 reports the binding affinities, expressed as pK_i values (−logK_i, M), of the enantiomeric pairs of compounds **6**−**9** and, as comparison terms, of (*S*)-**1** and (*S*)-**5** at the three cloned human α_1-AR subtypes and native 5-HT_{1A} serotoninergic receptor from rat hippocampus.

The affinities of the *S* enantiomers are always higher than those of their antipodes, and the most considerable eudismic indices are shown by **6**, **8**, and **9** at the three α_1-AR subtypes.

A dividing line can be drawn between the benzofuran and dihydrobenzofuran derivatives, (*S*)-**6** and (*S*)-**8**, and the indole and dihydroindole derivatives, (*S*)-**7** and (*S*)-**9**, the former pair exhibiting, at the four tested receptors, from high to moderate affinities, always higher than those of the latter pair. Furthermore, comparison of (*S*)-**6** and (*S*)-**8** with (*S*)-**1** indicates that only the α_{1a}- and the 5-HT_{1A} affinities of (*S*)-**6** are near to those of (*S*)-**1**, while the other affinity values are lower than that of (*S*)-**1**, though always less than by one pK_i unit at the α_1-AR subtypes. Therefore, the selectivity profiles of (*S*)-**6** and (*S*)-**8** substantially reproduce that of (*S*)-**1** but with more pronounced α_{1a} versus α_{1b} and α_{1d} selectivity in the case of (*S*)-**6** and significantly improved α_{1a}/5-HT_{1A} and α_{1d}/5-HT_{1A} selectivities in the case of (*S*)-**8**. Such a peculiarity of (*S*)-**8** becomes more evident in comparison with (*S*)-**6** and (*S*)-**5**, both conversely displaying 5-HT_{1A} affinity closer to those at the α_{1a}- and α_{1d}-AR. Another peculiarity distinguishes (*S*)-**8** from (*S*)-**6** and (*S*)-**5**: its $\alpha_{1a}/\alpha_{1b}/\alpha_{1d}$ selectivity profile is perfectly superimposable to that of (*S*)-**1**, its α_{1d} and α_{1a} affinities being nearly identical to each other as those of (*S*)-**1**, while both (*S*)-**6** and (*S*)-**5** display lower affinity at α_{1d}-AR than at α_{1a}-AR.

Compared to (*S*)-**6** and (*S*)-**8**, the N-analogues (*S*)-**7** and (*S*)-**9** have modest and moderate affinities, respectively. In particular, the dihydroindole derivative (*S*)-**9** exhibits α_1 and 5-HT_{1A} affinities a little lower than those of its dihydrobenzofuran analogue (*S*)-**8**, whereas a marked loss of affinity at all the receptors is effected by the replacement of benzofuran with indole as evidenced by comparing (*S*)-**6** to (*S*)-**7**.

In summary, the analysis of binding data indicates that replacement of one of the two *o*-methoxy substituents of **1** with benzo-condensed pyrrole or dihydropyrrole has a negative

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effect on the α_1 and 5-HT$_{1A}$ affinities, which becomes severely detrimental in particular when the 6-methoxy-7-indolyl residue replaces, as in (*S*)-**7**, the 2,6-dimethoxyphenyl portion of (*S*)-**1**. Quite different is the impact of furan and dihydrofuran benzo-condensation, which allows the two ortho oxygens of (*S*)-**1** to be maintained: the affinities and the selectivity profiles of (*S*)-**6** and (*S*)-**8** are not so far from those of (*S*)-**1** and (*S*)-**5**. This notwithstanding, (*S*)-**8** distinguishes itself from (*S*)-**6**, (*S*)-**5**, and (*S*)-**1** by its lower 5-HT$_{1A}$ affinity and from (*S*)-**6** and (*S*)-**5** by having identically high α_{1d} and α_{1a} affinities.

The functional assays were carried out only for the *S* enantiomers. Their antagonist affinities at α_1-AR subtypes and α_2-AR, expressed as pA_2, are listed in Table 3. The blocking activity was determined on different rat tissues: (a) by antagonism of (−)-noradrenaline (NA)-induced contraction of vas deferens prostatic portion (α_{1A}-AR)[20] and of thoracic aorta (α_{1D}-AR),[21] (b) by antagonism to (−)-phenylephrine-induced contraction of spleen (α_{1B}-AR),[21] (c) by antagonism to clonidine-inhibited twitch responses of the field-stimulated prostatic portion of vas deferens (α_2-AR). Furthermore, considering the demonstrated α_{1A}-AR involvement in maintaining prostate smooth muscle tone and the consequent therapeutic potential of agents reducing the latter for LUTS, the antagonist affinity was also evaluated in rat prostate.[22]

Inspection of the results reported in Table 3 reveals trends, which can be summarized as follows: (a) all the compounds exhibit high α_1/α_2 selectivity; (b) the α_1 antagonist affinities are always high (pA_2 > 8) with the exception of the α_{1A} antagonist affinities of (*S*)-**8** and (*S*)-**7** and of the α_{1B} antagonist affinity of the latter; (c) the maximum antagonist activities (subnanomolar pA_2) are shown by the two benzofuran and dihydrobenzofuran derivatives, (*S*)-**6** and (*S*)-**8**, at the α_{1D}-AR and by the dihydroindole derivative (*S*)-**9** at the α_{1B}-AR. The most interesting behavior is that of the dihydrobenzofuran (*S*)-**8**, which is a very potent (pA_2 = 9.58) and significantly selective (α_{1D}/α_{1A} = 50−130 and α_{1D}/α_{1B} = 12) α_{1D}-AR antagonist.

Matching of binding with functional tests indicates substantial divergences: all the α_{1B} and α_{1D} antagonist affinities are higher than the respective α_{1b} and α_{1d} binding affinities, while the α_{1A} antagonist affinity, compared to the α_{1a} binding affinity, decreases in the benzofuran and dihydrobenzofuran derivatives and increases in the two N-analogues. An analogous behavior to benzofuran and dihydrobenzofuran (*S*)-**6** and (*S*)-**8** was exhibited by the 2-methoxy-1-naphthoxy derivative (*S*)-**5**,[4] the α_{1B} and α_{1D} but not the α_{1A} antagonist affinities of which are remarkably higher than the corresponding binding affinities. In Table 4, the α_{1D}-AR selectivities of (*S*)-**5**, (*S*)-**6**, and (*S*)-**8** resulting from such a trend are compared with those of two literature α_{1D}-AR selective antagonists, **30** (SNAP 8719)[25] (Chart 1) and **2**,[3,26] which is commercially available. Also in Table 4, the 5-HT$_{1A}$ binding affinities are reported. The compound (*S*)-**8** shows the highest α_{1D}-AR antagonist affinity, associated with intermediate α_{1D}-AR selectivity between **2** and **30** and, similarly to the latter, with about 200-fold lower 5-HT$_{1A}$ binding affinity.

As previously for **1** and some of its derivatives and by the same semiquantitative assay, we verified if these antagonists behave as inverse agonists.[4,8] The results, reported in Table 3, indicate that (*S*)-**6** and (*S*)-**8** are neutral antagonists, thus differentiating them from (*S*)-**7** and (*S*)-**9** but also from (*S*)-**1** and (*S*)-**5**.

SAR analysis of these activity data would require the knowledge of the antagonist affinities of the wide number of ortho mono- and disubstituted phenoxy analogues of WB4101 we have previously studied only for their binding affinity.[10,11] In fact, frequent discrepancies between binding and functional tests have demonstrated poor prediction of activity by binding tests making comparison of these compounds with those hazardous. However, on the basis of the whole set of data that we previously reported, we can reasonably state that ortho disubstitution at the phenoxy moiety is strictly required for high binding and antagonist α_1-affinity probably because of stabilization of a properly extended conformation of the molecule and a properly oriented phenoxy moiety. The involvement of the two ortho substituents both in intramolecular (H-bond between O and NH$^+$ and/or hampered rotation of phenoxyl) and binding interactions is conceivable. Limiting the analysis to (*S*)-**1** and to the analogues, whose α_1-AR antagonist activities we have determined,[4] we notice that from high to very high α_1-AR antagonist activities are associated with the *o*-dimethoxy substitution at the phenoxyl or, alternatively, with the *o*-methoxy substitution combined with the presence of an aromatic ring, fused at the ortho and meta positions of phenoxyl or ortho linked, or of a heteroalicycle, condensed at the same positions and with the heteroatom superimposable on the oxygen of one of the two methoxy groups of **1** (Figure 1). When this pattern of ortho-disubstitution at the phenoxy residue was abandoned, as in the 2-methoxyphenoxy analogues bearing a phenyl in meta or in para position or in the 2-methoxytetrahydronaphthoxy analogue,[4] we observed a significant decrease or loss of antagonist activity. Hence, provided the ortho-disubstitution at the phenoxyl, the α_1-AR subtype selectivity is then achieved by modifying the benzodioxane portion. In particular, as shown in Figure 1, the 8-methoxylation of benzodioxane of (*S*)-**1** results in a selective α_{1B}-AR antagonist,[8] while a selective α_{1A}-AR antagonist is obtained by fusing a cyclohexane ring at the 6 and 7 positions of the benzodioxane of (*S*)-**5**.[4] On the other hand, a significant α_{1D}-AR selectivity is just shown by (*S*)-**5**, whose only modification, with respect to (*S*)-**1**, is the replacement of one of the two *o*-methoxy groups by a fused benzene ring.[4] Moreover, α_{1D}-AR-oriented activities, though with lower subtype selectivity, was also found for the analogues of (*S*)-**1** in which 2,6-dimethoxyphenoxyl is replaced by 2-methoxy-5,6,7,8-tetrahydronaphthoxyl or 2-methoxy-6-phenylphenoxyl.[4] These data indicate that the mere presence of an additional ring ortho-linked to phenoxyl or ortho-meta fused to it benefits the α_{1D}-AR antagonist activity, and such a suggestion is confirmed by the activity profile of the present compounds (Figure 1). These exhibit high and generally prevalent α_{1D}-AR antagonist activity

Table 4. Comparison of the Antagonists Affinities at α_1-AR Subtypes and of the Binding Affinity at the 5-HT$_{1A}$ Receptor of (*S*)-6 and (*S*)-8, the Two New Benzofuranoxy Analogues of 1, with Those of (*S*)-5, the Previously Reported Naphthoxy Analogue of 1, and of the Literature α_{1D}-AR Antagonists 2 and 30

compd	α_{1A}	α_{1B}	α_{1D}	α_{1D}/α_{1A}[c]	α_{1D}/α_{1B}[c]	5-HT$_{1A}$
(*S*)-**6**	8.26	8.68	9.01	6	2	8.59
(*S*)-**8**	7.47	8.49	9.58	129	12	7.33
(*S*)-**5**	8.96	9.69	10.68	52	10	7.95
2	6.94[a]	7.55[a]	8.34[a]	25	6	8.76
30	5.4[b]	6.7b	8.8b	2500	126	6.52

[a] pA_2 values taken from ref 26. [b] pK_B values taken from ref 25. [c] Antilog of ΔpA_2 or of ΔpK_B.

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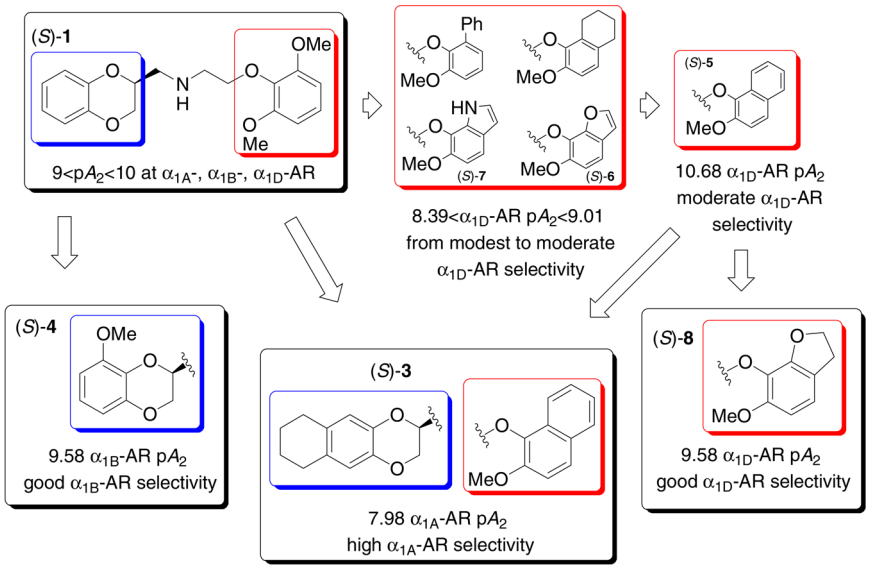

Figure 1. SAR milestones in the optimization of the hit compound (*S*)-**1**.

independently from the aromatic or nonaromatic nature of the heterocycle fused at the 5 and 6 positions of the 2-methoxyphenoxyl and from the HBA properties of its heteroatom (cf., benzofuran oxygen and indole nitrogen). In this set of α_{1D}-AR antagonists, the highest activity and the maximum α_{1D} selectivity of (*S*)-**8** would be due to the fact that only 6-methoxydihydrobenzofuran, among all the methoxylated bicycle systems replacing 2,6-dimethoxyphenoxyl and preferentially increasing the α_{1D} antagonist affinity, is a properly rigidified analogue of this portion of **1**, maintaining its electronic features and interaction potential unchanged.

CONCLUSION

We identified an analogue of **1**, (*S*)-**8**, which is a potent and selective α_{1D}-AR antagonist. Such a subtype selectivity was achieved simply by rigidifying the 2,6-dimethoxyphenoxyl substructure in the 2,3-dihydro-6-methoxy-7-benzofuranoxy system of (*S*)-**8**. In a lower degree, α_{1D} selectivity is also shown by the methoxybenzofuranoxy and methoxyindolyloxy analogues, (*S*)-**6** and (*S*)-**7**. Furthermore, (*S*)-**8** stands out for being a neutral antagonist and having modest 5-HT_{1A} binding affinity, markedly lower than that of (*S*)-**6** and the 2-methoxynaphthoxy analogue (*S*)-**5**. Compared to **2**, which is reported as the most reliable α_{1D}-AR antagonist and which acts as an inverse agonist,[2,3] and to **30**, the most α_{1D}-AR selective antagonist,[25] (*S*)-**8** is significantly more potent and displays a high α_{1D}-AR selectivity, intermediate between those of **2** and **30**.

Further advancements in the field of 2-aminomethyl-1,4-benzodioxanes as α_1-AR antagonists might include designing new derivatives by replacing the 2,6-dimethoxyphenoxy moiety with other aromatic heterobicyclic systems as well as by hybridizing the most productive modification at the benzodioxane substructure, namely replacement with tetrahydronaphthodioxane,[4] with those at the phenoxy moiety such as the here-described ortho-meta fusion with heterocycles. Moreover, the moderate 5-HT_{1A} selectivity of ortho monosubstituted analogues of **1** we have previously observed in binding experiments[10] deserves further investigation and might be matter for development of new 5-HT_{1A} selective ligands. However, at the present stage of our research, the major challenge is to have a deeper insight into the alternating nature between inverse agonism and neutral antagonism of these molecules, a dualism which should be reconsidered because the present-day concept of pharmacological efficacy at seven-transmembrane receptors is that all ligands with affinity have some form of efficacy and modify the receptor behavior.[27] According to these new models, the effects of both agonists and antagonists depend on their preferential interactions with, and stabilization of, a particular conformational state of the receptor, out of the different states the receptor may assume depending on the biological system. Thus, the discrepancies observed in this and previous studies[4,8] are not surprising, taking into account that, with binding assays, we are measuring the compound's affinity for the antagonist binding site on recombinant α_1-AR overexpressed in CHO cells, whereas functional assays provide the compound's potency in preventing a specific agonist-induced effect in native tissues.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were recorded, operating at 300 MHz and ^{13}C NMR at 75 MHz. Chemical shifts are given in parts per million relative to residual solvent ($CHCl_3$ or DMSO) as internal standard. Optical rotations were determined by a Jasco P-1010 polarimeter. Melting points were measured on a Buchi melting point apparatus and are uncorrected. Thermal analyses were performed on 2−5 mg samples in closed pans at 5 °C/min using a DSC 2010 (TA Instruments). Elemental analyses (CHN) are within ±0.40% of theoretical values. Purifications were performed using KP-Sil 32−63 μm 60 Å cartridges and Merck silica gel (particle size 40−63 μm). The results of elemental analyses indicated that the purity of all tested compounds was higher than 95%.

Ethyl (2,3-Dimethoxyphenoxy)acetate (10). A solution of (2,3-dimethoxy)phenol (16.95 mL, 130 mmol) in THF/DMSO (10 mL/10 mL) was added dropwise to a suspension of NaH (3.26 g, 136 mmol) in THF/DMSO (10 mL/10 mL) under nitrogen atmosphere at 0 °C. After 30 min, ethyl chloroacetate (15.4 mL, 143 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 15 h and then quenched in a cooled solution of 10% HCl (50 mL). The aqueous layer was extracted with dichloromethane (3 × 30 mL). The organic phases were combined, washed with water (2 × 50 mL), dried, and concentrated to give 30.86 g (98.8%) of **10** as an orange oil: ^{1}H NMR ($CDCl_3$): δ 1.23 (t, 3H), 3.80 (s, 3H), 3.85 (s, 3H), 4.19 (q, 2H), 4.64 (s, 2H), 6.45 (d, 1H), 6.57 (d, 1H), 6.90 (dd, 1H).

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(2,3-Dimethoxyphenoxy)acetic Acid (11). A 10% NaOH aqueous solution (300 mL) was added to a solution of **10** (30.86 g, 128 mmol) in methanol at 20 °C. After 1 h, methanol was removed under vacuum, and the residue was treated with 10% HCl (100 mL) and extracted with dichloromethane (3 × 30 mL). The organic phases were combined, washed with water, dried, and concentrated to give 24.47 g (90.1%) of **11** as a white solid: mp 103.3 °C. ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 3.92 (s, 3H), 4.70 (s, 2H), 6.59 (d, 1H), 6.68 (d, 1H), 7.01 (t, 1H).

(2,3-Dimethoxyphenoxy)acetyl Chloride (12). Oxalyl chloride (97.3 mL, 1.15 mol) was added dropwise to **11** (24.47 g, 115 mmol) at 0 °C. The reaction mixture was heated at 60 °C for 1 h and then concentrated to give 26.52 g (100%)of **12** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 6H), 5.00 (s, 2H), 6.53 (d, 1H), 6.67 (d, 1H), 6.97 (t, 1H).

7-Hydroxy-6-methoxy-3-oxo-2,3-dihydrobenzofuran (13). Under nitrogen, aluminum chloride (30.67 g, 230 mmol) was added to a solution of **12** (26.52 g, 115 mmol) in dichloroethane (120 mL) at 0 °C. The reaction mixture was stirred for 18 h at room temperature and then quenched in cooled 10% HCl (200 mL). The suspension was filtered, and the resultant solid crude product was crystallized from methanol (110 mL) to give 11.60 g (53.4%) of **13** as a light yellow solid: mp 218.0 °C. ^{1}H NMR (DMSO-d_6) δ 3.88 (s, 3H), 4.74 (s, 2H), 6.81 (d, 1H), 7.08 (d, 1H), 9.29 (s, 1H, exchangeable with D_2O).

Ethyl (6-Methoxy-3-oxo-2,3-dihydro-7-benzofuranoxy)-acetate (14). Under nitrogen, a solution of potassium *tert*-butoxide (8.67 g, 77.3 mmol) in anhydrous THF (75 mL) and DMSO (55 mL) was added dropwise to **13** (11.6 g, 64.4 mmol), previously dissolved in the same solvent mixture, at 0 °C. Subsequently, ethyl chloroacetate (7.60 mL, 70.8 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. THF was removed under vacuum. The residue was quenched in cooled 10% HCl (200 mL) and extracted twice with dichloromethane (2 × 100 mL). The combined organic phases were washed, in the following order, with 10% NaOH aqueous solution (2 × 100 mL), 10% HCl (100 mL), and water (2 × 100 mL), dried, and concentrated. The solid residue was crystallized from ethanol to give 12.43 g (72.4%) of **14** as a light yellow solid: mp 83.3 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H), 3.93 (s, 3H), 4.22 (q, 2H), 4.62 (s, 2H), 4.80 (s, 2H), 6.69 (d, 1H), 7.37 (d, 1H).

6-Methoxy-7-(2-hydroxyethoxy)benzofuran (15). Under nitrogen atmosphere at 0 °C, a solution of **14** (12.43 g, 46.7 mmol) in anhydrous THF was added to a suspension of $LiAlH_4$ (5.31 g, 140.1 mmol) in anydrous THF. The reaction mixture was refluxed for 5 h and then cooled to 0 °C. A 10% HCl (20 mL) solution was added dropwise, and then dichloromethane (50 mL) was added. The organic phase was separated, washed with 10% HCl (2 × 100 mL) and with brine (2 × 100 mL), dried, and concentrated to give a residue, which was purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate 80:20) to obtain 2.5 g (25.7%) of **15** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.13 (s, 1 H, exchangeable with D_2O), 3.81 (t, 2H), 3.93 (s, 3H), 4.39 (t, 2H), 6.71 (s, 1H), 6.92 (d, 1H), 7.24 (d, 1H), 7.55 (s, 1H).

6-Methoxy-7-(2-mesyloxyethoxy)benzofuran (16). Mesyl chloride (0.93 mL, 12.0 mmol) was added dropwise to a solution of **15** (2.50 g, 12.0 mmol) and triethylamine (1.71 mL, 12.0 mmol) in dichloromethane (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, and then dichloromethane (30 mL) was added. The resulting mixture was washed, in sequence, with 10% HCl (3 × 40 mL) and with brine (2 × 40 mL). The organic phase was separated, dried, and concentrated to give 2.87 g of crude product, which was crystallized from isopropyl ether/isopropyl alcohol (6:1; 35 mL) to give 2.71 g (78.8%) of **16** as white solid: mp 51.6 °C. ^{1}H NMR ($CDCl_3$) δ 3.14 (s, 3H), 3.91 (s, 3H), 4.60 (m, 4H), 6.71 (s, 1H), 6.91 (d, 1H), 7.23 (d, 1H) 7.54 (s, 1H).

6-Methoxy-7-(2-azidoethoxy)benzofuran (17). Sodium azide (8.09 g, 123.1 mmol) was added to a solution of **16** (2.71g, 9.5 mmol) in DMF/water (2:1, 75 mL). The reaction mixture was heated at 90 °C and, after 1 h, cooled to room temperature and extracted with dichloromethane (3 × 20 mL). The organic phases were combined, washed with brine (6 × 30 mL), dried, and concentrated to give 2.21 g (100%) of **17** as an orange oil: ^{1}H NMR ($CDCl_3$) δ 3.61 (m, 2H), 3.91 (s, 3H), 4.44 (m, 2H), 6.69 (s, 1H), 6.92 (d, 1H), 7.21 (d, 1H), 7.54 (s, 1H).

6-Methoxy-7-(2-aminoethoxy)benzofuran (18). Hydrazine hydrate (4.6 mL, 94.8 mmol) was added dropwise to a refluxing mixture of **17** (2.21 g, 9.5 mmol) and PdO (50 mg) in methanol (20 mL). The reaction was refluxed for 2 h. After cooling at room temperature, PdO was removed by filtration and the solvent evaporated. The residue was treated with dichloromethane (20 mL) and 10% HCl (45 mL). The aqueous layer was separated, washed with dichloromethane three times, made alkaline (pH 12) with NaOH, and extracted with ethyl acetate (4 × 20 mL). The organic phases were combined, dried, and concentrated to give 1.33 g (67.9%) of **18** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 2H), 3.00 (t, 2H), 3.91 (s, 3H), 4.32 (t, 2H), 6.69 (s, 1H), 6.91 (d, 1H), 7.20 (d, 1H), 7.53 (s, 1H).

6-Methoxy-7-(2-hydroxyethoxy)-2,3-dihydrobenzofuran (19). Pd/C (5%, 1.4 g) was added to a solution of **15** (6.09 g, 29.3 mmol) in absolute ethanol (200 mL). The mixture was vigorously shaken under hydrogen at room temperature for 24 h. Afterward, the catalyst was removed by filtration and the filtrate concentrated to give 6.15 g (100%) of **19** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.12 (t, 2H), 3.72 (t, 2H), 3.83 (s, 3H), 4.19 (t, 2H), 4.60 (t, 2H), 6.40 (d, 1H), 6.80 (d, 1H).

6-Methoxy-7-(2-mesyloxyethoxy)-2,3-dihydrobenzofuran (20). Obtained as a crude product from **19** (5.3 g, 25.2 mmol) as described for **16**. Crystallization from isopropyl ether/isopropyl alcohol (9:1) gave pure **20** (5.8 g, 80%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 3H), 3.09 (t, 2H), 3.75 (s, 3H), 4.34 (t, 2H), 4.52 (t, 2H), 4.53 (t, 2H), 6.32 (d, 1H), 6.77 (d, 1H).

***O*-Ethyl *N*-(2,3-Dimethoxyphenyl)carbamate (21).** Under nitrogen atmosphere at 0 °C, a THF solution of diphenylphosphoryl azide (19.6 mL, 90.8 mmol), ethanol (55 mL), and triethylamine (12.7 mL, 90.8 mmol) were added, in sequence, to a solution of 2,3-dimethoxybenzoic acid (16.55 g, 90.8 mmol) in anhydrous THF. After refluxing for 4 h, THF was removed and the residue was taken up by ethyl acetate (100 mL), washed with 10% aqueous solution of Na_2CO_3 (2 × 100 mL) and with brine (2 × 100 mL), dried, and concentrated. The resulting residue was purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate; 9:1) to obtain 15.74 g (76.9%) of **21** as a white solid: mp 45.1 °C. ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3H), 3.86 (s, 6H), 4.23 (q, 2H), 6.61 (d, 1H), 7.02 (t, 1H), 7.23 (m, 1H), 7.78 (m, 1H).

2,3-Dimethoxyaniline (22). A solution of compound **21** (15.74 g, 69.9 mmol) in ethanol was added to a suspension of KOH (11.76 g, 209.6 mmol) in ethanol (56 mL) and water (14 mL) at 10 °C. After refluxing for 3 h, the solvent was evaporated and the resulting residue was treated with dichloromethane (100 mL) and cooled 10% HCl (100 mL). The aqueous layer was separated, washed with dichloromethane (2 × 50 mL), made alkaline with 30% NaOH, and then extracted with dichloromethane (2 × 70 mL). The organic phases were combined, washed with 10% NaOH (2 × 50 mL), brine (2 × 50 mL), dried and concentrated to give 10.64 g (99.4%) of **22** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 6H), 6.35 (dd, 1H), 6.40 (dd, 1H), 6.85 (t, 1H).

2-Amino-3-hydroxy-4-methoxy-α-chloroacetophenone (23). Under nitrogen atmosphere at 0 °C, a solution of **22** (10.64 g, 69.5 mmol) in dichloroethane (50 mL) was added dropwise to a 1 M solution of BCl_3 in dichloromethane (278 mL). Subsequently, a solution of chloroacetonitrile (5.3 mL, 83.4 mmol) in dichloroethane was added. After refluxing for 3 h, the reaction mixture was poured into cooled phosphate buffer (18 g/L NaH_2PO_4, 72 g/L $gNaHPO_4$), and the separated aqueous layer was extracted with ethyl acetate (2 × 200 mL). The combined organic phases were washed with phosphate buffer (2 × 200 mL) and brine (2 × 200 mL), dried, and concentrated. Flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate; 8:2) of the resulting residue afforded 7.48 g (50%) of **23** as a yellow solid: mp 122.0 °C. ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 4.63 (s, 2H), 5.15 (br s, 1H), 6.31 (d, 1H), 7.24 (d, 1H).

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6-Methoxy-7-hydroxyindole (24). $NaBH_4$ (1.80 g, 47.58 mmol) was added to a solution of **23** (7.48 g, 29.7 mmol) in dioxane (150 mL) and water (15 mL) at 0 °C. The reaction mixture was heated at 90 °C for 3 h and then cooled to room temperature and concentrated. The resulting residue was treated with cooled 10% HCl (100 mL) and dichloromethane. The aqueous layer was separated and extracted with dichloromethane (50 mL). The combined organic phases were washed with 10% HCl (2 × 50 mL) and then with brine (50 mL) and dried. Solvent removal afforded 3.20 g (66.4%) of **24** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3H), 5.72 (s, 1H), 6.47 (m, 1H), 6.85 (d, 1H), 7.13 (m, 2H), 8.24 (br s, 1H).

6-Methoxy-7-(2-hydroxyethoxy)indole (25). A mixture of **24** (3.20 g, 19.6 mmol), ethylene carbonate (1.72 g, 19.61 mmol), and K_2CO_3 (2.7 g, 19.61 mmol) in dioxane (10 mL) was subjected to microwave irradiation for 60 min (150 °C, 150 W). The reaction mixture was cooled to room temperature, and dichloromethane (50 mL) and water (100 mL) were added. The organic phase was separated and washed with 10% HCl (3 × 50 mL). The aqueous layer was extracted with dichloromethane (50 mL) again, and the two organic phases were combined and washed with water (2 × 50 mL), dried, and concentrated. The resulting residue was purified by flash chromatography on silica gel (eluent cyclohexane/ethyl acetate, 6:4) to give 1.61 g (34.9%) of **25** as a red oil: ^{1}H NMR ($CDCl_3$) δ 3.20 (br s, 1H, exchangeable with D_2O), 3.91 (m, 5H), 4.27 (m, 2H), 6.47 (m, 1H), 6.84 (d, 1H), 7.11 (m, 1H), 7.31 (d, 1H), 8.90 (br s, 1H, exchangeable with D_2O).

6-Methoxy-7-(2-mesyloxyethoxy)indole (26). Mesyl chloride (1.15 g, 10.0 mmol) was added dropwise to a solution of **25** (1.61 g, 7.8 mmol) and triethylamine (1.4 mL, 10.0 mmol) in dichloromethane (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min, and then dichloromethane (20 mL) and 10% HCl (20 mL) were added. The aqueous layer was extracted with dichloromethane (2 × 20 mL). The organic phases were combined, washed with 10% HCl (2 × 20 mL) and water (2 × 20 mL), dried, and concentrated to give 2.15 g (97.0%) of **26** as a dark oil: ^{1}H NMR ($CDCl_3$) δ 3.05 (s, 3H), 3.91 (s, 3H), 4.46 (m, 2H), 4.54 (m, 2H), 6.46 (m, 1H), 6.83 (d, 1H), 7.15 (m, 1H), 7.31 (d, 1H), 8.87 (br s, 1H, exchangeable).

6-Methoxy-7-hydroxy-2,3-dihydroindole (27). Pd/C (5%, 580 mg) was added to a solution of **26** (2.94 g, 18.0 mmol) in acetic acid (50 mL), and the mixture was vigorously shaken under hydrogen atmosphere at room temperature for 16 h. Afterward, the catalyst was removed by filtration and the filtrate concentrated. The residue was treated with dichloromethane (120 mL) and 10% aqueous $NaHCO_3$ (80 mL). The aqueous layer was separated and extracted with dichloromethane (2 × 50 mL). The organic phases were combined, dried, and concentrated to give 2.78 g (94%) of **27** as a brown solid: mp 106.6 °C. ^{1}H NMR ($CDCl_3$) δ 2.99 (t, 2H), 3.59 (t, 2H), 3.83 (s, 3H), 6.30 (d, 1H), 6.63 (d, 1H).

***N*-Cbz-6-methoxy-7-hydroxy-2,3-dihydroindole (28).** Benzyl chloroformate (2.9 mL, 20.2 mmol) in dichloromethane (5 mL) was added dropwise to a solution of **27** (2.28 g, 16.8 mmol) in dichloromethane (50 mL) and pyridine (6.7 mL) at 0 °C. The reaction mixture was stirred at 10 °C for 3 h and then allowed to reach room temperature. Dichloromethane (50 mL) was added, and the resulting mixture was quenched in 10% aqueous $NaHCO_3$. The organic phase was separated, washed with 10% aqueous $NaHCO_3$ (80 mL) and then with brine (2 × 50 mL), and finally dried. The resulting dark solution was decolorized over charcoal by stirring for 30 min, filtered, and concentrated to give 4.77 g of crude product, which was crystallized from ethyl acetate to give 4.30 g (85.5%) of **28** as a white solid: mp 135.0 °C. ^{1}H NMR ($CDCl_3$) δ 3.01 (t, 2H), 3.86 (s, 3H), 4.05 (t, 2H), 5.27 (s, 2H), 6.60 (s, 2H), 7.41−7.26 (m, 5H) 10.94 (s, 1H).

***N*-Cbz-6-methoxy-7-(2-bromoethoxy)-2,3-dihydroindole (29).** A solution of **28** (3.78 g, 12.6 mmol) in THF/DMSO (19 mL/19 mL) was added dropwise to a suspension of NaH (757 mg, 31.5 mmol) in THF/DMSO (2 mL/2 mL) under nitrogen atmosphere at 0 °C. After 15 min, 1,2-dibromoethane (3.3 mL, 37.9 mmol) was added dropwise. The reaction mixture was refluxed for 16 h and then cooled to 0 °C. Aqueous HCl (10%, 15 mL) and diethyl ether (80 mL) were added. The aqueous phase was separated and extracted with diethyl ether (2 × 80 mL). The organic phases were combined, washed with brine (2 × 70 mL), dried, and concentrated to give 3.87 g of crude product, which was purified by flash chromatography on silica gel (eluent cyclohexane/ethyl acetate, 9.1) to yield 2.22 g of **29** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.93 (t, 2H), 3.48 (t, 2H), 3.83 (s, 3H), 4.10 (t, 2H), 4.31 (t, 2H), 5.23 (s, 2H), 6.60 (d, 1H), 6.84 (d, 1H), 7.31−7.44 (m, 5H).

(*S*)-2-[((2-(6-Methoxy-7-benzofuranoxy)ethyl)amino)-methyl]-1,4-benzodioxane Hydrochloride [(*S*)-6]. A mixture of (*R*)-2- mesyloxymethyl-1,4-benzodioxane (713 mg, 3.14 mmol) and **18** (650 mg, 3.14 mmol) in 2-methylpropanol (5 mL) was subjected to microwave irradiation for 45 min (120 °C, 100 W). After cooling the reaction mixture to room temperature, the solvent was evaporated and the resultant residue taken up in dichloromethane (20 mL) and washed with 10% aqueous $NaHCO_3$ (3 × 20 mL) and with brine (2 × 20 mL). The organic phase was dried and concentrated to give a residue, which was purified by chromatography on silica gel (eluent: dichloromethane/methanol/triethylamine; 98:2:1), yielding 373 mg of (*S*)-2-[((2-(6-methoxy-6-benzofuranoxy)ethyl)amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]_D^{25}$ = −16.1 (*c* 1, $CHCl_3$). The amine was dissolved in ethanol (4 mL), and 3 N ethanolic HCl (2 mL) was added. The solvent was removed, and the resulting crude product was treated with ethyl acetate (3 mL) to give a suspension, which was cooled to 0 °C and filtered, yielding 172 mg (42%, based on the starting amount of secondary amine) of (*S*)-**6** as a white solid: mp 123.7 °C; $[\alpha]_D^{25}$ = −39.4 (*c* 1, ethanol). ^{1}H NMR (DMSO-d_6) δ 3.43 (m, 4H), 3.84 (s, 3H), 4.10 (m, 1H), 4.42 (m, 3H), 4.68 (m, 1H), 6.89 (m, 5H), 7.06 (d, 1H), 7.32 (d, 1H), 7.88 (m, 1H), 9.35 (br s, 2H, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 47.29, 47.37, 57.57, 65.58, 69.2, 70.01, 107.56, 107.62, 110.71, 116.38, 117.85, 118.08, 122.43, 123.57, 132.84, 142.57, 143.42, 146.42, 147.52, 149.55. Anal. ($C_{20}H_{22}ClNO_5$) C, H, N, Cl.

(*R*)-2-[((2-(6-Methoxy-7-benzofuranoxy)ethyl)amino)-methyl]-1,4-benzodioxane Hydrochloride [(*R*)-6]. Obtained from (*S*)-2-mesyloxymethyl-1,4-benzodioxane (707 mg, 2.9 mmol) and **18** (600 mg, 2.9 mmol) as described for (*S*)-**6**: mp 124.2 °C; $[\alpha]_D^{25}$ = +37.9 (*c* 1, ethanol. ^{1}H NMR identical to that of (*S*)-**6**. Anal. ($C_{20}H_{22}ClNO_5$) C, H, N, Cl. (*R*)-2-[((2-(6-Methoxy-6-benzofuranoxy)ethyl)amino)methyl]-1,4-benzodioxane (free amine): $[\alpha]_D^{25}$ = +15.9 (*c* 1, $CHCl_3$).

(*S*)-2-[((2-(6-Methoxy-7-indolyloxy)ethyl)amino)methyl]-1,4-benzodioxane [(*S*)-7]. A mixture of (*S*)-2-aminomethyl-1,4-benzodioxane (672 mg, 4.1 mmol) and **26** (1.16 g mg, 4.1 mmol) in 2-methylpropanol (5 mL) was subjected to microwave irradiation for 1 h (120 °C, 100 W). The reaction mixture was cooled to room temperature and concentrated; the resultant residue was taken up in dichloromethane (20 mL) and washed with10% aqueous $NaHCO_3$ (3 × 20 mL) and with brine (2 × 20 mL). The organic phase was dried and concentrated and the residue purified by chromatography on silica gel (eluent: dichloromethane/methanol; 9:1), yielding 584 mg (40%) of (*S*)-7 as a light pink solid: mp 120.5 °C; $[\alpha]_D^{25}$ = −20.3 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 2.50 (br s, 1H, exchangeable with D_2O), 3.05 (m, 4H), 3.91 (s, 3H), 4.00 (dd, 1H), 4.29 (m, 3H), 4.44 (m, 1H), 6.43 (d, 1H), 6.90 (m, 5H), 7.10 (d, 1H), 7.29 (d, 1H), 10.15 (br s, 1H, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 49.83, 49.93, 57.94, 66.79, 72.94, 73.37, 102.01, 102.05, 108.80, 115.60, 117.56, 117.76, 121.75, 122.01, 125.27, 125.63, 131.24, 133.95, 143.78, 146.93. Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

(*R*)-2-[((2-(6-Methoxy-7-indolyloxy)ethyl)amino)methyl]-1,4-benzodioxane [(*R*)-7]. Obtained from (*R*)-2-aminomethyl-1,4-benzodioxane (726 mg, 4.4 mmol) and **26** (1.25 g, 4.4 mmol) as described for (*S*)-7: mp 122 °C; $[\alpha]_D^{25}$ = +17.6 (*c* 1, $CHCl_3$). ^{1}H NMR identical to that of (*S*)-7. Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

(*S*)-2-[((2-(6-Methoxy-2,3-dihydro-7-benzofuranoxy)ethyl)-amino)methyl]-1,4-benzodioxane Hydrochloride [(*S*)-8]. A mixture of (*S*)-2-aminomethyl-1,4-benzodioxane (691 mg, 4.2 mmol) and **20** (721 mg, 2.5 mmol) in 2-methylpropanol (4 mL) was subjected to microwave irradiation for 2 h (90 °C, 40 W). The

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reaction mixture was cooled to room temperature and concentrated. The residue was taken up in dichloromethane (30 mL) and washed with 10% aqueous $NaHCO_3$ (3 × 20 mL) and with brine (2 × 20 mL). The organic phase was dried and concentrated, and the residue was purified by chromatography on silica gel (eluent: ethyl acetate), yielding 488 mg of (*S*)-2-[((2-(6-methoxy-2,3-dihydro-7-benzofuranoxy)ethyl)amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]_D^{25} = -22.6$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in ethanol (4 mL), and 3 N ethanolic HCl (3 mL) was added. The solvent was removed and the resulting crude product treated with refluxing ethyl acetate. After cooling to 0 °C, the precipitate was isolated by filtration, yielding 470 mg (87%, based on the starting amount of secondary amine) of (*S*)-**8** as a white solid: mp 160.0 °C; $[\alpha]_D^{25} = -34.0$ (*c* 1, ethanol). ^{1}H NMR (DMSO-d_6) δ 3.08 (t, 2H), 3.43 (m, 4H), 3.72 (s, 3H), 4.10 (m, 1H), 4.20 (t, 2H), 4.40 (m, 1H), 4.57 (t, 2H), 4.68 (m, 1H), 6.48 (d, 1H), 6.89 (m, 5H), 9.28 (br s, 1H, exchangeable with D_2O), 9.7 (br s, 1H, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 29.53, 47.10, 47.47, 56.80, 65.56, 68.32, 69.96, 73.14, 105.18, 117.85, 117.92, 118.07, 120.16, 122.01, 122.43, 131.70, 142.56, 143.41, 152.55, 152.66. Anal. ($C_{20}H_{24}ClNO_5$) C, H, N, Cl.

(*R*)-2-[((2-(6-Methoxy-2,3-dihydro-7-benzofuranoxy)ethyl)-amino)methyl]-1,4-benzodioxane Hydrochloride [(*R*)-8]. Obtained from (*R*)-2-aminomethyl-1,4-benzodioxane (500 mg, 3.0 mmol) and **20** (865 mg, 3.0 mmol) as described for (*S*)-**8**: mp 158.3 °C; $[\alpha]_D^{25} = +29.9$ (*c* 1, ethanol). ^{1}H NMR identical to that of (*S*)-**6**. Anal. ($C_{20}H_{24}ClNO_5$) C, H, N, Cl. (*R*)-2-[((2-(6-Methoxy-2,3-dihydro-7-benzofuranoxy) ethyl)amino)methyl]-1,4-benzodioxane (free amine): $[\alpha]_D^{25} = +19.5$ (*c* 1, $CHCl_3$).

(*S*)-2-[((2-(6-Methoxy-2,3-dihydro-7-indolyloxy)ethyl)-amino)methyl]-1,4-benzodioxane Dihydrochloride [(*S*)-9]. A mixture of (*S*)-2-aminomethyl-1,4-benzodioxane (1.13 g, 6.82 mmol) and **29** (1.39 g, 3.41 mmol) in 2-methylpropanol (8 mL) was refluxed for 18 h. The solvent was evaporated and the resultant residue taken up in dichloromethane (35 mL) and washed with 10% aqueous $NaHCO_3$ (3 × 25 mL) and then with brine (3 × 20 mL). The organic phase was dried and concentrated and the residue purified by chromatography on silica gel (eluent: ethyl acetate), yielding 950 mg of (*S*)-2-[((2-(*N*-Cbz)-(6-methoxy-2,3-dihydro-7-indolyloxy)ethyl)-amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]_D^{25} = -19.6$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in methanol (70 mL), and 5% Pd/C (82 mg) was added. The reaction mixture was stirred under hydrogen atmosphere for 2 h. Afterward the catalyst was removed by filtration and the solvent evaporated. The resulting residue was taken up in dichloromethane (30 mL), washed with $NaHCO_3$ (2 × 15 mL), dried, and concentrated to give 660 mg of (*S*)-2-[((2-(6-methoxy-2,3-dihydro-7-indolyloxy)ethyl)amino)methyl]-1,4-benzodioxane: $[\alpha]_D^{25} = -23.6$ (*c* 1, $CHCl_3$). The diamine was dissolved in ethanol (1 mL), and 1.5 N ethanolic HCl (5 mL) was added. The resulting mixture was heated at 50 °C for 10 min and then stirred at 0 °C for 1 h. The suspension was filtered, obtaining 650 mg (82%, based on the starting amount of secondary amine) of (*S*)-**9** as a white solid: mp 238 °C; $[\alpha]_D^{25} = -46.3$ (*c* 1, methanol). ^{1}H NMR (DMSO-d_6) 3.11 (t, 2H), 3.34 (dd, 1H), 3.38–3.50 (m, 2H), 3.59 (dd, 1H), 3.68 (t, 2H), 3.81 (s, 3H), 4.11 (dd, 1H), 4.42–4.48 (m, 3H), 4.77–4.82 (m, 1H), 6.84–6.95 (m, 4H), 7.01 (d, 1H), 7.08 (d, 1H), 9.50 (br s, 1H, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ29.37, 46.11, 47.62, 48.02, 57.25, 65.54, 68.84, 69.87, 113.85, 117.82, 117.98, 118.15, 121.11, 122.41, 128.75, 131.56, 138.19, 142.54, 143.42, 151.94. Anal. ($C_{20}H_{26}Cl_2N_2O_4$) C, H, N, Cl.

(*R*)-2-[((2-(6-Methoxy-2,3-dihydro-7-indolyloxy)ethyl)-amino)methyl]-1,4-benzodioxane Dihydrochloride [(*R*)-9]. Obtained from (*R*)-2-aminomethyl-1,4-benzodioxane (812 mg g, 4.92 mmol) and **29** (1.0 g, 2.46 mmol) as described for (*S*)-**9**: mp 238 °C; $[\alpha]_D^{25} = +44.60$ (*c* 1, methanol). ^{1}H NMR identical to that of (*S*)-**9**. Anal. ($C_{20}H_{26}Cl_2N_2O_4$) C, H, N, Cl. (*R*)-2-[((2-(6-Methoxy-2,3-dihydro-7-indolyloxy)ethyl)amino)methyl]-1,4-benzodioxane (free amine): $[\alpha]_D^{25} = +23.2$ (*c* 1, $CHCl_3$).

Biology. According to previously reported procedures,[4,8] the pharmacological profile of compounds **6**–**9** was assessed: (a) by measuring the affinities of both the *S* and *R* enantiomers for α_{1a}, α_{1b}, α_{1d} AR-subtypes, and 5-HT_{1A} serotoninergic receptor with in vitro binding studies, (b) by determining the α_1-AR subtypes and α_2-AR blocking activity of the *S* enantiomers on different rat tissues, and (c) by evaluating the inverse agonist activity of the *S* enantiomers as an inhibition of calcium-induced increase in the resting tension (IRT) of calcium-depleted guinea pig thoracic aorta.

α_1-AR binding studies were carried out in membranes derived from Chinese Hamster Ovary (CHO) cells expressing α_1-AR subtypes were incubated for 30 min at 25 °C in Tris HCl, 50 mM (pH = 7.7, containing 10 μM pargyline and 0.1% ascorbic acid) with 0.5 nM [^{3}H]prazosin, in the absence or presence of different concentrations of the tested compounds. Prazosin, 1 μM, was used to determine nonspecific binding. 5-HT_{1A} receptor binding studies were carried out in crude membrane preparations from rat hippocampus, which were incubated for 30 min at 25 °C in Tris HCl (50 mM, pH = 7.7, containing 10 μM pargyline and 4 mM $CaCl_2$) with 1 nM [^{3}H]-8-OH-DPAT, in the absence or presence of different concentrations of the tested compounds. 5-HT, 1 μM, was used to determine nonspecific binding. Incubation was stopped by rapid filtration through GF/B fiber filters.

Saturation curves were analyzed using the one-site binding equation built into GraphPad Prism, giving IC_{50} values with the relative standard errors. Affinity constants were expressed as pK_i (−log K_i values, calculated using the Cheng and Prusoff equation).

Functional antagonism was determined on different tissues from Male Sprague–Dawley rats (Charles River, Italy). The required organ were isolated, freed from adhering connective tissue, and set up rapidly under resting tension in an organ bath (15 mL) containing physiological salt solution kept at appropriate temperature (see below) and gassed with 95% O_2 and 5% CO_2 at pH 7.4. Concentration–response curves were constructed by cumulative addition of agonist before and after incubation with antagonist. Parallel experiments in which tissues did not receive any antagonist were run to check any variation in sensitivity.

Prostates (from rats of 200–250 g) were used to assess α_{1A}-adrenoceptor antagonist activity.[22] Before the concentration curves were started, tissues were exposed to (−)-noradrenaline at a concentration of 1.0 μM. A minimum response of 0.5 g of tension was required for the tissue to be used for concentration–response curves. After a 90 min time period, a cumulative response curve to (−)-noradrenaline was constructed. After completion of the concentration–response curve, the tissue was washed for 90 min, and the antagonist was added and incubated for 30 min before a second cumulative concentration–response curve was obtained.

The vas deferens prostatic portion (from rats of 200–250 g) was used to assess α_{1A}-adrenoceptor antagonist activity.[20] Desipramine hydrochloride (0.01 μM) was present in the organ bath solution to prevent the neuronal uptake of (−)-noradrenaline. Contraction–response curves for isotonic contractions in response to (−)-noradrenaline were recorded at 30 min intervals, the first one being discarded and the second one taken as control. After the incubation with antagonist for 30 min, a third dose–response curve was obtained.

α_2-Adrenoreceptor antagonist activity was determined also on vas deferens prostatic portions. Propranolol hydrochloride (1 μM) and desipramine hydrochloride (0.01 μM) were present in the organ bath solution throughout the experiments to block β-adrenoreceptors and to prevent the neuronal uptake of (−)-noradrenaline, respectively. A first clonidine concentration–response curve, taken as control, was obtained cumulatively. The antagonist was allowed to equilibrate with the tissue for 30 min before obtaining a second dose–response curve.

Spleens (from rats of 250–300 g) were used to assess α_{1B}-adrenoceptor antagonist activity.[21] Desipramine hydrochloride (0.01 μM) and propranolol hydrochloride (1 μM) were added to the organ bath solution to prevent the neuronal uptake of (−)-noradrenaline and to block β-adrenoreceptors, respectively. The cumulative concentration–response curves to phenylephrine were measured isometrically and obtained at 30 min intervals, the first one being discarded and the second one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min, and then a new concentration–response curve to the agonist was constructed.

Thoracic aortas (from rats of 250−300 g) were used to assess α_{1D}-adrenoceptor antagonist activity.[21] Desipramine hydrochloride (0.01 μM)) and propranolol hydrochloride (1 μM) were added to the organ bath solution to prevent the neuronal uptake of (−)-noradrenaline and to block β-adrenoreceptors, respectively. Two helicoid strips were cut in strips from each aorta of about 1.5 cm length. The endothelium was removed by rubbing with filter paper: the functional loss of endothelial cells was confirmed by the absence of the relaxing response to acetylcholine. After an at least 1 h equilibration period under an optimal tension of 1 g, cumulative (−)-noradrenaline dose−response curves were recorded, the first two being discarded and the third one taken as a control. The antagonist was allowed to equilibrate with the tissue for 30 min before the generation of a fourth cumulative dose−response curve with (−)-noradrenaline.

The guinea pig thoracic aorta was used to assess the activity of α_1-antagonist as inverse agonist.[4,28] Aortic strips were isolated and cleaned as previously described and placed in an organ bath containing the Krebs solution maintained at 37 °C of the following composition (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 1.8; $MgCl_2$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; glucose, 11. Tissues were equilibrated for 1 h under an optimal tension of 1 g, and the effect of a single dose of (−)-noradrenaline (1 μM) was recorded. During 1 h of wash in Ca^{2+}-free Krebs solution containing EDTA (0.1 mM), the agonist was applied and washed with Ca^{2+}-free solution until no contraction was elicited, indicating depletion of internal Ca^{2+} stores sensitive to NA. After incubation with the antagonist for 30 min, addition of Ca^{2+} (1.8 mM) induced an increase in the resting tension (IRT). The magnitude of the inhibition was expressed as a percent decrease of the reference IRT, namely of the IRT induced by calcium (1.8 mM) in the absence of any agent.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR and ^{13}C NMR spectra and elemental analysis results for the final compounds **6**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AR, adrenoreceptor; Cbz, carbobenzyloxy; DCM, dichloromethane; DPPA, diphenylphosphoryl azide; HBA, hydrogen bond acceptor; IRT, increase in resting tension; LUTS, lower urinary tract symptoms; MsCl, mesyl chloride; SAR, structure−activity relationship; TEA, triethylamine

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