WB4101-Related Compounds: New, Subtype-Selective α_1-Adrenoreceptor Antagonists (or Inverse Agonists?)

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Our previous structure−affinity relationship study had considered the enantiomers of the naphthodioxane, tetrahydronaphthodioxane, and 2-methoxy-1-naphthoxy analogues (compounds **1**, **3**, and **2**, respectively) of 2-(2,6-dimethoxyphenoxyethylaminomethyl)-1,4-benzodioxane, the well-known α_1-adrenoceptor (α_1-AR) antagonist WB4101, showing that such modifications significantly modulate the affinity and selectivity profile for α_1-AR subtypes and 5-HT_{1A} receptor. Here, we extend investigations to antagonist activity enclosing new enantiomeric pairs, namely those of the methoxytetrahydronaphthoxy and methoxybiphenyloxy WB4101 analogues (**4** and **5**−**7**, respectively) and of a double-modified WB4101 derivative (**8**) resulting from hybridization between **2** and **3**. We found that (*S*)-**2** is a very potent (pA_2 10.68) and moderately selective α_{1D}-AR antagonist and the hybrid (*S*)-**8** is a potent (pA_2 7.98) and highly selective α_{1A}-AR antagonist. Both of these compounds and (*S*)-WB4101 seem to act as inverse agonists in a vascular model. The results, which generally validate the logic we followed in designing these eight compounds, are acceptably rationalized by comparative SAR analysis of binding and functional affinities.

Introduction

The tissue distribution and physiological role of the three α_1-adrenoreceptor (α_1-AR) subtypes (α_{1A}, α_{1B}, and α_{1D}) in humans have been intensively investigated in recent years as well as involvement of their overstimulation in the etiology of pathological disorders, in particular of hypertension and lower urinary tract symptoms (LUTS) secondary to benign prostatic hyperplasia (BPH).[1,2] The latest evidence indicates that the α_{1A} subtype and, with aging, the α_{1B} subtype would be the principal mediators of blood pressure control, while the α_{1A} and α_{1D} subtypes, predominating in prostatic stroma and bladder detrusor muscle, respectively, mediate LUTS. Owing to these effects, α_1-AR blockers were initially developed for the treatment of hypertension and, successively, of symptomatic BPH. Unfortunately, few ligands recognize only one α_1-AR subtype and such a lack of selectivity limits their administration in vivo and their therapeutic use. Although the relative importance of the various adrenergic receptor subtypes is still controversial, selectivity for the α_{1A} and/or α_{1D} subtypes over the α_{1B} subtype appears to be important for clinical "uroselectivity", that is, high effectiveness in relieving LUTS with low incidence of cardiovascular and hypotensive adverse effects.

Our research group has long been involved in designing new α_1-AR ligands structurally related to 2-(2,6-dimethoxyphenoxy)-ethylaminomethyl-1,4-benzodioxane (WB4101), a potent α_1 antagonist displaying a slight selectivity for α_{1A}- and, to a minor extent, for α_{1D}-ARs with respect to α_{1B}-AR and 5-HT_{1A} serotoninergic receptor.[3−8] Recently, we have demonstrated that it is possible to significantly modulate the affinity and selectivity profile of (*S*)-WB4101, the more potent enantiomer, simply fusing a cyclohexane or an additional benzene ring with its benzodioxane or phenoxy moiety.[6] Successively, by a wide number of ortho monosubstituted phenoxy analogues, we have proved that removal of one or both *o*-methoxy substituents adversely affects the affinity for the three α_1-AR subtypes, but not for the 5-HT_{1A} receptor, for which (*S*)-WB4101 analogues unsubstituted or suitably *o*-monosubstituted at the phenoxy moiety exhibit very high and significantly specific affinity.[7] Consistent with such indications, the affinity data for a series of new ortho disubstituted analogues we have lately studied show that *o*-disubstitution and the presence of at least one *o*-methoxyl play a crucial role for the interaction of the phenoxy moiety with the α_1-AR and, especially, with the α_{1a} subtype.[8] On these bases, we designed new WB4101 analogues **4**−**8** (Chart 1) in order to further investigate the consequences of different patterns of substitution, in particular with saturated or unsaturated carbocycles, at the phenoxy and benzodioxane moieties. The hypothesis was that such modifications, as those previously carried out and resulting in compounds **1**−**3**, would remarkably affect the affinity and selectivity profile and, hopefully, the α_1 activity spectrum. The ultimate aim was to obtain potent and selective α_{1A} and/or α_{1D} antagonists.

Here, we describe the synthesis of the *S* and *R* enantiomers of **4**−**8** and the biological profile of such novel compounds and of closely related congeners **1**−**3** (Chart 1) in binding and functional experiments at α_1-AR subtypes and the 5-HT_{1A} receptor. Furthermore, the nature of inverse agonist of WB4101 enantiomers and of the most potent and/or selective derivatives in the present series was studied and semiquantitatively determined by means of a vascular model. Finally, the SAFIR[a] and SAR data were comparatively analyzed.

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[a] Abbreviations: SAFIR, structure affinity relationship.

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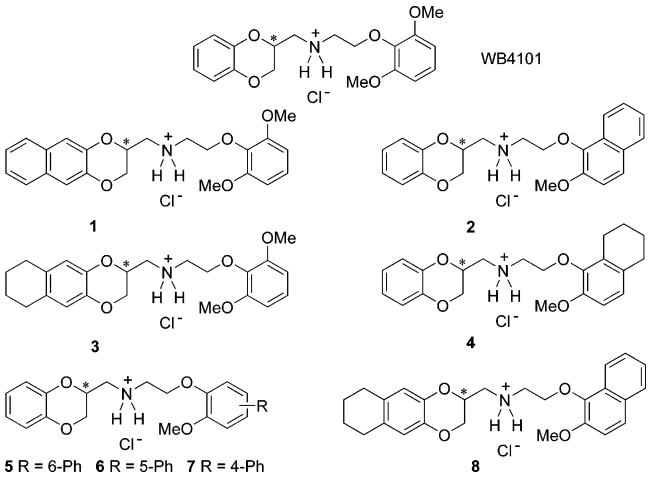
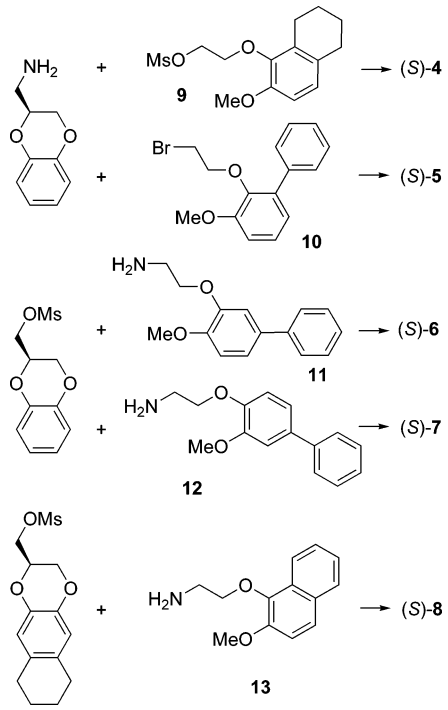
Chart 1



Scheme 1. Synthesis of Compounds (*S*)-**4**−(*S*)-**8**.

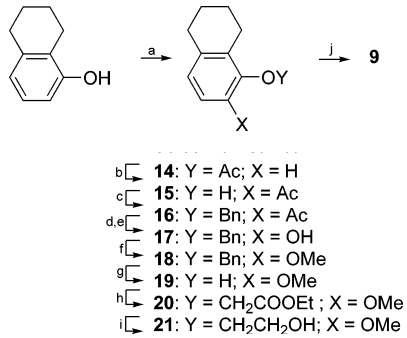


Chemistry

Of compounds **1**−**8**, which are required for the current study, **1**−**3** have been previously reported in both enantiomeric forms,[6] while **4**−**8** are new. The enantiomeric pairs of the latter were prepared by the same strategy as the former: enantiopure 2-aminomethyl- or 2-mesyloxymethyl-substituted benzodioxane or tetrahydronaphthodioxane, whose syntheses we have already described,[6,9,10] was reacted with 2-aryloxyethyl mesylate **9** or bromide **10** or with 2-aryloxyethylamines **11**−**13**, as exemplified in Scheme 1 for the *S* isomers.

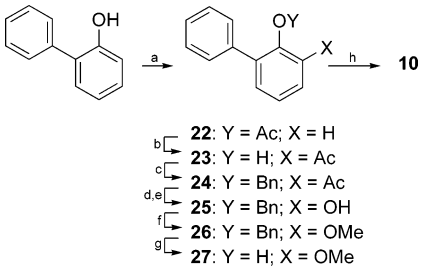
Mesylate **9** was prepared as outlined in Scheme 2. Esterification of 5,6,7,8-tetrahydro-1-naphthol with acetyl chloride, followed by Fries rearrangement, afforded **15**, which was benzylated and converted to 1-benzyloxy-5,6,7,8-tetrahydro-2-naphthol (**17**) by oxidation to acetate and ester hydrolysis. This was O-methylated and the resultant diether **18** debenzylated, yielding 2-methoxy-5,6,7,8-tetrahydro-1-naphthol (**19**), whose sodium salt was reacted with ethyl chloroacetate to obtain tetrahydronaphthoxyacetate **20**. Successive reduction of the ester to alcohol **21** and final mesylation of the latter led to **9**.

Scheme 2. Synthesis of Compound **9**[a]



[a] Reagents and conditions: (a) AcCl, Py, DCM; (b) $AlCl_3$, dichlorobenzene; (c) benzyl bromide, tetrabutylammonium bromide, 2.5 N NaOH, DCM; (d) *m*-CPBA, EtOAc; (e) 2.5 N NaOH, MeOH; (f) MeI, tetrabutylammonium bromide, 2.5 N NaOH, DCM; (g) H_2, Pd/C, EtOAc; (h) ethyl chloroacetate, NaH, DMSO; (i) $LiAlH_4$, THF; (j) mesyl chloride, Et_3N, DCM.

Scheme 3. Synthesis of Compound **10**[a]



[a] Reagents and conditions: (a) AcCl, Py, DCM; (b) $AlCl_3$, dichlorobenzene; (c) benzyl bromide, tetrabutylammonium bromide, 2.5 N NaOH, DCM; (d) *m*-CPBA, EtOAc; (e) 2.5 N NaOH, MeOH; (f) MeI, tetrabutylammonium bromide, 2.5 N NaOH, DCM; (g) H_2, Pd/C, acetone; (h) 1,2-dibromoethane, tetrabutylammonium bromide, 2.5 N NaOH, DCM.

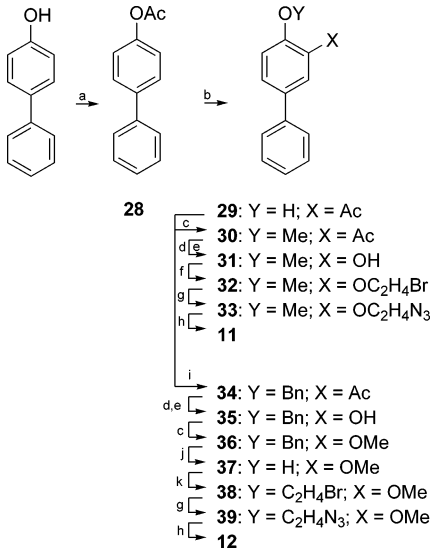
Bromide **10** was synthesized from 2-phenylphenol by the reaction sequence illustrated in Scheme 3: (a) esterification with acetyl chloride, (b) Fries rearrangement, (c) O-benzylation of 2-phenyl-6-acetylphenol (**23**), (d) oxidation to acetate and ester hydrolysis, (e) O-methylation of 2-benzyloxy-3-phenylphenol (**25**), (f) debenzylation, and (g) reaction with dibromoethane to give **10**.

The syntheses of *m*- and *p*-phenyl-substituted *o*-methoxyphenoxyethylamines, **11** and **12**, respectively, are shown in Scheme 4. They were both prepared from 2-acetyl-4-phenylphenol (**29**), which in turn was obtained from 4-phenylphenol by acetylation and subsequent Fries rearrangement. Phenol **29** was O-methylated, transformed by oxidation to the acetate and by ester hydrolysis into 2-methoxy-5-phenylphenol (**31**), and etherified with dibromoethane. The resultant 2-bromoethyl ether **32** was converted into azide **33** and finally reduced to amine **11**. Alternatively, phenol **29** was O-benzylated, oxidized to the acetate, and hydrolyzed to 2-benzyloxy-5-phenylphenol **35**, which was O-methylated and debenzylated to give **37**, the isomer of **31** with exchanged methoxyl and hydroxyl positions. Like the latter, **37** was etherified with dibromoethane, converted into the azide, and finally reduced, yielding amine **12**. Nucleophilic displacement of bromide by azide ion and successive reduction with hydrazine were also accomplished to synthesize amine **13** from 1-(2-bromoethoxy)-2-methoxynaphthalene, whose preparation has been previously reported.[6]

Biology

Binding Experiments. As previously reported for the enantiomers of WB4101 and of compounds **1**−**3**,[6] the pharmacologi-

Scheme 4. Synthesis of Compounds **11** and **12**[a]



[a] Reagents and conditions: (a) AcCl, Py, DCM; (b) $AlCl_3$, dichlorobenzene; (c) MeI, tetrabutylammonium bromide, 2.5 N NaOH, DCM; (d) *m*-CPBA, EtOAc; (e) 2.5 N NaOH, MeOH; (f) 1,2-dibromoethane, KOH, DMSO; (g) NaN_3, DMF; (h) hydrazine, PdO, MeOH; (i) benzyl bromide, tetrabutylammonium bromide, 2.5 N NaOH, DCM; (j) H_2, Pd/C, MeOH; (k) 1,2-dibromoethane, tetrabutylammonium bromide, 2.5 N NaOH, DCM.

cal profile of the enantiomeric pairs of **4**−**8** was initially evaluated by measuring affinities for cloned human α_{1a}-, α_{1b}-, and α_{1d}-AR subtypes and for native 5-HT_{1A} serotoninergic receptor from rat hippocampus. [^{3}H]prazosin was used to label cloned human α_1-ARs expressed in Chinese hamster ovary (CHO) cells, while [^{3}H]-8-OH-DPAT was the radioligand used in the binding assays at 5-HT_{1A} receptor, carried out in membrane preparations from rat hippocampus. The affinity constants, expressed as pK_i, of (*S*)- and (*R*)-WB4101 and of the enantiomers of **1**−**8** for these four receptors, are listed in Table 1.

Functional Assays. α_1-AR subtypes and α_2-AR blocking activity of both enantiomers of WB4101 and of **2** and of the *S* isomers of compounds **1** and **3**−**8** was determined on different rat tissues. In particular, α_1-AR subtypes blocking activity was assessed by antagonism of (−)-noradrenaline (NA)-induced contraction of vas deferens prostatic portion (α_{1A})[11] or thoracic aorta (α_{1D})[12] and by antagonism to (-)-phenylephrine-induced contraction of spleen (α_{1B}),[12] while α_2-adrenoreceptor blocking activity was determined by antagonism to clonidine-inhibited twitch responses of the field-stimulated prostatic portion of vas deferens. 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 for the lead compound, its enantiomers, and (*S*)-**8**, which is the most α_{1A}-AR selective compound in the series.[13] All the antagonist affinities, expressed as pA_2, of WB4101 and **2** enantiomers and of the *S* forms of **1** and **3**−**8** are listed in Table 2 together with those of WB4101 racemate, some of which have been previously reported.

For WB4101, its enantiomers, (*S*)-**2**, (*R*)-**2**, (*S*)-**5**, and (*S*)-**8**, inverse agonism was assessed by preincubating calcium-depleted guinea pig thoracic aorta with different concentrations of such antagonists and then evaluating the corresponding diminutions of successively Ca^{2+}-induced increase in the resting tone (IRT) of that tissue with respect to the IRT consequent on the same Ca^{2+}administration, but in the absence of antagonist.[14] The results are shown by the histogram represented in Figure 1.

Discussion

SARs. As previously reported,[6] binding tests of (*S*)-**1** and (*S*)-**3** at the three cloned human α_1-AR subtypes and 5-HT_{1A} receptor had indicated that both the naphthodioxane and tetrahydronaphthodioxane derivatives have almost equally (about 2 orders of magnitude) decreased α_{1a}, α_{1b}, and 5-HT_{1A} affinities with respect to (*S*)-WB4101, whereas, for α_{1d}, the loss of affinity is near 3 orders of magnitude. Such a trend slightly improves the α_{1a}/α_{1b} and α_{1a}/5-HT_{1A} selectivities of (*S*)-WB4101 and produces, what is noteworthy, a moderate α_{1a}/α_{1d} selectivity (α_{1a}/α_{1d} affinity ratio of 12.3 and 13.5, respectively), of which the lead compound is completely devoid (α_{1a}/α_{1d} affinity ratio of 1.3), so that (*S*)-**1** and (*S*)-**3** have still good α_{1a} affinity (pK_i = 7.47 and 7.60, respectively) and higher specificity for α_{1a}-AR than (*S*)-WB4101. Analogous enlargement had been accomplished at the phenoxy moiety of (*S*)-WB4101 by addition of a fused benzene ring, leading to (*S*)-**2**, which shows a significantly higher α_{1a} affinity (pK_i = 8.80) than (*S*)-**1** and (*S*)-**3**, with only slightly decreased α_{1a} specificity with respect to the latter [affinity ratios α_{1a}/α_{1b} of 10, α_{1a}/α_{1d} of 4, and α_{1a}/

Table 1. Affinity Constants, Expressed as pK_i (−log K_i, M), of WB4101 Enantiomers and of Compounds **1**−**8** for Cloned Human α_1-Adrenoceptor Subtypes and 5-HT_{1A} Receptor[a]

	pK_i (±ς EM)				affinity ratios[b]		
	α_{1a}	α_{1b}	α_{1d}	5-HT_{1A}	α_{1a}/α_{1b}	α_{1a}/α_{1d}	α_{1a}/5-HT_{1A}
S-WB4101	9.39 ± 0.06	8.24 ± 0.04	9.29 ± 0.11	8.61 ± 0.04	14.1	1.3	6.0
R-WB4101	7.95 ± 0.04	7.14 ± 0.06	7.98 ± 0.08	7.39 ± 0.03	6.5	0.9	3.6
(*S*)-**1**	7.47 ± 0.05	6.05 ± 0.04	6.38 ± 0.04	6.46 ± 0.04	26.3	12.3	10.2
(*R*)-**1**	6.99 ± 0.08	6.37 ± 0.04	6.53 ± 0.04	6.00 ± 0.04	4.2	2.9	9.8
(*S*)-**2**	8.80 ± 0.09	7.80 ± 0.07	8.18 ± 0.13	7.95 ± 0.06	10.0	4.2	7.1
(*R*)-**2**	8.34 ± 0.05	7.07 ± 0.07	7.65 ± 0.05	7.17 ± 0.04	18.6	4.9	14.8
(*S*)-**3**	7.60 ± 0.15	6.24 ± 0.03	6.47 ± 0.06	6.44 ± 0.03	22.9	13.5	14.5
(*R*)-**3**	7.00 ± 0.07	6.40 ± 0.05	6.69 ± 0.04	6.30 ± 0.03	4.0	2.0	5.0
(*S*)-**4**	8.55 ± 0.15	7.72 ± 0.07	7.84 ± 0.08	8.07 ± 0.08	6.8	5.1	3.0
(*R*)-**4**	7.94 ± 0.05	7.31 ± 0.03	7.80 ± 0.04	6.88 ± 0.04	4.3	1.3	11.5
(*S*)-**5**	8.92 ± 0.12	7.75 ± 0.05	8.47 ± 0.09	8.16 ± 0.07	14.8	2.8	5.8
(*R*)-**5**	8.03 ± 0.07	6.82 ± 0.06	7.54 ± 0.07	7.11 ± 0.06	16.2	3.1	8.3
(*S*)-**6**	8.25 ± 0.14	7.96 ± 0.14	7.82 ± 0.07	7.28 ± 0.04	1.9	2.7	9.3
(*R*)-**6**	7.79 ± 0.03	7.21 ± 0.07	7.27 ± 0.05	6.44 ± 0.04	3.8	3.3	22.4
(*S*)-**7**	7.06 ± 0.03	6.73 ± 0.12	6.67 ± 0.07	7.13 ± 0.02	2.1	2.5	0.9
(*R*)-**7**	6.63 ± 0.03	6.26 ± 0.15	6.44 ± 0.06	6.06 ± 0.06	2.3	1.5	3.7
(*S*)-**8**	7.18 ± 0.01	6.43 ± 0.04	6.83 ± 0.03	5.77 ± 0.02	5.6	2.2	25.7
(*R*)-**8**	6.49 ± 0.11	6.26 ± 0.10	6.02 ± 0.11	5.44 ± 0.06	1.7	3.0	11.2

[a] Data of WB4101 and **1**−**3** are reported in ref 6. [b] Antilog of ΔpK_i.

Table 2. Antagonist Affinities, Expressed as pA_2, of WB4101, Its Enantiomers, and Compounds **1**−**8** (*S* Isomers and, for **2**, Also the *R* Isomer) at α_1- and α_2-Adrenoceptors on Isolated Rat Tissues, Namely, Prostatic Vas Deferens (α_{1A} and α_2), Prostate (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D})

	pA_2[a]					affinity ratios[b]	
	α_{1A}				$\alpha_{2A/D}$		
compd	vas deferens	prostate	α_{1B} spleen	α_{1D} aorta	vas deferens	α_{1a}/α_{1b}	α_{1a}/α_{1d}
WB4101	9.36 ± 0.04	9.18 ± 0.02	8.21 ± 0.02	8.60 ± 0.02	6.59 ± 0.03	14.1	5.8
S-WB4101	9.98 ± 0.01	9.49 ± 0.05	9.17 ± 0.01	9.20 ± 0.06	6.92 ± 0.02	6.5	6.0
R-WB4101	7.79 ± 0.03	7.70 ± 0.03	7.64 ± 0.01	7.65 ± 0.04	6.34 ± 0.05	1.4	1.4
(*S*)-**1**	5.46 ± 0.02		5.61 ± 0.04	6.74 ± 0.01	<5	0.7	0.05
(*S*)-**2**	8.96 ± 0.05		9.69 ± 0.09	10.68 ± 0.06	5.87 ± 0.04	0.2	0.02
(*R*)-**2**	7.23 ± 0.07		7.83 ± 0.04	8.00 ± 0.03	6.20 ± 0.01	0.25	0.17
(*S*)-**3**	<5		5.58 ± 0.06	5.36 ± 0.07		<0.3	<0.4
(*S*)-**4**	7.04 ± 0.08		8.35 ± 0.09	8.39 ± 0.06		0.05	0.04
(*S*)-**5**	8.21 ± 0.03		8.12 ± 0.01	8.99 ± 0.05		1.2	0.2
(*S*)-**6**	<5		6.87 ± 0.04	7.21 ± 0.01		<0.01	<0.006
(*S*)-**7**	6.12 ± 0.05		6.88 ± 0.02	6.97 ± 0.08		0.17	0.14
(*S*)-**8**	7.98 ± 0.09	7.37 ± 0.03	<5	5.59 ± 0.08	<5	>955	245

[a] pA_2 values ± SEM ($n = 5-7$) were calculated from Schild plots,[15] constrained to a slope of −1.0, unless otherwise specified.[16] pA_2 is the positive value of the intercept of line derived by plotting log(DR − 1) vs log[antagonist]. The log(DR − 1) was calculated at 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 of the agonist (EC_{50}) in the presence of the antagonist and in its absence. The parallelism of dose−response curves was checked by linear regression, and the slopes were tested for significance ($p < 0.05$). [b] Antilog of ΔpA_2.

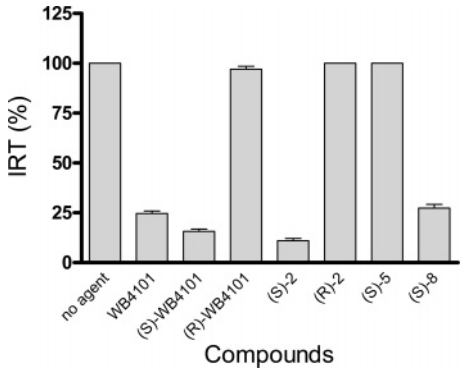


Figure 1. Magnitude of Ca^{2+} (1.8 mM)-induced increase in the resting tension (IRT) of calcium-depleted guinea pig thoracic aorta in the presence of WB4101 (10 nM), (*S*)-WB4101 (10 nM), (*R*)-WB4101 (100 nM), (*S*)-**2** (10 nM), (*R*)-**2** (10 nM), (*S*)-**5** (10 nM), and (*S*)-**8** (100 nM) expressed as a percentage of Ca^{2+} (1.8 mM)-induced IRT in the absence of any agent. Data represent the mean ± SEM from three to five experiments.

$5HT_{1A}$ of 7 versus 26, 12, and 10, and 23, 13, and 14, respectively, for (*S*)-**1** and (*S*)-**3**]. Then, considering that tetrahydronaphthodioxane (*S*)-**3** exhibits a similar profile to that of naphthodioxane (*S*)-**1**, namely analogous α_{1a} affinity and selectivity, it seemed worthwhile to verify if the conversion of naphthoxy derivative (*S*)-**2** into tetrahydronaphthoxy analogue (*S*)-**4** would be also nondetrimental or even productive. Conversely, (*S*)-**4** exhibits lower and more leveled affinities than (S)-**2**, partly losing the α_{1a} specificity of the latter. These results could be interpreted considering the reported binding models of WB4101 with α_{1a}-AR, which propose the interaction between two hydrophobic/aromatic regions of the antagonist-binding site with the benzodioxane system and the 2,6-dimethoxyphenoxy fragment respectively.[17−19] According to such models, the pocket interacting with this fragment is characterized by the presence of more aromatic amino acid residues, which might explain why both receptor regions tolerate the addition of a fused cycle to the respective aromatic counterpart of the ligand molecule, but with a preference for a benzene over a cyclohexane in the case of the phenoxy enlargement. In other words, due to the different nature of the two subsites, the aromaticity of the added ring would be determinant for the interaction of the aryloxy moiety and only optional for that of the benzodioxane system.

This reasonable hypothesis induced us to consider the substitution at the phenoxy moiety with a phenyl residue hoping that less rigid biphenyloxy systems could allow a better interaction of the additional aromatic ring than the naphthyloxy residue. We then explored the replacement of 2,6-dimethoxyphenoxyl by 6-, 5-, and 4-phenyl-substituted 2-methoxyphenoxyl. Interestingly, the binding affinities of (*S*)-**5**, bearing a 2-methoxy-6-phenylphenoxy moiety, are nearly identical to those of (*S*)-**2**, suggesting analogous binding interactions for the additional benzene ring, whether 6-positioned or 5,6-fused on the 2-methoxyphenoxy fragment. Phenyl shifting from the 6- to the 5-position and then to the 4-position gradually decreases the affinity at all the tested receptors, except the α_{1b} subtype. It is noteworthy that two of the three derivatives, (*S*)-**5** and (*S*)-**6**, show high affinity values (pK_i ranges of 8.92−7.75 and 8.25−7.28, respectively), substantially similar to those of (*S*)-**2** and markedly higher than those of (*S*)-**1** and (*S*)-**3**, while the third derivative, (*S*)-**7**, is sensibly outdistanced, its pK_i values ranging from 7.13 to 6.67. The affinity decreases consequent on phenyl shifting from ortho to meta and para positions of 2-methoxyphenoxyl would further support the already demonstrated importance of its ortho disubstitution[7,8] and confirm the hypothesized negative effects of its para substitution.[20] Indeed, considering the affinity values of (*S*)-**5**, (*S*)-**6**, and (*S*)-**7**, it can be stated that a phenyl substituent on the 2-methoxyphenoxy group is well-tolerated, if 6- or even 5-positioned but not 4-positioned, and it cannot be excluded that such a substituent contributes to the postulated interaction with that binding pocket lined by a cluster of aromatic residues.

Finally, we decided to hybridize a structure modified at the phenoxy moiety with one modified at the benzodioxane system. On the basis of the previous affinity and selectivity data, it seemed promising to combine the tetrahydronaphthodioxane system, the presence of which had produced the maximum gain of α_{1a} specificity relative to (*S*)-WB4101 with only moderate loss of α_{1a} affinity [see (*S*)-**3**], with the 2-methoxy-1-naphthoxy moiety, the introduction of which, vice versa, had given the closest α_{1a} affinity to (*S*)-WB4101 with a slight increase of α_{1a} specificity [see (*S*)-**2**]. As shown in Table 1, the results of such a hybridization disappointed our expectations: the affinities of (*S*)-**8** at the three α_1-AR subtypes and the 5-HT_{1A} receptor are

from moderate (α_{1a} pK_i = 7.18) to modest (5-HT$_{1A}$ pK_i = 5.77), much lower than those of (*S*)-**2**, and its α_{1a} specificity, compared to that of (*S*)-**3**, did not significantly improve.

In the same table, the affinities of the *R* isomers of **1**−**8** are also reported. Except the cases of the low α_{1b} and α_{1d} affinities displayed by the enantiomeric pairs of **1** and **3**, they are always lower than those of the corresponding *S* isomers. It is noteworthy that (*R*)-**2** and (*R*)-**5** show very similar affinities like their *S* antipodes and, compared with the latter, analogous degrees of α_{1a} specificity.

For WB4101, its enantiomers, (*S*)-**1**−(*S*)-**8**, and (*R*)-**2**, the antagonist affinities at α_1-AR subtypes were determined on isolated rat tissues. The results are listed in Table 2. Most of them diverge from the binding data (Table 1), representing different trends, which can be summarized as follows. (*S*)-WB4101, due to the remarkable increase of α_{1B} antagonist affinity with respect to α_{1b} binding affinity (pA_2 = 9.17 versus pK_i = 8.24), becomes near equipotent (9 < pA_2 < 10) at the three α_1-AR subtypes. An analogous trend is shown by (*R*)-WB4101, namely α_{1B} antagonist affinity increase (pA_2 = 7.64 versus pK_i = 7.14) and consequent lost of any α_1 subtype selectivity (α_{1A} pA_2 = 7.79, α_{1B} pA_2 = 7.64, and α_{1D} pA_2 = 7.65). For (*S*)-**2**, (*R*)-**2**, (*S*)-**4**, and (*S*)-**5**, the α_{1D} and the α_{1B} pA_2 values are higher, sometimes to a great extent, than the corresponding pK_i values, while, at α_{1A}, there is a slight difference [see (*S*)-**2**] or moderate decrease [see (*R*)-**2**, (*S*)-**4**, and (*S*)-**5**]. On the whole, these four compounds can be considered potent α_1 antagonists, especially for the α_{1D} subtype, displaying from modest to moderate α_{1D} selectivity. In particular, (*S*)-**2** stands out for its impressive antagonist affinity at the α_{1D}-AR and its significant α_{1D} specificity. Otherwise, for (*S*)-**1**, (*S*)-**3**, (*S*)-**6**, and (*S*)-**7**, the pA_2 values, except for (*S*)-**1** and (*S*)-**7** at α_{1D}-AR and for (*S*)-**7** at α_{1B}-AR, are markedly lower than the corresponding pK_i values. In particular, for the α_{1A} subtype, the drop reaches 2 orders of magnitude and more. In this context, (*S*)-**8**, developed by hybridization of (*S*)-**2** with (*S*)-**3**, forms a positive exception. In fact, it is the only derivative that shows a significant α_{1A} pA_2 increase relative to α_{1a} pK_i (7.98 vs 7.18) associated with α_{1B} and α_{1D} antagonist affinities even lower than the corresponding binding affinities (pA_2 < 5 and = 5.59 vs pK_i = 6.43 and 6.83, respectively).

Comparative SAR analysis of the binding and functional affinities gives the following indications. (a) Modifications at the phenoxy moiety producing the maximum affinities at the three α_1 subtypes, associated with a moderate α_{1a} specificity, generally result in still higher α_1 antagonist affinities with shifted selectivity toward α_{1D}-AR. Such modifications are the replacement of phenoxyl by naphthoxyl [(*S*)-**2** and (*R*)-**2**], tetrahydronaphthoxyl [(*S*)-**4**], and 2-phenylphenoxyl [(*S*)-**5**]. (b) Modifications at the benzodioxane system and at the phenoxy moiety producing lower affinities at the three α_1 subtypes, associated with sometimes significant α_{1a} selectivity, result in even lower, often negligible, α_1 antagonist affinities. Such modifications are the replacement of the benzodioxane system by naphthodioxane [(*S*)-**1**] and tetrahydronaphthodioxane [(*S*)-**3**] and of phenoxyl by phenylphenoxy moieties unsubstituted at one of the ortho positions of phenoxyl [(*S*)-**6** and (*S*)-**7**]. (c) Hybridization of the naphthoxy derivative (*S*)-**2** with the tetrahydronaphthodioxane derivative (*S*)-**3**, detrimental for the binding affinity profile, conversely corresponds to a successful combination of the low α_{1B} and α_{1D} antagonist affinities of (*S*)-**3** with the high α_{1A} antagonist affinity of (*S*)-**2**, leading to a potent (pA_2 = 7.98) and very selective (α_{1A}/α_{1B} >1000 and α_{1A}/α_{1D} = 245 activity ratios) α_{1A}-AR antagonist [(*S*)-**8**].

These highly positive, but contradictory, results prompted us to substantiate the inverse agonist hypothesis, already demonstrated for WB4101, prazosin, and benoxatian and proposed for some of their derivatives[14,21,22] to justify differences in binding and antagonist affinities. On the basis of the theory, antagonist affinity assessed in functional experiments should not be different from that determined in binding assays using both native and recombinant receptors, but the results often appear inconsistent. Such a discrepancy might be explained by the fact that so-called neutral antagonists really behave as inverse agonists in the interaction with receptor[23,24] or by the phenomenon of receptor dimerization. Many G-protein-coupled receptors, comprising the α_1-AR subtypes, may exist in a spontaneously active form in the absence of agonist.[25,26] According to the two-state receptor model,[27] some antagonists, called inverse agonists or negative antagonists, preferentially lead to the inactive conformational state, reducing its basal activity. Their affinity values may not be system-independent, but different according to the relative proportion (*L*) of receptor in the resting state and receptor in the active state typical of the system employed for the determination. Since it has been demonstrated that classical α_1-AR antagonists, such as prazosin, benoxatian, and WB4101, act as inverse agonists,[22] we hypothesized that also our compounds are inverse agonists if tested in the appropriate model. So we decided to assess their ability to inhibit the increase in the resting tone (IRT) of guinea pig thoracic aorta induced by Ca^{2+}.[14] In particular, we tested seven compounds: WB4101 and its enantiomers; (*S*)-**2**, namely, the most potent α_{1D} antagonist; its antipode, (*R*)-**2**; (*S*)-**8**, which is the most selective α_{1A} antagonist; and, finally, (*S*)-**5**, the most potent α_{1A} antagonist with α_{1A} antagonist affinity lower than α_{1a} binding affinity (Figure 1). In order to verify if discrepancies between the results of binding and functional studies could be due to inherent species difference, (*S*)-**2** and (*S*)-**8**, which show the highest positive and negative difference, respectively, between α_{1D} pA_2 (antagonist affinity at α_{1D}-AR from rat aorta) and α_{1d} pK_i (binding affinity at recombinant human α_{1D}-AR), were previously submitted to binding studies on recombinant rat α_{1D}-AR, too. The finding of the same binding affinity ratio [i.e., (*S*)-**2** affinity 20-fold higher than that of (*S*)-**8**)] induced us to exclude a confounding species effect. After ascertaining that such an effect was not involved, we carried out the tests of IRT inhibition in guinea pig thoracic aorta.

Some experimental evidence[28] suggests that contraction of this vessel is mediated by the α_{1L}-AR subtype, which is pharmacologically similar to α_{1A}-AR of rat vas deferens and human prostate and urethra.[1,29] Due to this similarity, guinea pig thoracic aorta may be a suitable tool to test new α_1-AR antagonists as inverse agonists therapeutically advantageous in the treatment of urinary tract obstruction (e.g., in BPH). As shown in Figure 1, guinea pig thoracic aorta, depleted of internally stored Ca^{2+} until irresponsive to noradrenaline administration and then incubated with 10 nM WB4101, shows, after addition of Ca^{2+} (1.8 mM), an IRT equal to 25% of IRT produced by the same quantity of Ca^{2+} administered after depleting internal Ca^{2+} but in the absence of WB4101. Such a decrease of response to Ca^{2+} seems imputable to the *S* enantiomer, which, at the same concentration as the racemate, lowers Ca^{2+}-induced IRT to 15%, whereas the *R* enantiomer has quite negligible effects at 100 nM concentration. Both (*S*)-**2** and (*S*)-**8** behave as inverse agonists, the former with a nearly equal potency to (*S*)-WB4101 (10% IRT at 10 nM concentration) and the latter to a minor but still high degree (27% IRT at 100 nM). No inverse agonism was shown by (*R*)-**2** at the same

concentration as its antipode and by (*S*)-**5** at 10 nM concentration. These observations indicate that (a) for the two tested enantiomeric pairs (WB4101 and **2**), configuration inversion from *S* to *R* results in a change from inverse agonist to neutral antagonist; (b) considering the four tested *S* derivatives [(*S*)-WB4101, (*S*)-**2**, (*S*)-**5** and (*S*)-**8**], inverse agonism is a very frequent, but not a general feature of these compounds; (c) all the detected inverse agonist activities in guinea pig thoracic aorta correspond to α_{1A} pA_2−α_{1a} pK_i differences of the same sign, since inverse agonism behavior was found for compounds with higher α_{1A} pA_2 than α_{1a} pK_i [(*S*)-WB4101, (*S*)-**2** and (*S*)-**8**] and not for those showing the reverse difference [(*R*)-WB4101, (*R*)-**2** and (*S*)-**5**]. We do not know if (*S*)-**2** and (*S*)-**8** act as inverse agonists on α_{1D}-AR, too. Anyway, the respective differences between α_{1D} pA_2 and α_{1d} pK_i could not be both ascribed to a hypothetic inverse agonism at this receptor subtype, since they are opposite [10.68 vs 8.18 for (*S*)-**2** and 5.59 vs 6.83 for (*S*)-**8**]. This notwithstanding, on the basis of the present data, it is likely that many of the observed discrepancies are due to the nature of inverse agonists, whose binding and antagonist affinities, differently from neutral antagonists, increase or decrease depending on the receptor distribution between the active and resting state in the system employed for the determinations.

Finally, it is to be underlined that, as shown in Table 2, (*S*)-**8** is virtually devoid of α_2-AR antagonistic affinity and, for (*S*)-**2**, this is from 3 to 5 orders of magnitude lower than those at α_1-AR subtypes.

Conclusion

Through a planned short sequence of modifications, consisting of introducing an additional or fused benzene or cyclohexane ring into the benzodioxanic or phenoxy portion of WB4101 enantiomers and finally hybridizing two of these modifications, we have identified a new, potent, and highly specific α_{1A}-AR antagonist, (*S*)-**8**, which presents a tetrahydronaphthodioxane system and a 2-methoxy-1-naphthoxy residue in place, respectively, of the benzodioxane and 2,6-dimethoxyphenoxy of the lead compound. The new selective α_{1A}-AR antagonist hybridizes the high α_{1A} antagonist affinity of one parent compound, the 2-methoxy-1-naphthoxy derivative (*S*)-**2**, with the very low α_{1B} and α_{1D} antagonist affinities of the other, the tetrahydronaphthodioxane derivative (*S*)-**3**.

Part of the differences between binding and functional affinities have been tentatively explained by the inverse agonist rather than neutral antagonist nature of some of these compounds, as demonstrated and semiquantitatively determined, in a vascular model, for (*S*)-**8**, (*S*)-**2**, and (*S*)-WB4101. Furthermore, for these two latter derivatives, it has been shown that configuration inversion changes inverse agonism into neutral antagonism.

Experimental Section

Chemistry. Melting points were measured on a Büchi melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded operating at 300 MHz. Chemical shifts, in parts per million relative to residual solvent ($CHCl_3$ or DMSO) as internal standard, are reported in the Supporting Information. Optical rotations were determined by a Perkin-Elmer 241 polarimeter at 25 °C. Elemental analyses (CHClN) are within 0.40% of theoretical values. Purifications were performed by flash chromatography using silica gel (particle size 40−63 μm, Merck).

5,6,7,8-Tetrahydro-1-naphthyl Acetate (14). Acetyl chloride (28.2 mL, 396 mmol) was added dropwise to a solution of 5,6,7,8-tetrahydronaphthol (50 g, 330 mmol) and pyridine (29.4 mL, 363 mmol) in dichloromethane (250 mL). After 3 h, the mixture was washed, in sequence, with water (250 mL), 10% HCl (250 mL), water (120 mL), and a saturated solution of $NaHCO_3$ (250 mL). The organic phase was dried and concentrated to give 62.7 g (100%) of **14** as a yellow oil.

2-Acetyl-5,6,7,8-tetrahydro-1-naphthol (15). Aluminum chloride (44 g, 330 mmol) was added to a solution of **14** (62.7 g, 330 mmol) in dichlorobenzene. After being warmed to 80 °C for 12 h, the reaction mixture was allowed to cool to room temperature, added with dichloromethane (250 mL), and poured into 10% HCl cooled to 0 °C. The organic phase was separated, treated with 10% HCl (2 × 200 mL) again, washed with water (200 mL), dried, and concentrated. Dichlorobenzene was removed by distillation under vacuum (1 mbar) at 100 °C yielding 62.7 g (100%) of **15** as a brown oil.

1-Benzyloxy-2-acetyl-5,6,7,8-tetrahydronaphthalene (16). Tetrabutylammonium bromide (4.76 g, 14.8 mmol) and 2.5 N NaOH (118 mL) were added to a solution of **15** (28.08 g, 148 mmol) in dichloromethane (250 mL). Benzyl bromide (26.5 mL, 221 mmol) was added dropwise to the mixture while it was vigorously stirred. After 24 h at room temperature, the reaction mixture was poured into 10% HCl (150 mL). The organic phase was separated, washed with water (100 mL), dried, and concentrated. Benzyl bromide was removed by distillation under vacuum (1.5 mbar) at 120 °C yielding 41.3 g (100%) of **16** as a brown oil.

1-Benzyloxy-5,6,7,8-tetrahydro-2-naphthol (17). 3-Chloroperoxybenzoic acid (50.9 g, 295 mmol) was added in small portions to a solution of **16** (41.3 g, 147 mmol) in ethyl acetate (420 mL) at 0 °C. The reaction mixture was stirred for 24 h at room temperature and then concentrated. The residue was added with dichloromethane (250 mL), treated with a saturated solution of $NaHCO_3$ (3 × 100 mL), and washed with water (100 mL). The organic phase was dried and concentrated to give an oil, which was dissolved in methanol (420 mL) and added with 2.5 N NaOH (67 mL). After stirring for 2 h at room temperature, methanol was evaporated and the residue treated with dichloromethane (250 mL) and 10% HCl (100 mL). The organic phase was separated, washed with 10% HCl (100 mL) again and then with water (100 mL), dried, and finally concentrated, yielding 36.2 g (97%) of **17** as a brown oil.

1-Benzyloxy-2-methoxy-5,6,7,8-tetrahydronaphthalene (18). Tetrabutylammonium bromide (4.84 g, 15 mmol) and 2.5 N NaOH (120 mL) were added to a solution of **17** (36.2 g, 142.5 mmol) in dichloromethane (390 mL). Iodomethane (10.3 mL, 165 mmol) was added dropwise to the mixture while vigorously stirring. After 6 days, during which aliquots of iodomethane (10 mL in all) were occasionally added, the reaction mixture was poured into 10% HCl (200 mL). The organic phase was separated, washed with water (120 mL), dried, and concentrated. Column chromatography on silica gel (eluent cyclohexane/ethyl acetate, 90/10) of the resulting residue allowed the isolation of 19.69 g (48.9%) of **18** as an orange oil.

2-Methoxy-5,6,7,8-tetrahydro-1-naphthol (19). A solution of **18** (19.7 g, 73.4 mmol) in ethyl acetate (200 mL) was added with 5% Pd/C (3.9 g) and vigorously shaken under hydrogen at room temperature for 24 h. The catalyst was removed by filtration and the filtrate concentrated to give a waxy residue (12.45 g), which was crystallized from cyclohexane (62 mL), yielding 3.37 g (25.6%) of **19** as a white solid: mp 93.9 °C.

Ethyl 2-(2-Methoxy-5,6,7,8-tetrahydro-1-naphthoxy)acetate (20). A solution of **19** (2.37 g, 13.3 mmol) in DMSO (15 mL) was added dropwise to a suspension of sodium hydride (0.34 g. 13.3 mmol) in DMSO. Ethyl chloroacetate (1.42 mL, 13.3 mmol) was then added dropwise and the reaction mixture was stirred for 2 h, cooled to 0 °C, and treated with 10% HCl (40 mL). The aqueous phase was extracted with ethyl ether (2 × 75 mL), and the organic extracts were combined, washed with 10% KOH (70 mL) and with water (70 mL), dried, and concentrated, yielding 2.69 g (76.4%) of **20** as an orange oil.

2-(2-Methoxy-5,6,7,8-tetrahydronaphthoxy)ethanol (21). A solution of **20** (2.69 g, 10.2 mmol) in THF (5 mL) was added

dropwise to a suspension of $LiAlH_4$ (0.42 g, 11.2 mmol) in THF (5 mL) at 0 °C. The mixture was stirred for 24 h at room temperature. After cooling to 0 °C, 10% HCl (70 mL) and dichloromethane (100 mL) were added. The organic phase was separated, washed with water (70 mL), dried, and concentrated to give a residue (1.92 g), which was purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate, 80/20), yielding 1.2 g (52.8%) of **21** as a yellow oil.

2-(2-Methoxy-5,6,7,8-tetrahydro-1-naphthoxy)ethyl Mesylate (9). Mesyl chloride (0.84 mL, 10.8 mmol) was added to a solution of **21** (1.2 g, 5.4 mmol) and triethylamine (1.5 mL, 10.8 mmol) in dichloromethane at 0 °C. After stirring for 2 h at room temperature, dichloromethane (50 mL) and a saturated aqueous solution of $NaHCO_3$ were added. The organic phase was separated, washed with water (30 mL), dried, and concentrated, yielding 1.62 g (100%) of **9** as an orange oil.

2-Biphenyl acetate (22) was quantitatively obtained from 2-phenylphenol and acetyl chloride as a white solid by the same procedure described for **14**: mp 62.8 °C.

2-Hydroxy-3-phenylacetophenone (23) was obtained from **22** in 74.5% yield as a brown oil by treatment with aluminum chloride in dichlorobenzene at 100 °C for 24 h according to the procedure described for **15**. 3-Phenyl-4-hydroxyacetophenone side product was partly eliminated by filtering off the precipitate formed during the HCl/dichloromethane extraction and definitively removed by treating the crude product resultant from dichlorobenzene distillation with 7 parts of cyclohexane, in which the *p*-hydroxy ketone is insoluble.

2-Benzyloxy-3-phenylacetophenone (24) was obtained from **23** and benzyl bromide in 63% yield as a yellow oil according to the procedure described for **16**, followed by chromatographic purification on silica gel (eluent cyclohexane/ethyl acetate, 80/20).

2-Benzyloxy-3-phenylphenol (25) was obtained from **24** in 67% yield as an oil following the procedure described for **17** with a few changes (reaction with 2.4 mol of *m*-CPBA per substrate mole for 72 h and final chromatographic purification of the phenol on silica gel eluting with 80/20 cyclohexane/ethyl acetate).

2-Benzyloxy-3-methoxybiphenyl (26) was obtained from **25** in 74% yield as a colorless oil according to the procedure described for **18**, but reducing the amount of iodomethane to 1.1 mol per substrate mole and the reaction time to 24 h. The crude product was purified by chromatography on silica gel eluting with 80/20 cyclohexane/ethyl acetate.

2-Methoxy-6-phenylphenol (27) was quantitatively obtained from **26** as an oil following the procedure described for **19**, but replacing ethyl acetate with acetone. Purification of the crude product was unnecessary.

2-(2-Bromoethoxy)-3-phenylanisole (10). 1,2-Dibromoethane (4.4 mL, 51.5 mmol) was added dropwise to a vigorously stirred mixture of **27** (2.6 g, 13 mmol), tetrabutylammonium bromide (0.42 g, 1.3 mmol), 2.5 N NaOH (20.6 mL), and dichloromethane (60 mL). After 24 h, the organic phase was separated, washed with 10% HCl (2 × 30 mL) and then with water (30 mL), dried, and concentrated. The resultant residue was purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate, 95/5), yielding 2.0 g (51.2%) of **10** as a colorless oil.

4-Biphenyl acetate (28) was quantitatively obtained from 4-phenylphenol as a white solid by the same procedure described for **14**: mp 88.6 °C.

2-Hydroxy-5-phenylacetophenone (29) was obtained from **28** in 50% yield as a pink solid by treatment with aluminum chloride in dichlorobenzene at 100 °C for 24 h according to the procedure described for **15**. The crude product was purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate, 80/20): mp 59.5 °C.

2-Methoxy-5-phenylacetophenone (30) was quantitatively obtained, after overnight reaction and without chromatographic purification, from **29** as a white solid according to the procedure described for **18**: mp 62.8 °C.

2-Methoxy-5-phenylphenol (31) was obtained from **30** in 48% yield as a yellow solid following the procedure described for **17** with a few changes (reaction with 4 mol of *m*-CPBA per substrate mole for 72 h and final crystallization of the phenol from methanol): mp 108.6 °C.

2-(2-Bromoethoxy)-4-phenylanisole (32). 1,2-Dibromoethane (3.2 mL, 37.2 mmol) was added dropwise to a stirred mixture of **31** (1.86 g, 9.3 mmol), powdered KOH (2.08 g, 37.2 mmol), and DMSO (18 mL). After 24 h, the reaction mixture was acidified with 10% HCl and extracted with dichloromethane (50 mL). The organic phase was separated, washed with a saturated aqueous solution of $NaHCO_3$, dried, and concentrated, yielding 2.1 g (75%) of **32** as a white solid: mp 97.2 °C.

2-(2-Azidoethoxy)-4-phenylanisole (33). A solution of **32** (2.1 g, 7 mmol) and NaN_3 (5.78 g, 88 mmol) in DMF (40 mL) and water (20 mL) was warmed to 90 °C for 4 h. After cooling to room temperature, dichloromethane (100 mL) and water (30 mL) were added. The organic phase was separated, washed with a saturated aqueous solution of NaCl (10 × 30 mL), dried, and concentrated to give 1.6 g (85%) of **33** as a very viscous oil.

2-(2-Methoxy-5-phenylphenoxy)ethylamine (11). Hydrazine hydrate (2.8 mL, 59 mmol) was added dropwise to a refluxing mixture of **33** (1.6 g, 5.9 mmol) and PdO (160 mg) in methanol (16 mL). The reaction mixture was refluxed for 3 h and, after cooling to room temperature, filtered and poured into dichloromethane/10% HCl. The aqueous phase was separated, made alkaline by addition of 2.5 N NaOH, and extracted with dichloromethane (50 mL). The organic phase was separated, dried, and concentrated. The residue was added to 2-propanol (6 mL) and the resultant precipitate filtered off. The propanolic filtrate was concentrated to give 1.1 g (76%) of **11** as a waxy solid.

2-Benzyloxy-5-phenylacetophenone (34) was obtained from **29** and benzyl bromide in 66% yield as a beige solid according to the procedure described for **16**, followed by crystallization of the crude product from 5 parts of cyclohexane: mp 80 °C.

2-Benzyloxy-5-phenylphenol (35) was obtained from **34** in 50.5% yield as a yellow crystalline solid following the procedure described for **17**. The crude phenol was crystallized from cyclohexane: mp 108 °C.

2-Benzyloxy-5-phenylanisole (36) was quantitatively obtained, after overnight reaction and without chromatographic purification, from **35** as a crystalline solid according to the procedure described for **18**: mp 79.3 °C.

2-Hydroxy-5-phenylanisole (37) was quantitatively obtained from **36** as an oil following the procedure described for **19**, but replacing ethyl acetate with methanol. Purification of the crude product was unnecessary.

2-(2-Bromoethoxy)-5-phenylanisole (38) was obtained from **37** in 67.4% yield as a white solid according to the procedure described for **10**. The crude product was purified by chromatography on silica gel eluting with 90/10 cyclohexane/ethyl acetate: mp 75.9 °C.

2-(2-Azidoethoxy)-5-phenylanisole (39) was obtained from **38** in 91.5% yield as a colorless oil according to the procedure described for **33**.

2-(2-Methoxy-4-phenylphenoxy)ethylamine (12) was obtained from **39** in 47% yield as an oil according to the procedure reported for **11**, but protracting reaction for 72 h and isolating the product by chromatography on silica gel (eluent dichloromethane/methanol/TEA, 95/5/0.1).

1-(2-Azidoethoxy)-2-methoxynaphthalene (40). A solution of 1-(2-bromoethoxy)-2-methoxynaphthalene (6.24 g, 22.2 mmol) and NaN_3 (18.75 g, 288.5 mmol) in DMF (60 mL) and water (60 mL) was refluxed for 5 h. After cooling to room temperature, water (120 mL) was added and the aqueous phase extracted with hexane (5 × 100 mL). The organic extracts were combined, dried, and concentrated, yielding 4.93 g (91.3%) of **40** as an orange oil.

1-(2-Aminoethoxy)-2-methoxynaphthalene (13). Hydrazine hydrate (9.9 mL, 203 mmol) was added dropwise to a refluxing mixture of **40** (4.93 g, 20.3 mmol) and PdO (250 mg) in methanol (50 mL). The reaction mixture was refluxed for 24 h, cooled to room temperature, and filtered. The filtrate was concentrated and the residue treated with dichloromethane (100 mL) and 2.5 N NaOH

(70 mL). The organic phase was separated, washed with water (70 mL), dried, and concentrated to give 4.32 g (98.2%) of **13** as an orange oil.

(*S*)-2-[((2-(2-Methoxy-5,6,7,8-tetrahydronaphthoxy)ethyl)amino)methyl]-1,4-benzodioxane Hydrochloride [(*S*)-4]. A mixture of (*S*)-2-aminomethyl-1,4-benzodioxane (1.5 g, 7.8 mmol) and **9** (1.15 g, 3.8 mmol) in 2-methylpropanol (5 mL) was submitted to microwave irradiation for 60 min (120 °C, 100 W). The solvent was evaporated and the residue purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate/triethylamine, 50/50/0.1), yielding 491 mg of (*S*)-2-[((2-(2-methoxy-5,6,7,8-tetrhydronaphthoxy)ethyl)amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]^{25}_D = -30.2$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in ethanol (1.2 mL), and 1.4 N HCl/EtOH (0.7 mL) was added. The resulting precipitate was isolated and dried, yielding 280 mg (17%, based on the starting amount of **9**) of (*S*)-**4** as a white solid: mp 122.6 °C; $[\alpha]^{25}_D = -43.5$ (*c* 1, ethanol). Anal. ($C_{22}H_{28}ClNO_4$) C, H, Cl, N.

(*R*)-2-[((2-(2-Methoxy-5,6,7,8-tetrahydronaphthoxy)ethyl)amino)methyl]-1,4-benzodioxane hydrochloride [(*R*)-4] was obtained from (*R*)-2-aminomethyl-1,4-benzodioxane and **9** as described for (*S*)-**4**: mp 121.8; $[\alpha]^{25}_D = +36.2$ (*c* 1, ethanol); ^{1}H NMR identical to that of (*S*)-**4**. Anal. ($C_{22}H_{28}ClNO_4$) C, H, Cl, N.

(*S*)-2-[((2-(2-Methoxy-6-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane Hydrochloride [(*S*)-5]. A mixture of (*S*)-2-aminomethyl-1,4-benzodioxane (590 mg, 3.6 mmol) and **10** (1 g, 3.25 mmol) in 2-methylpropanol (2.7 mL) was submitted to microwave irradiation for 60 min (120 °C, 100 W). The solvent was evaporated and the resultant residue treated with dichloromethane and saturated aqueous solution of $NaHCO_3$. The organic phase was separated, dried, and concentrated and the residue purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate/triethylamine, 50/50/0.1), yielding 400 mg of (*S*)-2-[((2-(2-methoxy-6-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]^{25}_D = -15.4$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in ethyl acetate (10 mL), and 2.3 N HCl/EtOH (0.5 mL) was added. The resulting precipitate was isolated and dried, yielding 347 mg (27%, based on the starting amount of **10**) of (*S*)-**5** as a white solid: mp 144.8 °C; $[\alpha]^{25}_D = -45.1$ (*c* 1, ethanol). Anal. ($C_{24}H_{26}ClNO_4$) C, H, Cl, N.

(*R*)-2-[((2-(2-Methoxy-6-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane hydrochloride [(*R*)-5] was obtained from (*R*)-2-aminomethyl-1,4-benzodioxane and **10** as described for (*S*)-**5**: mp 144.6; $[\alpha]^{25}_D = +47.3$ (*c* 1, ethanol); ^{1}H NMR identical to that of (*S*)-**5**. Anal. ($C_{24}H_{26}ClNO_4$) C, H, Cl, N.

(*S*)-2-[((2-(2-Methoxy-5-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane Hydrochloride [(*S*)-6]. A mixture of (*R*)-2-mesyloxymethyl-1,4-benzodioxane (630 mg, 2.5 mmol) and **11** (700 mg, 2.9 mmol) in 2-methylpropanol (5 mL) was refluxed for 24 h. The solvent was evaporated and the resultant residue treated with dichloromethane (30 mL) and a saturated aqueous solution of $NaHCO_3$ (15 mL). The organic phase was separated, washed with a saturated aqueous solution of $NaHCO_3$ again (2 × 15 mL) and then with water, dried, and concentrated. The residue was purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate/2-methylpropanol, 50/50/0.3), yielding 470 mg of (*S*)-2-[((2-(2-methoxy-5-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]^{25}_D = -19.8$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in ethanol (5 mL), and 1.4 N HCl/EtOH (3 mL) was added. The resulting precipitate was isolated and dried, yielding 250 mg (23.4%, based on the starting amount of mesylate) of (*S*)-**6** as a white solid: mp 159.6 °C; $[\alpha]^{25}_D = -38.6$ (*c* 1, ethanol). Anal. ($C_{24}H_{26}ClNO_4$) C, H, Cl, N.

(*R*)-2-[((2-(2-Methoxy-5-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane hydrochloride [(*R*)-6] was obtained from (*S*)-2-mesyloxymethyl-1,4-benzodioxane and **11** as described for (*S*)-**6**: mp 159.6; $[\alpha]^{25}_D = +34.3$ (*c* 1, ethanol); ^{1}H NMR identical to that of (*S*)-**6**. Anal. ($C_{24}H_{26}ClNO_4$) C, H, Cl, N.

(*S*)-2-[((2-(2-Methoxy-4-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane Hydrochloride [(*S*)-7]. A mixture of (*R*)-2-mesyloxymethyl-1,4-benzodioxane (300 mg, 1.2 mmol) and **12** (374 mg, 1.3 mmol) in 2-methylpropanol (5 mL) was submitted to microwave irradiation for 60 min (120 °C, 100 W). The solvent was evaporated and the resultant residue purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate/triethylamine, 50/50/0.1), yielding 240 mg of (*S*)-2-[((2-(2-methoxy-4-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane as a colorless oil: $[\alpha]^{25}_D = -26.4$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in ethanol (2 mL) and 1.4 N HCl/EtOH (0.2 mL) was added. The resulting precipitate was isolated and dried, yielding 130 mg (25.3%, based on the starting amount of mesylate) of (*S*)-**7** as a white solid: mp 140.9 °C; $[\alpha]^{25}_D = -41.6$ (*c* 1, ethanol). Anal. ($C_{24}H_{26}ClNO_4$) C, H, Cl, N.

(*R*)-2-[((2-(2-Methoxy-4-phenylphenoxy)ethyl)amino)methyl]-1,4-benzodioxane hydrochloride [(*R*)-7] was obtained from (*S*)-2-mesyloxymethyl-1,4-benzodioxane and **12** as described for (*S*)-**7**: mp 140.6; $[\alpha]^{25}_D = +35.8$ (*c* 1, ethanol); ^{1}H NMR identical to that of (*S*)-**7**. Anal. ($C_{24}H_{26}ClNO_4$) C, H, Cl, N.

(*S*)-2-[((2-(2-Methoxy-1-naphthoxy)ethyl)amino)methyl]-2,3,6,7,8,9-hexahydronaphtho[2,3-*b*][1,4]dioxine Hydrochloride [(*S*)-8]. A mixture of (*R*)-2-mesyloxymethyl-2,3,6,7,8,9-hexahydronaphtho[2,3-*b*][1,4]dioxine (520 mg, 1.7 mmol) and **13** (410 mg, 1.9 mmol) in 2-methylpropanol (5 mL) was submitted to microwave irradiation for 60 min (120 °C, 100 W). The solvent was evaporated and the resultant residue purified by chromatography on silica gel (eluent cyclohexane/ethyl acetate/triethylamine, 50/50/0.1), yielding 335 mg of (*S*)-2-[((2-(2-methoxy-1-naphthoxy)-ethyl)amino)methyl]-2,3,6,7,8,9-hexahydronaphtho[2,3-*b*][1,4]-dioxine as a colorless oil: $[\alpha]^{25}_D = -26.3$ (*c* 1, $CHCl_3$). The secondary amine was dissolved in ethyl ether (2 mL) and 1.8 N HCl/Et_2O (0.23 mL) was added. The resulting precipitate was isolated and dried, yielding 190 mg (24.5%, based on the starting amount of mesylate) of (*S*)-**8** as a white solid: mp 255.5 °C; $[\alpha]^{25}_D = -63.7$ (*c* 1, ethanol). Anal. ($C_{26}H_{30}ClNO_4$) C, H, Cl, N.

(*R*)-2-[((2-(2-Methoxy-1-naphthoxy)ethyl)amino)methyl]-2,3,6,7,8,9-hexahydronaphtho[2,3-*b*][1,4]dioxine hydrochloride [(*R*)-8] was obtained from (*S*)-2-mesyloxymethyl-2,3,6,7,8,9-hexahydronaphtho[2,3-b][1,4] dioxine and **13** as described for (*S*)-**8**: mp 255.0 °C; $[\alpha]^{25}_D = +61.8$ (*c* 1, ethanol); ^{1}H NMR identical to that of (*S*)-**8**. Anal. ($C_{26}H_{30}ClNO_4$) C, H, Cl, N.

Biology. Radioligand Binding Assays. Affinities for α_{1a}, α_{1b}, α_{1d} AR-subtypes and 5-HT_{1A} serotoninergic receptor were measured by in vitro binding studies. Briefly, membranes derived from Chinese hamster ovary (CHO) cells expressing α_1-AR subtypes (prepared as described by Testa et al.[30]) were resuspended in 50 mM Tris HCl, pH 7.7, containing 10 μM pargyline and 0.1% ascorbic acid and incubated for 30 min at 25 °C with 0.5 nM [^{3}H]-prazosin (NEN, 80.5 Ci/mmol) in the absence or presence of different concentrations of the tested compounds. Prazosin (1 μM) was routinely used to determine nonspecific binding, although our data indicate that identical levels of nonspecific binding could be obtained by using α_1-AR ligands chemically distinct from [^{3}H]-prazosin, such as WB-4101 or the other compounds tested in the present study (data not shown). Binding studies at 5-HT_{1A} receptors were carried out using crude membrane preparations from rat hippocampus, which were resuspended in 50 mM Tris HCl, pH 7.7, with 10 μM pargyline and 4 mM $CaCl_2$ and incubated for 30 min at 25 °C 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. Incubations were stopped by rapid filtration, through GF/B filters, which were then washed, dried, and counted in a Wallac 1409 rack β-liquid scintillation spectrometer. At least three different experiments, in triplicate, were carried out for each compound, and usually each compound was tested simultaneously on the different α_1-AR subtypes. Prazosin or 5-HT were always tested in parallel, as reference drugs. The percentage inhibitory effects obtained in the different experiments were pooled together and the inhibition curves were analyzed using the "one-site competition" equation built into GraphPad Prism 4.0 (GraphPAD Softwaree, San Diego, CA). This analysis gives the IC_{50} (i.e., the drug concentration inhibiting specific binding by 50%), calculated with the relative standard error.

K_i values were then calculated by IC_{50} using the Cheng and Prusoff equation in which the K_d of [^{3}H]prazosin for α_{1a}, α_{1b}, α_{1d} AR-subtypes were 0.4, 0.4, and 0.7 nM, respectively, whereas the K_d of [^{3}H]-8-OH-DPAT for 5-HT_{1A} receptors was 1.2 nM.

Functional Antagonism in Isolated Rat Tissues. Male Sprague−Dawley rats (Charles River, Italy) were killed by cervical dislocation under ketamine anaesthesia, and the organ required was isolated, freed from adhering connective tissue, and set up rapidly under resting tension in an organ bath (15 mL) containing a physiological salt solution kept at appropriate concentration (see below) and gassed with 95%O_2 and 5%CO_2 at pH 7.4. Concentration−response curves were constructed by cumulative addiction of agonist. The concentration of agonist in the organ bath was increased approximately 5-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady.

All experimental data were recorded by means of isometrically or isotonically FT.03 Grass force transducers using Power Lab software (AD-Instruments Pty Ltd, Castle Hill, Australia). In addition, parallel experiments in which tissues did not receive any antagonist were run in order to check for any variation in sensitivity.

Prostate. This tissue (from rats of 200-250 g) was used to assess α_{1A}-adrenoceptor antagonist activity.[13] Prostatic strips measuring 8−10 mm in length and 1−2 mm in width were placed under a resting tension of 2 g in modified Krebs solution of the following composition (mM): NaCl, 118.0; KCl, 4.7; $CaCl_2 \cdot H_2O$, 2.5; $MgSO_4 \cdot 7H_2O$ 1.18; $NaHCO_3$, 25.0; KH_2PO_4, 1.18; glucose, 5.5. The preparations were equilibrated for 60 min; during this time the bathing solution was changed every 20 min. 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.

Vas Deferens Prostatic Portion. This tissue (from rats of 200−250 g) was used to assess α_{1A}-adrenoceptor antagonist activity.[11] Prostatic portions of 2 cm length were set up in Tyrode solution of the following composition (mM): NaCl, 130.0; KCl, 2.0; $CaCl_2 \cdot 2H_2O$, 1.8; $MgCl_2$ 0.89; $NaHCO_3$, 25.0; $NaH_2PO_4 \cdot 2H_2O$, 0.42; glucose, 5.6. Desipramine hydrochloride (0.01 μM) was added to prevent the neuronal uptake of (−)-noradrenaline. The medium was maintained at 37 °C The preparation were equilibrated for 1 h under a resting tension of 0.35 g. The preparations were equilibrated for 45−60 min, and during this time the bathing solution was changed every 10 min. 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 concentration for 30 min, a third dose−response curve was obtained.

α_2-Adrenoreceptor antagonist activity was determined also on prostatic portions of 1.5−2 cm length which were set up in an organ bath containing a Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.52; $MgSO_4$, 0.6; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. Propranolol hydrochloride (1 μM) and desipramine hydrochloride (0.01 μM) were present in the above-described Krebs solutions throughout the experiments to block β-adrenoreceptors and to prevent the neuronal uptake of (−)-noradrenaline, respectively. The physiological salt solution was kept at 37 °C. Field stimulation of the tissues was carried out by means of two platinum electrodes, connected to a Grass S88 stimulator, placed near the top and bottom of the vas deferens at 0.1 Hz, using square pulses of 3 ms duration at voltage of 20−40 V. A 1-h equilibration period under a resting tension of 0.35 g was allowed. A first clonidine concentration−response curve, taken as control, was obtained cumulatively. The antagonist concentration was allowed to equilibrate with the tissue for 30 min before obtaining a second dose−response curve.

Spleen. This tissue (from rats of 250−300 g) was used to assess α_{1B}-adrenoceptor antagonist activity.[12] The spleens were bisected transversally into two strips and were suspended in organ baths maintained at 37 °C and containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$ 1.2; $NaHCO_3$, 25.0; $NaH_2PO_4 \cdot 2H_2O$, 1.2; glucose, 11.7. Desipramine hydrochloride (0.01 μM) and (±)-propranolol hydrochloride (1 μM) were added to prevent the neuronal uptake of (-)-phenylephrine and to block β-adrenoreceptors, respectively. The spleen strips were placed under 1 g of resting tension and equilibrated for 1 h. 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 Aorta. This tissue (from rats of 250−300 g) was used to assess α_{1D}-adrenoceptor antagonist activity.[12] The thoracic portion of aorta was cleaned from extraneous connective tissue and placed in an organ bath containing Krebs solution maintained at 37 °C of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$ 1.2; $NaHCO_3$, 25.0; NaH_2PO_4, 1.2; glucose, 11.7. Desipramine hydrochloride (0.01 μM)) and (±)-propranolol hydrochloride (1 μM) were added to prevent the neuronal uptake of (-)-noradrenaline and to block β-adrenoreceptors, respectively. Two helicoids 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 at least a 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.

Inverse Agonism. The guinea pig thoracic aorta was used to assess the activity of α_1-antagonist as inverse agonist.[14] 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 tone (IRT). The magnitude of the IRT in presence of each concentration of each compound was expressed as a percentage of the reference IRT in the absence of any agent.

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Supporting Information Available: ^{1}H NMR data for all synthesized compounds; ^{1}H NMR spectra of compounds (*S*)-**4**, (*S*)-**5**, (*S*)-**6**, (*S*)-**7**, and (*S*)-**8**; and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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