WB 4101-Related Compounds. 2.[1] Role of the Ethylene Chain Separating Amine and Phenoxy Units on the Affinity for α_1-Adrenoreceptor Subtypes and 5-HT_{1A} Receptors

Maria L. Bolognesi,[†] Roberta Budriesi,[†] Andrea Cavalli,[†] Alberto Chiarini,[†] Roberto Gotti,[†] Amedeo Leonardi,[‡] Anna Minarini,[†] Elena Poggesi,[‡] Maurizio Recanatini,[†] Michela Rosini,[†] Vincenzo Tumiatti,[†] and Carlo Melchiorre*,[†]

Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy, and Research and Development Division, Recordati S.p.A., Via Civitali 1, 20148 Milano, Italy

Received April 23, 1999

WB 4101 (**1**)-related benzodioxanes were synthesized by replacing the ethylene chain separating the amine and the phenoxy units of **1** with a cyclopentanol moiety, a feature of 6,7-dihydro-5-[[(*cis*-2-hydroxy-*trans*-3-phenoxycyclopentyl)amino]methyl]-2-methylbenzo[*b*]thiophen-4(5*H*)-one that was reported to display an intriguing selectivity profile at α_1-adrenoreceptors. This synthesis strategy led to 4 out of 16 possible stereoisomers, which were isolated in the case of (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** and whose absolute configuration was assigned using a chiral building block for the synthesis of (−)-**3** starting from (+)-(2*R*)-2,3-dihydro-1,4-benzodioxine-2-carboxylic acid ((+)-**9**) and (1*S*,2*S*,5*S*)-2-amino-5-phenoxycyclopentan-1-ol ((+)-**10**). The aim of this project was to further investigate whether it is possible to differentiate between these compounds with respect to their affinity for α_1-adrenoreceptor subtypes and the affinity for 5-HT_{1A} receptors, as **1** binds with high affinity at both receptor systems. The biological profiles of reported compounds at α_1-adrenoreceptor subtypes were assessed by functional experiments in isolated rat vas deferens (α_{1A}), spleen (α_{1B}), and aorta (α_{1D}) and by binding assays in CHO and HeLa cells membranes expressing the human cloned α_1-adrenoreceptor subtypes and 5-HT_{1A} receptors, respectively. Furthermore, the functional activity of (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** toward 5-HT_{1A} receptors was evaluated by determining the induced stimulation of [^{35}S]-GTPγS binding in cell membranes from HeLa cells transfected with human cloned 5-HT_{1A} receptors. The configuration of the cyclopentane unit determined the affinity profile: a 1*R* configuration, as in (+)-**3** and (−)-**4**, conferred higher affinity at α_1-adrenoreceptors, whereas a 1*S* configuration, as in (−)-**3** and (+)-**4**, produced higher affinity for 5-HT_{1A} receptors. For the enantiomers (+)-**4** and (−)-**4** also a remarkable selectivity was achieved. Functionally, the stereoisomers displayed a similar α_1-selectivity profile, that is $\alpha_{1D} > \alpha_{1B} > \alpha_{1A}$, which is different from that exhibited by the reference compound **1**. The epimers (−)-**3** and (+)-**4** proved to be agonists at the 5-HT_{1A} receptors, with a potency comparable to that of 5-hydroxytryptamine.

Introduction

The finding that receptor systems are comprised of multiple subtypes has stimulated an intense research to clarify the structural elements that allow a ligand to selectively bind to one receptor subtype rather than to another.[2] In the field of α_1-adrenoreceptors, it is now accepted that they can be divided into at least three subtypes, named α_{1a} (α_{1A}), α_{1b} (α_{1B}), and α_{1d} (α_{1D}), with upper and lower case subscripts being used to designate native or recombinant receptors, respectively.[3−5] It should be noted, however, that an additional α_1-adrenoreceptor subtype, named α_{1L}, may exist although efforts to clone it have been unsuccessful so far.[6−8] Whereas it has been claimed that α_{1A}-adrenoreceptor antagonists can be useful in the treatment of benign prostatic hyperplasia,[2] a potential therapeutic use of either α_{1B}- or α_{1D}-subtype antagonists has not been defined yet.

The goal of this project was to discover WB 4101-related compounds, which display an improved selectivity for α_1-adrenoreceptor subtypes in comparison to the prototype WB 4101 (**1**). It was also our aim to investigate further the possibility to differentiate between the affinity for α_1-adrenoreceptors on the one hand and the affinity for 5-HT_{1A} receptors on the other, as it is known that **1** binds with high affinity at both receptors.[9] In a previous project we observed that the structural modifications performed on the benzodioxane moiety as well as on the *N*-substituent of **1** resulted in a parallel effect on the affinity for both α_1-adrenoreceptors and 5-HT_{1A} receptors.[10]

The starting point for the search described in this paper was the observation that 6,7-dihydro-5-[[(*cis*-2-hydroxy-*trans*-3-phenoxycyclopentyl)amino]methyl]-2-methylbenzo[*b*]thiophen-4(5*H*)-one (**2**) displayed an intriguing selectivity profile.[11] It turned out to be a rather potent antagonist ($pA_2 = 8.13$) in blocking the vasoconstrictor effects of phenylephrine in rabbit aorta, while not showing any antagonism at α_2-adrenoreceptors in the rat vas deferens. Since it is now clear that rabbit aorta[7] and rat vas deferens[12] contain different α_1-adrenoreceptor subtypes, we thought that **2** might

[†] University of Bologna.
[‡] Recordati S.p.A.

10.1021/jm991065j CCC: $18.00
Published on Web 09/23/1999

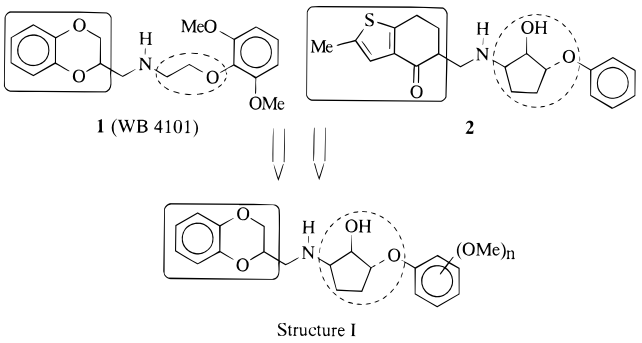


Figure 1. Design strategy for the synthesis of compounds **3**−**8** (structure I) by replacing the oxyethylene chain of **1** with the trisubstituted cyclopentane unit of **2**.

represent a lead for the design of related compounds, which, hopefully, are endowed with high affinity and selectivity for α_1-adrenoreceptor subtypes.

An analysis of Dreiding stereomodels of reference compounds **1** and **2** reveals that they can be superimposed on to each other. It derives that the 6,7-dihydro-2-methylbenzo[*b*]thiophen-4(5*H*)-one unit of **2** can be replaced by the benzodioxane moiety of **1**. To this end, we have already demonstrated that the two oxygen atoms at positions 1 and 4 of **1** are not essential for affinity at α_1-adrenoreceptors and can be replaced by a carbonyl and a methylene, respectively, without affecting the potency.[13] Thus we designed structure I (Figure 1), which is a hybrid structure of both prototypes **1** and **2**. Since it was demonstrated that the other stereoisomers of **2**, having a different relationship among the substituents on the cyclopentane unit, were at the best less potent at α_1-adrenoreceptors,[11] we decided to keep constant in hybrid structure I and related compounds the same spatial arrangement as in **2** to possibly achieve the best fit with α_1-adrenoreceptor subtypes. Considering the fact that the enantiomers of **1** have different affinity for α_1-adrenoreceptors,[14,15] it is of interest to investigate whether the 4 of 16 possible stereoisomers of structure I derived from the combination of the chiral moieties of **1** and **2** ((+)-**3**, (−)-**3**, (+)-**4**, and (−)-**4**) might be able to better discriminate among α_1-adrenoreceptor subtypes and 5-HT_{1A} receptors.

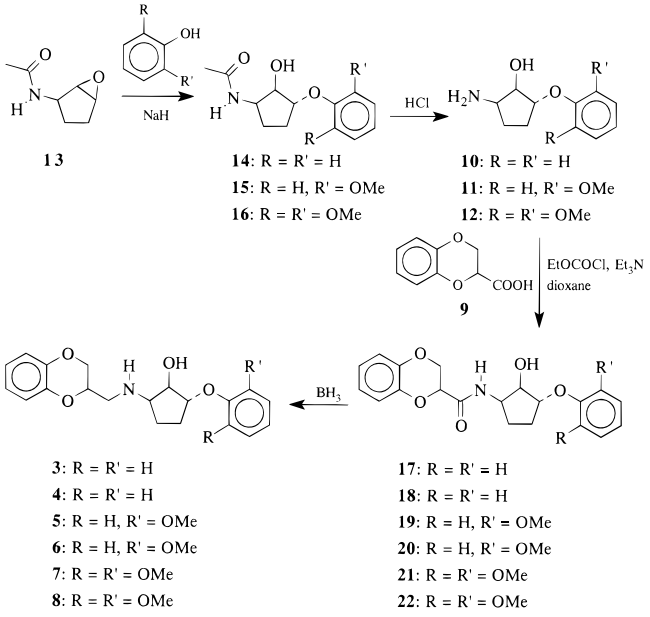
Finally, we included in this work compounds **5**−**8** to investigate whether the insertion of methoxy groups on the phenoxy moiety of structure I might affect differently the affinity for α_1-adrenoreceptors and 5-HT_{1A} receptors.

Chemistry

The compounds used in the present investigation were synthesized by standard procedures and characterized by ^{1}H NMR and elemental analysis.

The key compounds for the synthesis of stereoisomers **3**−**8** were 2,3-dihydro-1,4-benzodioxine-2-carboxylic acid (**9**) and *cis*-2-amino-*trans*-5-phenoxycyclopentan-1-ol (**10**) or its methoxy analogues **11** and **12**. The latter two compounds were synthesized by following an adapted procedure reported for **10** (Scheme 1).[16] The opening of epoxide **13** by appropriate sodium phenoxides in DMF at 100 °C gave the corresponding cyclopentanols **14**−**16**, which, upon treatment with 3 N HCl−methanol, afforded amines **10**−**12** bearing the aryloxy moiety in a trans relationship with both the hydroxy and the

Scheme 1

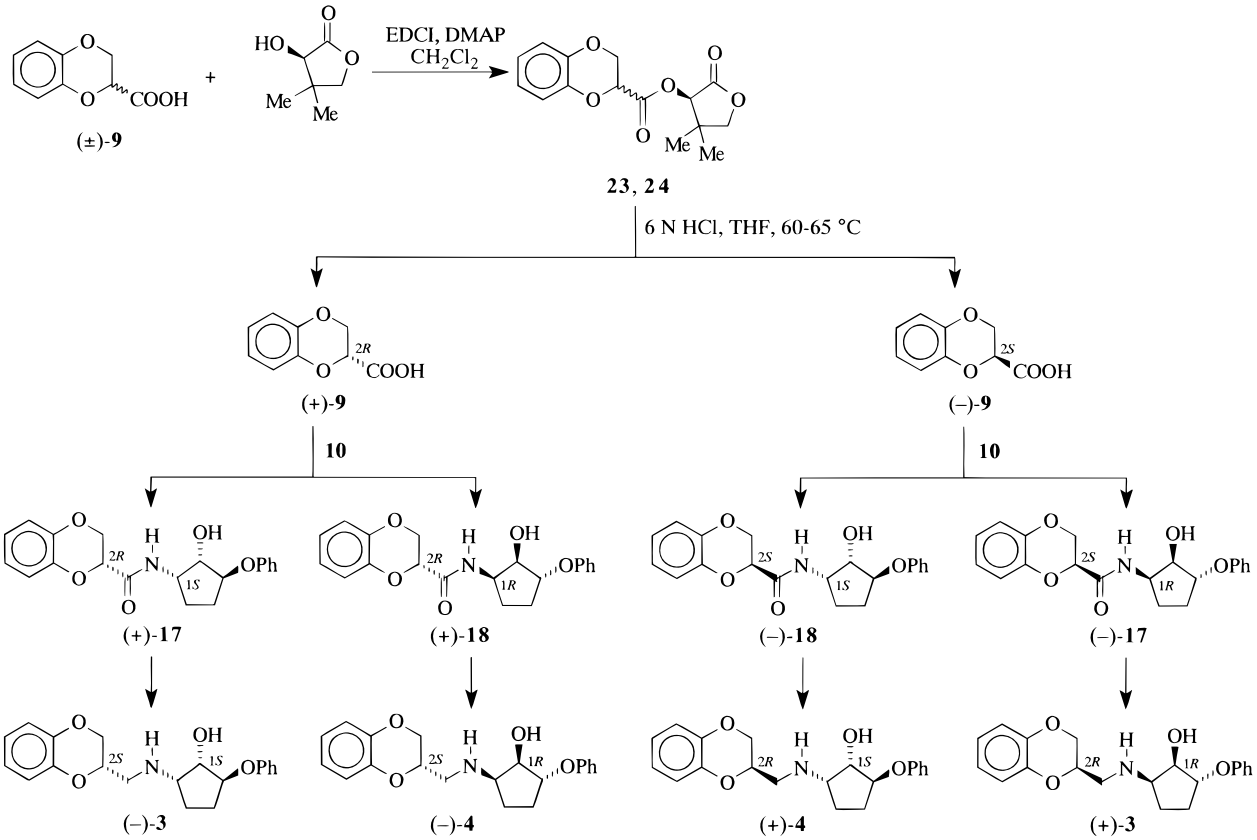


amine group. 2,3-Dihydro-1,4-benzodioxine-2-carboxylic acid (**9**) was amidated with amines **10**−**12** to give a 1:1 mixture of the corresponding diastereomeric amides **17** and **18**, **19** and **20**, and **21** and **22**, which were separated by flash chromatography and reduced with borane methyl sulfide complex to give racemic **3**−**8** (Scheme 1).

Attempts to separate the enantiomers of racemates **3** and **4** through the formation of diastereomeric mixture by reaction with *N*-tosyl-*S*-proline chloride or (+)-diacetyl-L-tartaric anhydride were unsuccessful. Thus we turned our attention to a different synthetic scheme in which enantiomers (+)-**9** and (−)-**9** were used instead of the racemic (±)-**9**. This different approach gave us the possibility to obtain, through an amidation reaction, a mixture of diastereomeric amides (+)-**17** and (+)-**18** or (−)-**17** and (−)-**18**, which could be easily separated by chromatography. Although enantiomers (+)-**9** and (−)-**9** have been already described,[17] we preferred to obtain them by using an adapted procedure described for 7-methoxy-2,3-dihydro-1,4-benzodioxine-2-carboxylic acid[18] (Scheme 2). Thus, racemic **9** was treated with (−)-(*R*)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone ((−)-(*R*)-pantolactone) to afford a mixture of diastereomeric esters **23** and **24**, which were isolated by flash chromatography followed by their hydrolysis with 6 N HCl in THF to afford (+)-**9** and (−)-**9**, respectively. Their optical purity was assessed by capillary electrophoresis using a citric acid/Tris base (pH 8.0) buffer containing heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin (DMCD) as chiral selector; baseline enantioresolution was achieved with short analysis time (migration time of the first eluting peak: 11 min). The enantiomeric excess amounted to 98.2% for (−)-**9** and to 98.0% for (+)-**9**.

Enantiomers (+)-**9** and (−)-**9** were separately amidated with racemic **10** to give a mixture of diastereomeric amides (+)-**17** and (+)-**18** or (−)-**17** and (−)-**18**, respectively, which were isolated by flash chromatography. These amides were reduced with borane methyl sulfide complex to afford the corresponding (−)-**3**, (−)-**4**, (+)-**3**, and (+)-**4** (Scheme 2). The enantiomeric excess determined by capillary electrophoresis employing an

Scheme 2



acidic running buffer (0.1 M phosphoric acid−triethanolamine (pH 3.0) containing 10 mM hydroxypropyl-β-cyclodextrin (HPCD)) turned out to be 97.6% and 97.8% for (−)-**4** and (+)-**4**, respectively (Figure 2), and 97.4% and 98.0% for (−)-**3** and (+)-**3**, respectively.

The absolute configuration of compounds (+)-**3**, (−)-**3**, (+)-**4**, and (−)-**4** was assigned using a chiral building block for the synthesis of (+)-**17** starting from (+)-(2*R*)-2,3-dihydro-1,4-benzodioxine-2-carboxylic acid ((+)-**9**) and (1*S*,2*S*,5*S*)-2-amino-5-phenoxycyclopentan-1-ol ((+)-**10**). The latter was synthesized from (1*S*)-2-cyclopenten-1-amine[19] (**25**) following the procedure reported for racemic **10** (Scheme 3). Acylation of **25** gave *N*1-[(1*S*)-2-cyclopentenyl]acetamide (**26**). Epoxidation of **26** with *m*-chloroperbenzoic acid gave *N*1-[(1a*S*,2*S*,4a*R*)tetrahydro-1a*H*-cyclopenta[*b*]oxiren-2-yl]acetamide (**27**), which, upon treatment with sodium phenoxide, afforded *N*1-[(1*S*,2*S*,3*S*)-2-hydroxy-3-phenoxycyclopentyl]acetamide (**28**). Hydrolysis of **28** gave amine (+)-**10**, which, following a reaction with (+)-**9**, led to (+)-**17**. This amide was identical to a sample obtained by separation through flash chromatography of a diastereomeric mixture of (+)-**17** and (+)-**18** as described above. Assuming that reactions proceeded without racemization, it reasonably follows that (+)-**17** has a (1*S*,2*S*,3*S*)(2*R*) configuration and, consequently, (+)-**18**, which is the other diastereoisomer obtained through the reaction between (+)-**9** and racemic **10**, has a (1*R*,2*R*,3*R*)(2*R*) configuration. Diastereoisomers (−)-**17** and (−)-**18**, which are the enantiomers of (+)-**17** and (+)-**18**, respectively, have a (1*R*,2*R*,3*R*)(2*S*) and (1*S*,2*S*,3*S*)(2*S*) configuration. Finally, it derives that (+)-**3**, (+)-**4**, (−)-**4**, and (−)-**3**, which were obtained from (−)-**17**, (−)-**18**, (+)-**18**, and (+)-**17**, respectively, have a (1*R*,2*R*,5*R*)(2*R*), (1*S*,2*S*,5*S*)-(2*R*), (1*R*,2*R*,5*R*)(2*S*), and (1*S*,2*S*,5*S*)(2*S*) configuration.

Biology

Functional Studies. The pharmacological profile of racemates **3**−**8** and enantiomers (+)-**3**, (−)-**3**, (+)-**4**, and (−)-**4** was evaluated at α_1-adrenoreceptors on different isolated tissues using WB 4101 (**1**) and BMY-7378 as reference compounds (Figure 3). α_1-Adrenoreceptor subtype blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of rat prostatic vas deferens (α_{1A})[12] or thoracic aorta (α_{1D})[20] and by antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B}).[20] Furthermore, the agonist efficacy of the enantiomers of **3** and **4** toward 5-HT_{1A} receptors was assessed by determining the induced stimulation of [^{35}S]-GTPγS binding in cell membranes from HeLa cells transfected with human cloned 5-HT_{1A} receptors[21] using 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), 5-hydroxytryptamine (5-HT), and 5-carboxamidotryptamine (5-CT) as reference compounds (Figure 3).

Binding Experiments. Receptor subtype selectivity of selected WB 4101-related benzodioxanes was further determined by employing receptor binding assays using WB 4101 (**1**), BMY-7378, and 8-OH-DPAT as reference compounds. [^{3}H]Prazosin was used to label cloned human α_1-adrenoreceptors expressed in Chinese hamster ovary (CHO) cells.[22] Furthermore, [^{3}H]rauwolscine, [^{3}H]spiperone, and [^{3}H]ketanserin were used to label α_2-adrenoreceptors in rat cortex,[23] D_2 receptors in rat striatum,[24] and 5-HT_{2A} receptors in rat cortex,[25] respectively, whereas [^{3}H]8-OH-DPAT was the radioligand to

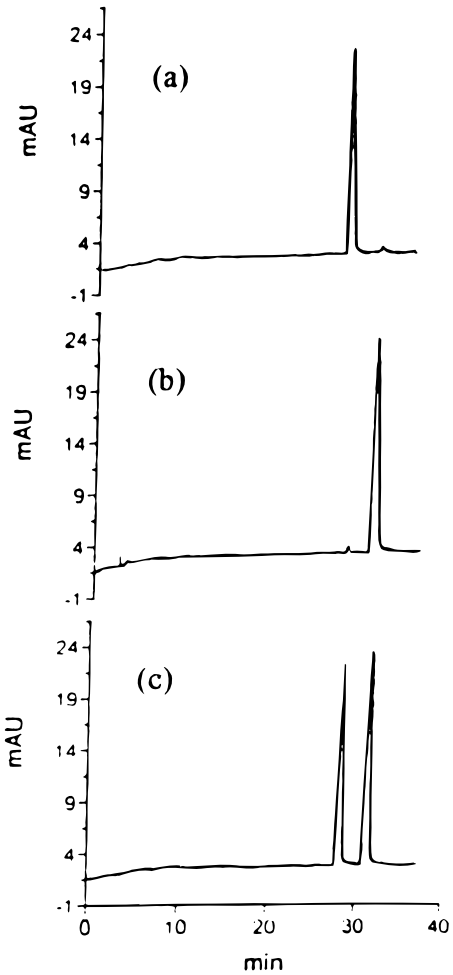
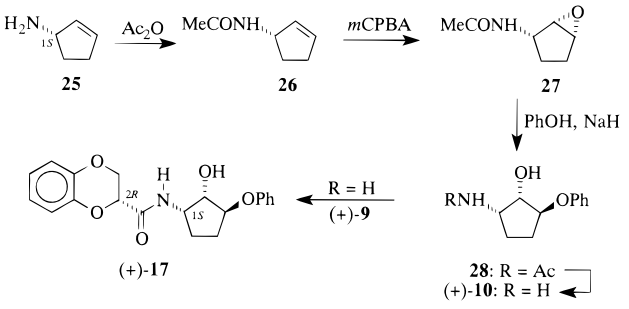


Figure 2. Analytical capillary electrophoresis enantioseparation of (−)-**4** (a), (+)-**4** (b), and racemic mixture (±)-**4** (c). Conditions: fused-silica capillary 48.5 cm in length (40 cm effective length) × 50 mm i.d.; applied voltage 25 kV; detection wavelength 220 nm; temperature 15 °C; hydrodynamic injection (pressure 5 kPa) 10 s; running buffer 10 mM hydroxypropyl-*β*-cyclodextrin in 100 mM phosphoric acid adjusted to pH 3.0.

Scheme 3



label cloned human 5-HT_{1A} receptors which were expressed in HeLa cells.[26, 27]

Results and Discussion

The biological activity, expressed as pK_b and pD_2 values, at α_1-adrenoreceptor subtypes and 5-HT_{1A} receptors, respectively, of compounds used in the present study is shown in Tables 1 and 2. Prototype **1** and the reference compounds BMY-7378, 8-OH-DPAT, 5-HT, and 5-CT were included for comparison. All the compounds behaved as antagonists at α_1-adrenoreceptor subtypes, whereas the enantiomers of **3** and **4** proved to be agonists at 5-HT_{1A} receptors.

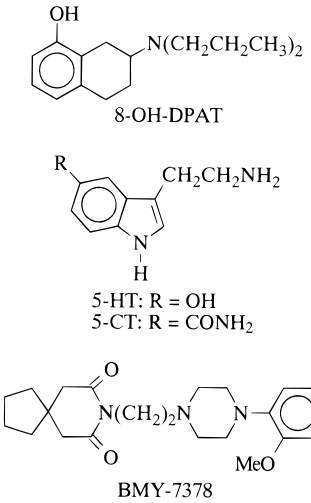
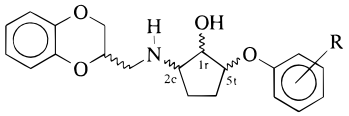


Figure 3. Chemical structures of the reference compounds 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), and BMY-7378.

Table 1. Antagonist Affinities, Expressed as Apparent pK_b Values, of **1**, **3**−**8**, and BMY-7378 at α_1-Adrenoreceptors in Isolated Rat Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D})

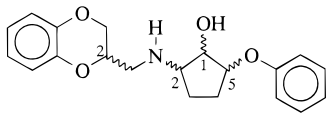


		pK_b[a]		
no.[b]	R	α_{1A}	α_{1B}	α_{1D}
1		9.36 ± 0.04	8.21 ± 0.02	8.60 ± 0.02
3	H	5.48 ± 0.03	6.45 ± 0.03	7.66 ± 0.04
4	H	5.70 ± 0.02	6.47 ± 0.05	7.85 ± 0.01
5	2-MeO	5.68 ± 0.10	6.24 ± 0.03	7.15 ± 0.01
6	2-MeO	6.56 ± 0.02	6.80 ± 0.01	7.30 ± 0.03
7	2,6-$(MeO)_2$	5.89 ± 0.02	6.27 ± 0.01	7.32 ± 0.03
8	2,6-$(MeO)_2$	5.79 ± 0.09	6.17 ± 0.01	6.94 ± 0.04
BMY-7378		6.94 ± 0.02	7.55 ± 0.10	8.34 ± 0.08

[a] Apparent pK_b values ± SE were calculated according to Arunlakshana and Schild[35] with the following equation: pK_b = −log K_b = log (DR − 1) − log [B]. The log (DR − 1) was calculated from 2 to 3 different antagonist concentrations, and each concentration [B] of antagonist was tested four 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. [b] The relationship among the substituents linked to the cyclopentane ring was always cis between the hydroxy group at position 1, taken as the reference atom (1r), and the amine function at position 2 (2c) and trans between both hydroxy and amine moieties and the phenoxy group at position 5 (5t).

An inspection of the results reveals that the inclusion of the ethylene chain separating the amine and the phenoxy moieties of **1** into a cyclopentanol unit (Figure 1), affording **3**−**8**, caused a dramatic effect in the affinity profile for α_1-adrenoreceptor subtypes and 5-HT_{1A} receptors. The two diastereomeric racemates **3** and **4** displayed a similar selectivity profile, which was, however, remarkably different from that of **1** at α_1-adrenoreceptor subtypes. Compounds **3** and **4** displayed a significant selectivity toward the α_{1D}-adrenoreceptor while being weak antagonists for both α_{1A}- and α_{1B}-subtypes, though affinity was a bit higher at the latter.

Table 2. Antagonist Affinities and Agonist Efficacies, Expressed as pK_b and pD_2 Values, Respectively, of the Enantiomers of **3** and **4** at α_1-Adrenoreceptors in Isolated Rat Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D}) and at 5-HT_{1A} Receptors in HeLa Cells (binding [^{35}S]GTP) in Comparison to 8-OH-DPAT, 5-HT, 5-CT, and BMY-7378



		α_{1A}		α_{1B}		α_{1D}		5-HT_{1A}		
no.	config	pK_b[a]	ER[b]	pK_b[a]	ER[b]	pK_b[a]	ER[b]	pD_2[c]	ER[b]	% max
(−)-**3**	(1*S*,2*S*,5*S*)(2*S*)	5.65 ± 0.06		5.79 ± 0.03		6.92 ± 0.01		7.30		82
			3		11		7		27	
(+)-**3**	(1*R*,2*R*,5*R*)(2*R*)	6.10 ± 0.09		6.83 ± 0.06		7.78 ± 0.08		5.87		23
(−)-**4**	(1*R*,2*R*,5*R*)(2*S*)	6.55 ± 0.05		7.13 ± 0.01		8.17 ± 0.08		5.87		100
			>35		49		33		123	
(+)-**4**	(1*S*,2*S*,5*S*)(2*R*)	<5[d]		5.44 ± 0.01		6.65 ± 0.02		7.96		96
8-OH-DPAT		—[e]		—		—		7.60		100
5-HT		—		—		—		7.30		100
5-CT		—		—		—		8.45		96
BMY-7378		6.94 ± 0.02		7.55 ± 0.10		8.34 ± 0.08		9.27		26

[a] Apparent pK_b values ± SE were calculated according to Arunlakshana and Schild[35] with the following equation: $pK_b = -\log K_b = \log (DR - 1) - \log [B]$. The log (DR − 1) was calculated from three different antagonist concentrations, and each concentration [B] of antagonist was tested four 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. [b] The eudismic ratio (ER) is the antilog of the difference between the pK_b and pD_2 values of the eutomers and the corresponding distomers. [c] pD_2 values are the negative logarithm of the agonist concentration required to obtain 50% of the maximal stimulation of [^{35}S]GTPγS binding and were calculated from 2 to 3 experiments, which agreed within ±20%. Each experiment was performed in triplicate. [d] Inactive up to a 10 μM concentration. [e] Not tested.

On the other hand, prototype **1** was about 4500−7500-, 55−58-, and 6−8-fold more potent than **3** and **4** at α_{1A}-, α_{1B}-, and α_{1D}-adrenoreceptors, respectively, displaying selectivity for the α_{1A}-subtype.

Next, we investigated the effect of methoxy groups on the biological profile of **5**−**8** as it is known that two methoxy groups at positions 2 and 6 of the phenoxy unit confer optimum affinity in prototype **1**.[28] Surprisingly, the insertion of one, as in **5** and **6**, or two as in **7** and **8**, methoxy groups did not modify the selectivity profile at α_1-adrenoreceptor subtypes. Instead, it caused a decrease in the affinity for the α_{1D}-subtype relative to the unsubstituted compounds **3** and **4**. This finding clearly suggests that the methoxy groups play a different role in **1** and related cyclopentane-bearing compounds.

To verify whether the stereochemistry of the cyclopentane unit may be important, we investigated the two couples of enantiomers (−)-**3** and (+)-**3**, and (−)-**4** and (+)-**4** at both α_1-adrenoreceptor subtypes and 5-HT_{1A} receptors. It has already been demonstrated that the 2*S* enantiomer of **1** is significantly more potent than the 2*R* enantiomer at α_1-adrenoreceptors[14,15] and at 5-HT_{1A} receptors as well.[9] The results shown in Table 2, though not in total disagreement with previous finding, deserve comment. Epimers (+)-**3** and (−)-**4** have the opposite configuration at the carbon at position 2 of the benzodioxan unit, namely *R* and *S*, respectively, while having the same configuration at the cyclopentane nucleus. They are more potent at all α_1-adrenoreceptor subtypes and remarkably less potent at 5-HT_{1A} receptors, as compared with their corresponding enantiomers (−)-**3** and (+)-**4**. This finding suggests clearly that the stereochemistry of the cyclopentane unit has a greater influence on affinity than that of the benzodioxane moiety. Interestingly enough, a 1*R* configuration, as in (+)-**3** and (−)-**4**, conferred higher affinity at α_1-adrenoreceptors, whereas a 1*S* configuration produced higher affinity for 5-HT_{1A} receptors, which indicates that the two receptor systems have different stereochemical requirements. Enantioselectivity was more pronounced for enantiomers (−)-**4** and (+)-**4** than for (−)-**3** and (+)-**3** at α_1-adrenoreceptors and 5-HT_{1A} receptors as well (Table 2).

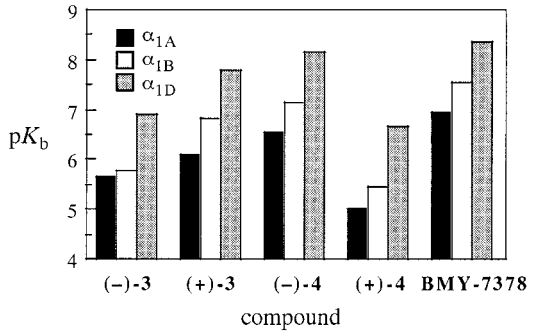


Figure 4. Affinity constants (pK_b) in rat prostatic vas deferens (α_{1A}), spleen (α_{1B}), and aorta (α_{1D}) α_1-adrenoreceptor subtypes of stereoisomers (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** in comparison with BMY-7378.

It is evident that stereoisomers (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** all have, though to a different extent, the same selectivity profile, that is $\alpha_{1D} > \alpha_{1B} > \alpha_{1A}$, which is similar to that displayed by BMY-7378. Among them, (−)-**4** displayed a significant α_{1D} selectivity versus the other subtypes, which was slightly higher than that of BMY-7378 as graphically shown in Figure 4. Its selectivity versus the 5-HT_{1A} receptor is more than 2 log units.

Finally, epimers (−)-**3** and (+)-**4** proved to be agonists at 5-HT_{1A} receptors with a potency comparable to or even higher than that of 5-HT as revealed by their pD_2 value (Table 2), while being 27- and 123-fold more potent than the corresponding enantiomers (+)-**3** and (−)-**4**. Enantiomers (+)-**4** and (−)-**4** behaved as full agonists, whereas (+)-**3** and (−)-**3** showed to be partial agonists, in analogy with BMY-7378.

The binding affinities, expressed as pK_i values, at human cloned α_1-adrenoreceptor subtypes and 5-HT_{1A}

Table 3. Affinity Constants, Expressed as pK_i (−log K_i, M), of Compounds **1**, **3**−**8**, 8-OH-DPAT, and BMY-7378 for Human Recombinant α_1-Adrenoreceptor Subtypes and 5-HT_{1A} Receptors and for Rat Native α_2, D_2, and 5-HT_2 Receptors[a]

	pK_i, human cloned receptors				pK_i, native receptors (rat brain)		
no.	α_{1a}	α_{1b}	α_{1d}	5-HT_{1A}	α_2	D_2	5-HT_2
1	9.37	8.0	9.29	8.68	7.83	6.91	6.0
(−)-**3**	6.41	5.74	6.70	9.08	6.23	5.62	<6
(+)-**3**	7.11	7.03	7.79	7.65	6.27	5.21	−
(−)-**4**	7.44	7.33	8.10	8.26	6.05	5.62	<6
(+)-**4**	6.02	<6	6.03	8.43	6.21	5.95	−
5	−[b]	−	−	7.61	−	−	−
6	−	−	−	7.57	−	−	−
7	−	−	−	7.30	−	−	−
8	−	−	−	7.47	−	−	−
BMY-7378	6.42	7.16	8.84	9.03	5.93	7.31	6.31
8-OH-DPAT	<6	<6	<6	8.46	6.08	<6	<6

[a] Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng−Prusoff equation.[37] The affinity estimates were derived from displacement of [^{3}H]prazosin, [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin, [^{3}H]rauwolscine, [^{3}H]ketanserin, and [^{3}H]spiperone binding for α_1-adrenoreceptors, 5-HT_{1A} receptors, α_2-adrenoreceptors, 5-HT_2 receptors, and D_2 receptors, respectively. Each experiment was performed in triplicate. K_i values were from 2 to 3 experiments, which agreed within ±20%. [b] Not determined.

receptors and at native α_2-adrenoreceptors, D_2, and 5-HT_2 receptors of selected compounds are shown in Table 3 together with those of prototype **1** and reference compounds BMY-7378 and 8-OH-DPAT.

It can be seen that, while binding affinities of reference compound BMY-7378 are qualitatively and quantitatively comparable with pK_b values derived from functional experiments, those observed for (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** are not in complete agreement in particular for the α_{1a}-subtype, where functional affinities were lower. Very recently,[29] we discussed the possibility that if an antagonist does not adhere perfectly to the concept of neutral antagonism in the interaction with the receptor but behaves as an inverse agonist, then a discrepancy between affinity values estimated in functional assays and in binding experiments may not represent a surprise, because the estimated affinities of inverse agonists are system-dependent. Interestingly, the prototype of the present study, WB 4101 (**1**), was shown to be an inverse agonist in a vascular model containing α_{1D}-adrenoreceptors.[30] Thus, the 5-fold difference observed for **1** between binding and functional affinity at $\alpha_{1d/D}$-adrenoreceptors might be explained by the fact that **1** is an inverse agonist at this subtype and, as a consequence, its affinity is not system-independent. It derives that a similar explanation might apply for the discrepancy observed between binding and functional affinities for stereoisomers (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** at α_{1a}-adrenoreceptors.

A further analysis of the results reveals that stereoisomers (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** displayed a lower affinity not only at all α_1-adrenoreceptor subtypes but also at α_2-adrenoreceptors and D_2 receptors as well in comparison to **1**.

Similarly to the trend observed at α_{1a}-adrenoreceptors, stereoisomers (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** displayed a radioreceptor binding affinity higher than that observed in functional assays at 5-HT_{1A} receptors. Furthermore, the eudismic ratio between (−)-**3** and (+)-**3** enantiomers was identical to that observed in functional assays, whereas for (−)-**4** and (+)-**4** it was much lower. However, a difference in potency observed for the agonists in different assays is not surprising, since the affinity is system-dependent, as assumed by theory.

With regard to the 5-HT_{1A} receptor, enantiomer (+)-**4** proved functionally the most selective, in analogy with (−)-**4** at the α_{1D} subtype.

It can be seen that the insertion of methoxy groups in the phenoxy moiety, as in **5**−**8**, did not increase the affinity for 5-HT_{1A} receptors, following the trend observed in functional experiments at α_1-adrenoreceptors.

In conclusion, a most intriguing finding of the present investigation was the observation that the replacement of the carbon chain separating the amine and the phenoxy groups of **1** with a cyclopentane ring afforded stereoisomers (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** that retained good affinity for α_1-adrenoreceptor subtypes or 5-HT_{1A} receptors according to the configuration of the cyclopentane unit. In particular for the enantiomers of **4**, a 1*R* configuration imparted high affinity and selectivity for α_{1D}-adrenoreceptors, whereas the reverse applied for high affinity and selectivity at 5-HT_{1A} receptors. The trend noted above might help in developing relevant structure−activity relationships to differentiate and to understand the structural elements which confer selective interaction at α_1-adrenoreceptor subtypes and at the 5-HT_{1A} receptor.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi 530 apparatus and are uncorrected. Electron impact (EI) mass and ^{1}H NMR spectra were recorded on VG 7070E and Varian VXR 300 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet), or m (multiplet). Optical activity was measured at 20 °C with a Perkin-Elmer 241 polarimeter. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. When the elemental analysis is not included, crude compounds were used in the next step without further purification. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm; Merck) by flash chromatography. Petroleum ether refers to the fraction with a boiling point of 40−60 °C. The term "dried" refers to the use of anhydrous sodium sulfate. All the electrophoretic separations were performed on a HP 3D capillary electrophoresis system with real-time UV−visible diode array detection (Hewlett-Packard GmbH, Waldbronn, Germany). The system was controlled by a HP Vectra 486 PC using the 3DCE-ChemStation software and macro programs based on established algorithms.

***N*1-[2-*cis*-Hydroxy-3-*trans*-(2-methoxyphenoxy)cyclopentyl]acetamide (15).** This was synthesized following an

adapted procedure described for **14**.[16] A solution of 2-methoxyphenol (5.14 g, 41.4 mmol) in dry DMF (8 mL) was added to a cooled (5 °C) suspension of 60% NaH in mineral oil (0.86 g, 21.2 mmol) in dry DMF (8 mL) followed, after the gas evolution was ceased, by the addition of a solution of **13**[16] (3.0 g, 21.2 mmol) in dry DMF (15 mL). The resulting mixture was heated at 100 °C for 16 h. After cooling, excess NaH was destroyed by cautious addition of water. Extraction with EtOAc, followed by washing with aqueous saturated NaCl solution, drying, and evaporation of the extracts gave **15**: 30% yield; mp 118−120 °C; ^{1}H NMR ($CDCl_3$) δ 1.51−1.69 (m, 1), 1.79−1.96 (m, 1), 2.01 (s, 3), 2.15−2.34 (m, 2), 2.62 (br s, 1, exchangeable with D_2O), 3.83 (s, 3), 4.23−4.33 (m, 1), 4.36−4.50 (m, 1), 4.52−4.59 (m, 1), 5.92 (br s, 1, exchangeable with D_2O), 6.80−7.00 (m, 4).

***N*1-[3-*trans*-(2,6-Dimethoxyphenoxy)-2-*cis*-hydroxycyclopentyl]acetamide (16).** This was synthesized from **13** (3.0 g, 21.2 mmol) and 2,6-dimethoxyphenol (6.38 g, 41.4 mmol) following the procedure described for **15**: yield 35%; mp 152−155 °C; ^{1}H NMR ($CDCl_3$) δ 1.52−1.69 (m, 1), 1.81−1.95 (m, 1), 1.98 (s, 3), 2.10−2.32 (m, 2), 2.81 (br s, 1, exchangeable with D_2O), 3.82 (s, 6), 4.20−4.28 (m, 1), 4.30−4.39 (m, 1), 4.41−4.54 (m, 1), 5.98 (br s, 1, exchangeable with D_2O), 6.59 (d, 2), 7.01 (t, 1).

2-*cis*-Amino-5-*trans*-(2-methoxyphenoxy)cyclopentan-1-ol Hydrochloride (11). This was synthesized following an adapted procedure described for **10**.[16] A solution of **15** (1.70 g, 6.4 mmol) in MeOH (25 mL) and 3 N HCl (20 mL) was heated to reflux for 15 h under a stream of dry nitrogen. After cooling to room temperature, the formed solid was collected and then dissolved in water. Resulting solution was washed with ether, made basic with NaOH pellets, and extracted with $CHCl_3$. Removal of dried solvent gave **11** as the free base, which was transformed into the hydrochloride salt: yield 56%; mp 190−191 °C; ^{1}H NMR (DMSO-d_6) δ 1.57−1.78 (m, 2), 1.99−2.17 (m, 1), 2.19−2.36 (m, 1), 3.46−3.60 (m, 1), 3.75 (s, 3), 4.10−4.15 (m, 1), 4.56−4.58 (m, 1), 6.04 (br s, 1, exchangeable with D_2O), 6.87−7.26 (m, 4), 8.01 (br s, 3, exchangeable with D_2O).

2-*cis*-Amino-5-*trans*-(2,6-dimethoxyphenoxy)cyclopentan-1-ol Hydrochloride (12). This was synthesized from **16** (1.86 g, 6.4 mmol) following the procedure described for **11**: yield 52%; mp 180−181 °C; ^{1}H NMR (DMSO-d_6) δ 1.54−1.77 (m, 2), 1.99−2.17 (m, 2), 3.62−3.75 (m, 1), 3.73 (s, 6), 3.98−4.01 (m, 1), 4.40−4.45 (m, 1), 6.66 (d, 2), 7.0 (t, 1), 8.0 (br s, 3, exchangeable with D_2O).

Diastereomeric *N*2-[2-*cis*-Hydroxy-3-*trans*-phenoxycyclopentyl]-2,3-dihydro-1,4-benzodioxine-2-carboxamides (17 and 18). Ethyl chlorocarbonate (0.47 mL, 4.96 mmol) was added dropwise to a cooled (5 °C) solution of racemic **9**[31] (0.89 g, 4.96 mmol) and Et_3N (0.69 mL, 4.96 mmol) in anhydrous dioxane (60 mL) followed, after stirring for 15 min, by the addition of a solution of racemic **10**[16] (0.96 g, 4.96 mmol) in anhydrous dioxane (40 mL). After standing overnight at room temperature, the solvent was evaporated to give a residue, which was purified by chromatography eluting with cyclohexanes−EtOAc (13:3). The first fraction was **17**: 0.71 g (40% yield); mp 137−138 °C (from EtOAc/cyclohexane); ^{1}H NMR ($CDCl_3$) δ 1.69−1.85 (m, 2), 2.22−2.40 (m, 2 + 1 exchangeable with D_2O), 4.20−4.30 (m, 2), 4.46−4.61 (m, 3), 4.75 (dd, 1), 6.83−7.05 (m, 7 + 1, the latter is exchangeable with D_2O), 7.20−7.31 (m, 2).

The second fraction was **18**: 0.76 g (43% yield); mp 134−135 °C (from EtOAc/cyclohexane); ^{1}H NMR ($CDCl_3$) δ 1.68−1.85 (m, 2), 2.12−2.33 (m, 2), 2.40 (br s, 1, exchangeable with D_2O), 4.15−4.22 (m, 1), 4.31 (m, 1), 4.41−4.52 (m, 2), 4.54−4.58 (m, 1), 4.71 (dd, 1), 6.86−7.04 (m, 7 + 1, the latter was exchangeable with D_2O), 7.25−7.32 (m, 2); EI MS *m*/*z* 355 (M^+).

Diastereomeric *N*2-[2-*cis*-Hydroxy-3-*trans*-(2-methoxyphenoxy)cyclopentyl]-2,3-dihydro-1,4-benzodioxine-2-carboxamides (19 and 20). These were synthesized from acid **9** and amine **11** as described for **17** and **18**. The first fraction was **19**: 40% yield; ^{1}H NMR ($CDCl_3$) δ 1.65−1.87 (m, 2), 2.23−2.35 (m, 2), 3.0 (d, 1, exchangeable with D_2O), 3.81 (s, 3), 4.13−4.21 (m, 2), 4.45−4.55 (m, 3), 4.66 (dd, 1), 6.85−6.98 (m, 8), 7.11 (d, 1, exchangeable with D_2O).

The second fraction was **20**: 43% yield; ^{1}H NMR ($CDCl_3$) δ 1.62−1.85 (m, 2), 2.15−2.31 (m, 2), 2.99 (br s, 1, exchangeable with D_2O), 3.84 (s, 3), 4.16−4.22 (m, 1), 4.34 (m, 1), 4.43−4.56 (m, 3), 4.66 (dd, 1), 6.86−6.98 (m, 8), 7.09 (d, 1, exchangeable with D_2O).

Diastereomeric *N*2-[3-*trans*-(2,6-Dimethoxyphenoxy)-2-*cis*-hydroxycyclopentyl]-2,3-dihydro-1,4-benzodioxine-2-carboxamides (21 and 22). These were synthesized from acid **9** and amine **12** as described for **17** and **18**. The first fraction was **21**: 40% yield; mp 194−195 °C; ^{1}H NMR ($CDCl_3$) δ 1.83−1.98 (m, 2), 2.30−2.43 (m, 2), 3.87 (s, 6), 4.17−4.24 (m, 2), 4.35−4.43 (m, 1), 4.54−4.65 (m, 2), 4.70 (dd, 1), 6.59−6.62 (m, 2), 6.87−7.06 (m, 5 + 1, tha latter was exchangeable with D_2O).

The second fraction was **22** as an oil: 44% yield; ^{1}H NMR ($CDCl_3$) δ 1.83−1.92 (m, 2), 2.10−2.36 (m, 2), 2.68 (br s, 1, exchangeable with D_2O), 3.87 (s, 6), 4.09−4.39 (m, 5), 4.57 (m, 1), 5.11 (br s, 1, exchangeable with D_2O), 6.58−6.62 (m, 2), 6.83−7.05 (m, 5).

Diastereomeric 2-*cis*-[(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)amino]-5-*trans*-phenoxycyclopentan-1-ol Hydrochlorides (3 and 4). A solution of 10 M $BH_3{\cdot}CH_3SCH_3$ (3.6 mL) in dry diglyme (10 mL) was added dropwise at room temperature to a solution of **17** (0.71 g, 2.0 mmol) in dry diglyme (20 mL) with stirring under a stream of dry nitrogen. When the addition was completed, the reaction mixture was heated at 100 °C for 7 h. After cooling at 0 °C, excess borane was destroyed by cautious dropwise addition of MeOH (5 mL). The resulting mixture was left to stand overnight at room temperature, cooled at 0 °C, treated with HCl gas for 10 min, and then heated at 120 °C for 3 h. After cooling, the solid was filtered and washed with ether to give **3**: 90% yield; mp 227−230 °C; ^{1}H NMR (CD_3OD) δ 1.79−2.09 (m, 2), 2.12−2.51 (m, 2), 3.31−3.46 (m, 2), 3.79−3.89 (m, 1), 4.01−4.07 (m, 1), 4.30−4.37 (m, 2), 4.53−4.61 (m, 1), 4.67−4.69 (m, 1), 6.84−6.97 (m, 7), 7.24−7.30 (m, 2); EI MS *m*/*z* 341 (M^+). Anal. ($C_{20}H_{24}ClNO_4$) C, H, N.

Diastereomeric **4** was synthesized from **18** following the procedure described for **3**: 80% yield; mp 207−209 °C; ^{1}H NMR (CD_3OD) δ 1.78−2.04 (m, 2), 2.22−2.56 (m, 2), 3.25−3.49 (m, 2), 3.80−3.90 (m, 1), 3.99−4.08 (m, 1), 4.29−4.37 (m, 2), 4.52−4.65 (m, 1), 4.69−4.71 (m, 1), 6.81−6.98 (m, 7), 7.25−7.32 (m, 2); EI MS *m*/*z* 341 (M^+). Anal. ($C_{20}H_{24}ClNO_4$) C, H, N.

Diastereomeric 2-*cis*-[(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)amino]-5-*trans*-(2-methoxyphenoxy)cyclopentan-1-ol Hydrochlorides (5 and 6). These were synthesized from **19** and **20**, respectively, following the procedure described for **3**. Compound **5**: 56% yield; mp 230−233 °C; ^{1}H NMR (CD_3OD) δ 1.82−2.03 (m, 2), 2.26−2.45 (m, 2), 3.30−3.49 (m, 2), 3.80 (s, 3), 3.90−3.98 (m, 1), 4.02−4.09 (m, 1), 4.32−4.37 (m, 2), 4.55−4.63 (m, 1), 4.66 (d, 1), 6.84−6.94 (m, 4), 6.96−7.02 (m, 4). Anal. ($C_{21}H_{26}ClNO_5$) C, H, N.

Compound **6**: 60% yield; mp 188−192 °C; ^{1}H NMR (CD_3OD) δ 1.85−1.99 (m, 2), 2.31−2.47 (m, 2), 3.30−3.48 (m, 2), 3.80 (s, 3), 3.92−3.98 (m, 1), 4.01−4.07 (m, 1), 4.32−4.36 (m, 2), 4.56−4.63 (m, 1), 4.67 (d, 1), 6.81−6.95 (m, 5), 6.97−6.99 (m, 3); EI MS *m*/*z* 371 (M^+). Anal. ($C_{21}H_{26}ClNO_5$) C, H, N.

Diastereomeric 2-*cis*-[(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)amino]-5-*trans*-(2,6-dimethoxyphenoxy)cyclopentan-1-ol Hydrochlorides (7 and 8). These were synthesized from **21** and **22**, respectively, following the procedure described for **3**. Compound **7**: 16% yield; mp 173−175 °C; ^{1}H NMR (CD_3OD) δ 1.86−1.97 (m, 2), 2.17−2.40 (m, 2), 3.35−3.48 (m, 2), 3.82 (s, 6), 4.04−4.13 (m, 2), 4.27−4.30 (m, 1), 4.34−4.40 (m, 1), 4.56−4.65 (m, 2), 6.56−6.59 (d, 2), 6.87−6.92 (m, 3), 6.99−7.08 (m, 2). Anal. ($C_{22}H_{28}ClNO_6$) C, H, N.

Compound **8**: 22% yield; mp 214−216 °C; ^{1}H NMR (CD_3OD) δ 1.83−1.98 (m, 2), 2.21−2.48 (m, 2), 3.48−3.72 (m, 2), 3.82 (s, 6), 4.07−4.14 (m, 2), 4.31−4.33 (m, 1), 4.40 (dd, 1), 4.58−4.65 (m, 2), 6.70 (d, 2), 6.95−6.98 (m, 3), 7.0−7.09 (m, 2). Anal. ($C_{22}H_{28}ClNO_6$) C, H, N.

(3*R*)-4,4-Dimethyl-2-oxotetrahydro-3-furanyl (2*R*)-2,3-Dihydro-1,4-benzodioxine-2-carboxylate (23) and (3*R*)-4,4-Dimethyl-2-oxotetrahydro-3-furanyl (2*S*)-2,3-Dihydro-1,4-benzodioxine-2-carboxylate (24). 1-Ethyl-3-[3-(dimethyl-

amino)propyl]carbodiimide hydrochloride (EDCI; 1.89 g, 9.85 mmol) was added to a stirred and cooled (0 °C) solution of racemic **9** (1.50 g, 9.02 mmol), *N*,*N*-(dimethylamino)pyridine (0.60 g, 4.9 mmol), and (−)-(*R*)-pantolactone (1.47 g, 11.3 mmol). After stirring 2 h at 0 °C and overnight at room temperature, the mixture was added with water and the organic layer was separated and washed with aqueous $NaHCO_3$ saturated solution and finally with water. Removal of dried solvent gave a residue, which was purified by chromatography with methylene chloride as the eluting solvent. The first fraction was **23**: 42% yield; mp 99−101 $[\alpha]_D$ +27.2° (*c* 1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3), 1.10 (s, 3), 4.0 (s, 2), 4.38 (dd, 1), 4.57 (dd, 1), 5.04 (m, 1), 5.39 (s, 1), 6.86−6.94 (m, 3), 7.0−7.04 (m, 1).

The second fraction was **24** as an oil: ^{1}H NMR ($CDCl_3$) δ 1.12 (s, 3), 1.20 (s, 3), 4.05 (s, 2), 4.38 (dd, 1), 4.55 (dd, 1), 5.09 (m, 1), 5.44 (s, 1), 6.88−6.94 (m, 3), 7.01−7.04 (m, 1).

(+)-(2*R*)-2,3-Dihydro-1,4-benzodioxine-2-carboxylic Acid ((+)-9). A solution of **23** (1.06 g, 3.62 mmol) and 6 N HCl (32 mL) in THF (24 mL) was heated at 60−65 °C for 2.5 h. After THF removal under reduced pressure, aqueous solution was made basic with saturated K_2CO_3 solution, washed with methylene chloride to remove nonacidic materials, acidified with concentrated HCl, and finally extracted with methylene chloride. Removal of dried solvent gave **(+)-9**: 75% yield; mp 104−105 °C; $[\alpha]_D$ +63° (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 4.37−4.49 (m, 2), 4.90−4.95 (m, 1), 6.89−7.03 (m, 4), 10.33 (br s, 1, exchangeable with D_2O).

(−)-(2*S*)-2,3-Dihydro-1,4-benzodioxine-2-carboxylic Acid ((−)-9). This was synthesized from **24** following the procedure described for **(+)-9**: 78% yield; mp 105−107 °C; $[\alpha]_D$ −63.5° (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 4.37−4.49 (m, 2), 4.90−4.95 (m, 1), 6.89−7.03 (m, 4), 10.33 (br s, 1, exchangeable with D_2O).

(+)-*N*2-[(1*S*,2*S*,3*S*)-2-Hydroxy-3-phenoxycyclopentyl]-(2*R*)-2,3-dihydro-1,4-benzodioxine-2-carboxamide ((+)-17) and (+)-*N*2-[(1*R*,2*R*,3*R*)-2-Hydroxy-3-phenoxycyclopentyl]-(2*R*)-2,3-dihydro-1,4-benzodioxine-2-carboxamide ((+)-18). These were synthesized from **(+)-9** (0.49 g, 2.72 mmol) and racemic **10** (0.96 g, 4.96 mmol) following the procedure described for racemic **17** and **18** and separated by chromatography (eluting system: cyclohexanes−ETOAc, 13:3). The first fraction was **(+)-17**, which was further purified by crystallization from EtOAc−cyclohexane: 0.18 g (19% yield); mp 141−142 °C; R_f 0.36 (EtOAc−cyclohexane, 7:3); $[\alpha]_D$ +54.7 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.63−1.79 (m, 2), 2.07 (br s, 1, exchangeable with D_2O), 2.12−2.34 (m, 2), 4.13 (m, 1), 4.18−4.24 (m, 1), 4.38−4.48 (m, 2), 4.50−4.53 (m, 1), 4.67 (dd, 1), 6.79−6.96 (m, 7 + 1, the latter was exchangeable with D_2O).

The second fraction was **(+)-18**, which was further purified by crystallization from EtOAc−cyclohexane: 0.2 g (21% yield); mp 137−140 °C; R_f 0.25 (EtOAc−cyclohexane, 7:3); $[\alpha]_D$ +25.4 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.67−1.82 (m, 2), 2.13−2.35 (m, 2), 2.48 (br s, 1, exchangeable with D_2O), 4.16−4.22 (m, 1), 4.31 (m, 1), 4.41−4.51 (m, 2), 4.54−4.59 (m, 1), 4.70 (dd, 1), 6.87−7.04 (m, 7 + 1, the latter was exchangeable with D_2O), 7.25−7.31 (m, 2).

Amide **(+)-17** was synthesized also starting from **(+)-9** and **(+)-10**. It was identical to that obtained by a different method (see above).

(−)-*N*2-[(1*R*,2*R*,3*R*)-2-Hydroxy-3-phenoxycyclopentyl]-(2*S*)-2,3-dihydro-1,4-benzodioxine-2-carboxamide ((−)-17) and (+)-*N*2-[(1*S*,2*S*,3*S*)-2-Hydroxy-3-phenoxycyclopentyl]-(2*S*)-2,3-dihydro-1,4-benzodioxine-2-carboxamide ((−)-18). These were synthesized from **(−)-9** and racemic **10** following the procedure described for racemic **17** and **18**. The first fraction was **(−)-17**, which was further purified by crystallization from EtOAc−cyclohexane: 21% yield; mp 142−143 °C; R_f 0.36 (EtOAc−cyclohexane, 7:3); $[\alpha]_D$ −55.5° (*c* 0.5, MeOH). The ^{1}H NMR spectra was identical to that of **(+)-17**.

The second fraction was **(−)-18**, which was further purified by crystallization from EtOAc−cyclohexane: 20% yield; mp 136−138 °C; R_f 0.25 (EtOAc−cyclohexane, 7:3); $[\alpha]_D$ −25.9° (*c* 0.5, MeOH). The ^{1}H NMR was identical to that of **(+)-18**.

(−)-(1*S*,2*S*,5*S*)-2-{[(2*S*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethyl]amino}-5-phenoxycyclopentan-1-ol Hydrochloride ((−)-3). This was synthesized from **(+)-17** following the procedure described for racemic **3**: 53% yield; mp 228−230 °C (from MeOH/2-PrOH); $[\alpha]_D$ −26.2° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 1.81−1.94 (m, 1), 1.96−2.06 (m, 1), 2.23−2.33 (m, 1), 2.37−2.51 (m, 1), 3.35−3.48 (m, 2), 3.81−3.89 (m, 1), 4.03−4.09 (m, 1), 4.32−4.39 (m, 2), 4.57−4.63 (m, 1), 4.69−4.71 (m, 1), 6.86−6.98 (m, 7), 7.26−7.31 (m, 2); EI MS *m*/*z* 341 (M^+). Anal. ($C_{20}H_{24}ClNO_4$) C, H, N.

(+)-(1*R*,2*R*,5*R*)-2-{[(2*R*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethyl]amino}-5-phenoxycyclopentan-1-ol Hydrochloride ((+)-3). This was synthesized from **(−)-17** following the procedure described for racemic **3**: 88% yield; mp 207−210 °C (from MeOH/2-PrOH); $[\alpha]_D$ +26.9° (*c* 0.5, MeOH). The ^{1}H NMR was identical to that of **(−)-3**. Anal. ($C_{20}H_{24}ClNO_4$) C, H, N.

(−)-(1*R*,2*R*,5*R*)-2-{[(2*S*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethyl]amino}-5-phenoxycyclopentan-1-ol Hydrochloride ((−)-4). This was synthesized from **(+)-18** following the procedure described for racemic **3**: 68% yield; mp 209−212 °C (from MeOH/2-PrOH); $[\alpha]_D$ −59.4° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 1.83−2.01 (m, 2), 2.29−2.37 (m, 1), 2.41−2.52 (m, 1), 3.36−3.47 (m, 2), 3.83−3.90 (m, 1), 4.01−4.07 (m, 1), 4.31−4.37 (m, 2), 4.57−4.63 (m, 1), 4.70−4.72 (m, 1), 6.82−6.99 (m, 7), 7.27−7.32 (m, 2); EI MS *m*/*z* 341 (M^+). Anal. ($C_{20}H_{24}ClNO_4$) C, H, N.

(+)-(1*S*,2*S*,5*S*)-2-{[(2*R*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethyl]amino}-5-phenoxycyclopentan-1-ol Hydrochloride ((+)-4). This was synthesized from **(−)-18** following the procedure described for racemic **3**: 73% yield; mp 232−235 °C (from MeOH/2-PrOH); $[\alpha]_D$ +60.2° (*c* 0.5, MeOH). The ^{1}H NMR was identical to that of **(−)-4**. Anal. ($C_{20}H_{24}ClNO_4$) C, H, N.

(−)-*N*1-[(1*S*)-2-Cyclopentenyl]acetamide (26). This was synthesized from **25**[19] (0.44 g, 5.3 mmol) following the procedure described for racemic **26**[32] and purified by chromatography. Eluting with petroleum ether−EtOAc (3:7) gave **26**: 0.53 g (80% yield); $[\alpha]_D$ −92.9° (*c* 0.5, $CHCl_3$).

(−)-*N*1-[(1a*S*,2*S*,4a*R*)Tetrahydro-1a*H*-cyclopent[*b*]oxiren-2-yl]acetamide (27). This was synthesized from **26** following the procedure described for racemic **27**[32] and purified by chromatography. Eluting with EtOAc−EtOH (9.8:0.2) gave **27**: 60% yield; mp 89−91 °C; $[\alpha]_D$ −98.0° (*c* 0.5, $CHCl_3$).

***N*1-[(1*S*,2*S*,3*S*)-2-Hydroxy-3-phenoxycyclopentyl]acetamide (28).** This was synthesized from **27** following the procedure described for racemic **14**[16] and purified by chromatography. Eluting with EtOAc−$CHCl_3$ (1.5:8.5) gave **28**: 17% yield; ^{1}H NMR ($CDCl_3$) δ 1.86−2.07 (m, 2), 2.26 (s, 3), 2.39−2.61 (m, 2), 3.33 (br s, 1, exchangeable with D_2O), 4.47−4.49 (m, 1), 4.60−4.69 (m, 1), 4.82−4.85 (m, 1), 6.35 (br s, 1, exchangeable with D_2O), 7.12−7.21 (m, 3), 7.49−7.54 (m, 2).

(+)-(1*S*,2*S*,5*S*)-2-Amino-5-phenoxycyclopentan-1-ol ((+)-10). This was synthesized from **28** following the procedure described for racemic **10**: 43% yield; $[\alpha]_D$ +16.6 (*c* 0.5, MeOH).

Capillary Electrophoresis. Determination of Stereochemical Purity. Fused-silica capillaries (Supelco, Milan, Italy) 48.5 cm in length (40 cm effective length) × 50 μm i.d. were used. The applied voltage was held constant at 25 kV, and the detection wavelength was adjusted to 220 nm. All the electrophoretic runs were realized at 15 °C; the samples were introduced hydrodynamically for 10 s (injection pressure 5 kPa). The sample solutions were prepared in water at a concentration of 0.1 mg/mL and stored at ambient temperature. All carrier electrolytes were filtered through 0.45-mm Millex-HV filter units (Millipore, Milford, MA). The capillary was rinsed (3 min) between runs with the separation electrolyte. Chiral analysis of diastereoisomers (+)-**3**, (−)-**3**, (+)-**4**, and (−)-**4** was performed using as running buffer 0.1 M phosphoric acid solution adjusted to pH 3.0 with triethanolamine and containing 10 mM HPCD.[33] The enantiomeric purity control of (+)-**9** and (−)-**9** was realized using, as running buffer,

a 25 mM citric acid solution adjusted to pH 8.0 with Tris base and containing 20 mM DMCD. The quantification limit for both couples of enantiomers, expressed as the signal-to-noise ratio of 10, was evaluated by progressive dilution of enantiomer solutions (over 98% purity) and it corresponded to 0.2 μg/mL.

Biology. Functional Antagonism in Isolated Rat Tissues. Male Sprague−Dawley rats (Charles River, Italy) were killed by cervical dislocation under ketamine anesthesia, and the organs required were isolated, freed from adhering connective tissue, and set up rapidly under a suitable resting tension in 15-mL organ baths containing physiological salt solution kept at appropriate temperature (see below) and aerated with 5% CO_2:95% O_2 at pH 7.4. Concentration−response curves were constructed by cumulative addition 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. Contractions were recorded by means of a force displacement transducer (FT.03 Grass and 7003 Basile) connected to a four-channel pen recorder (Battaglia-Rangoni KV 380). In addition parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

Vas Deferens Prostatic Portion. This tissue (from rats of 200−230 g) was used to assess α_{1A}-adrenergic antagonism.[12] Prostatic portions of 2-cm length were mounted under 400−450 g tension at 37 °C 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 preparations were equilibrated for 45−60 min. During this time the bathing solution was changed every 10 min. Concentration−response curves for isotonic contractions in response to (−)-noradrenaline were 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, then a new concentration−response curve to the agonist was obtained. (−)-Noradrenaline solutions contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

Spleen. This tissue (from rat of 250−300 g) was employed to determine α_{1B}-adrenoreceptor antagonist potency.[20] The spleen was removed and bisected longitudinally into two strips which were suspended in tissue baths 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, 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 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.

Aorta. This tissue (from rats of 250−300 g) was used to assess α_{1D}-adrenoreceptor antagonist potency.[20] Thoracic aorta was cleaned from extraneous connective tissue and placed in 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, 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 helicoidal strips (15 mm × 3 mm) were cut from each aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of acetylcholine-induced relaxation was taken as an indicator that vessel was denuded successfully. Vascular strips were then tied with surgical thread and suspended in a jacketed tissue bath containing Tyrode solution. Strips were secured at one end to Plexiglas hooks and connected to transducer for monitoring changes in isometric contraction. After at least a 1-h equilibration period under an optimal tension of 1 g, cumulative (−)-noradrenaline concentration−response curves were recorded at 30-min intervals: the first two being discarded and the third one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min before the generation of the fourth cumulative concentration−response curve to (−)-noradrenaline.

Radioligand Binding Assays. Native Receptors. Binding studies on native α_2-adrenoreceptors, 5-HT_{2A}, and D_2 receptors were carried out in membranes of rat cerebral cortex (α_2 and 5-HT_{2A})[23,25] and striatum (D_2).[24] Male Sprague−Dawley rats (200−300 g; Charles River, Italy) were killed by cervical dislocation, and different tissues were excised and immediately frozen and stored at −70 °C until use. For α_2 and 5-HT_{2A} membrane preparations, cerebral cortex was homogenized (2 × 20 s) in 50 volumes of cold Tris-HCl buffer, pH 7.4, using a Polytron homogenizer (speed 7). Homogenates were centrifuged at 49000*g* for 10 min, resuspended in 50 volumes of the same buffer, incubated at 37 °C for 15 min, and centrifuged and resuspended two more times. The final pellets were suspended in 100 volumes of Tris-HCl buffer, pH 7.4, containing 10 μM pargiline and 0.1% ascorbic acid. Membranes were incubated in a final volume of 1 mL for 30 min at 25 °C with 0.5−1.5 nM [^{3}H]rauwolscine (α_2-adrenoreceptors) or for 20 min at 37 °C with 0.7−1.3 nM [^{3}H]ketanserin (5-HT_{2A} receptors), in the absence or presence of competing drugs. For D_2 membrane preparations, rat striata were homogenized (2 × 20 s) in 30 volumes of cold Tris-HCl buffer, pH 7.4, using a Polytron homogenizer (speed 7) and centrifuged at 49000*g* for 10 min. The final pellets were suspended in 200 volumes of Tris-HCl incubation buffer containing 10 μM pargiline, 0.1% ascorbic acid, and the following saline concentrations: NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; then membranes were incubated for 15 min at 37 °C with 0.2−0.6 nM [^{3}H]spiperone. Nonspecific binding was determined in the presence of 10 μM phentolamine (α_2-adrenoreceptors), 2 μM ketanserin (5-HT_{2A} receptors), and 1 μM (+)-butaclamol (D_2 receptors). The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters. The filters were then washed with ice-cold buffer, and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

Cloned Receptors. Binding to cloned human α_1-adrenoreceptor subtypes was performed in membranes from CHO (chinese hamster ovary) cells transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoreceptor subtype. Cloning and stable expression of the human α_1-adrenoreceptor gene was performed as previously described.[22] CHO cell membranes (30 μg of protein) were incubated in 50 mM Tris-HCl, pH 7.4, with 0.1−0.4 nM [^{3}H]prazosin, in a final volume of 1.02 mL for 30 min at 25 °C, in the absence or presence of competing drugs (1 pM−10 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters. Genomic clone G-21 coding for the human 5-HT_{1A} receptor was stably transfected in a human cell line (HeLa).[26] HeLa cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum and gentamicin (100 μg/mL), 5% CO_2 at 37 °C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lysed in ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4). Homogenates were centrifuged at 40000*g* for 20 min, and pellets were resuspended in a small volume of ice-cold Tris (5 mM) and EDTA (5 mM) buffer (pH 7.4) and immediately frozen and stored at −70 °C until use. On the experimental day, cell membranes were resuspended in binding buffer: 50 mM Tris-HCl (pH 7.4), 2.5 mM $MgCl_2$, 10 μM pargiline.[27] Membranes were incubated in a final volume of 1 mL for 30 min at 30 °C with 0.7−1.4 nM [^{3}H]8-

OH-DPAT, in the absence or presence of competing drugs. Nonspecific binding was determined in the presence of 10 μM 5-HT. The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

Stimulation of [^{35}S]GTPγS Binding at Cloned 5-HT$_{1A}$ Receptors. The effects of the compounds tested with [^{35}S]-GTPγS binding were evaluated according to the method of Stanton and Beer[21] with minor modifications. On the experimental day, cell membranes from HeLa cells transfected with human cloned 5-HT$_{1A}$ receptors, prepared as above-described, were resuspended in buffer containing 20 mM HEPES, 3 mM $MgSO_4$, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 μM GDP and decreasing concentrations of test drugs (from 100 μM to 0.1 nM) or decreasing concentrations of 5-HT, from 100 μM to 0.1 nM (reference curve), for 20 min at 30 °C in a final volume of about 0.5 mL. Samples were then transferred to ice, added with [^{35}S]GTPγS (150−250 pM), and then incubated for a further 30 min at 30 °C. Nonspecific binding was determined in the presence of 10 μM GTPγS. The incubation was stopped by addition of ice-cold HEPES and rapid filtration on Schleicher & Schuell GF52 filters, using a Brandel cell harvester. The filters were washed three times with a total of 5 mL of the same buffer. Radioactivity was counted by liquid scintillation spectrometry at an efficiency of >90%. All assays were carried out in triplicate on 2−3 separate sessions.

Data Analysis. In functional studies responses were expressed as percentage of the maximal contraction observed in the agonist concentration−response curve taken as a control. The agonist concentration−response curves were analyzed by pharmacological computer programs.[34] pK_b values were calculated according to Arunlakshana and Schild[35] by the formula: $pK_b = \log([B]/(DR - 1))$, where B is the antagonist concentration and the dose ratio (DR) is the ratio of the potency of the agonist (EC_{50}) in the presence of the antagonist and in its absence. DR values were obtained for 2−3 different antagonist concentrations, and each concentration was tested four times.

Binding data were analyzed using the nonlinear curve-fitting program Allfit.[36] Scatchard plots were linear in all preparations. All pseudo-Hill coefficients (n^H) were not significantly different from unity ($p > 0.05$). Equilibrium inhibition constants (K_i) were derived from the Cheng−Prusoff equation:[37] $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. pK_i values (Table 2) are the mean ± SE of 2−3 separate experiments performed in triplicate.

Stimulation of [^{35}S]GTPγS binding induced by the compounds tested was expressed as percent increase in binding above basal value, being the maximal stimulation observed with 5-HT taken as 100%. The concentration−response curve of the agonistic activity was analyzed by the nonlinear fitting program Allfit.[36] The maximal stimulation of [^{35}S]GTPγS binding (E_{max}) achieved for each drug and the concentration required to obtain 50% of E_{max} (pD_2 value) were evaluated.

Acknowledgment. This work was supported by a grant from MURST and the University of Bologna.

References

(1) For part 1, see ref 10.

(2) (a) Kenny, B.; Ballard, S.; Blagg, J.; Fox, D. Pharmacological options in the treatment of benign prostatic hyperplasia. *J. Med. Chem.* **1997**, *40*, 1293−1315. (b) Leonardi, A.; Testa, R.; Motta, G.; De Benedetti, P. G.; Hieble, P.; Giardinà, D. α_1-Adrenoceptors: subtype- and organ-selectivity of different agents. In *Perspective in Receptor Research*; Giardinà, D., Piergentili, A., Pigini, M., Eds.; Elsevier: Amsterdam, 1996; pp 135−152. (c) Ruffolo, R. R., Jr.; Bondinell, W.; Hieble, J. P. α- and β-Adrenoceptors. From the gene to the clinic. 2. Structure−activity relationships and therapeutic applications. *J. Med. Chem.* **1995**, *38*, 3681−3716. (d) Matyus, P.; Horvath, K. α-Adrenergic approach in the medical management of benign prostatic hyperplasia. *Med. Res. Rev.* **1997**, *17*, 523−535.

(3) (a) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R., Jr.; Trendelenburg, U. IV. International union of pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* **1994**, *46*, 121−136. (b) Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. International union of pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. *Pharmacol. Rev.* **1995**, *47*, 267−270.

(4) Faure, C.; Pimoule, C.; Arbilla, S.; Langer, S. Z.; Graham, D. Expression of α_1-adrenoceptor subtypes in rat tissues: implications for α_1-adrenoceptor classification. *Eur. J. Pharmacol. (Mol. Pharmacol. Sect.)* **1994**, *268*, 141−149.

(5) Ford, A. P. D. W.; Williams, T. J.; Blue, D. R.; Clarke, D. E. α_1-Adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol. Sci.* **1994**, *15*, 167−170.

(6) For a review, see: Docherty, J. R. Subtypes of functional α_1- and α_2-adrenoreceptors. *Eur. J. Pharmacol.* **1998**, *361*, 1−15.

(7) Testa, R.; Guarneri, L.; Angelico, P.; Poggesi, E.; Taddei, C.; Sironi, G.; Colombo, D.; Sulpizio, A. C.; Naselsky, D. P.; Hieble, J. P.; Leonardi, A. Pharmacological characterization of the uroselective *alpha*-1 antagonist Rec 15/2739 (SB 216469): role of the *alpha*-1L adrenoceptor in tissue selectivity. Part II. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 1284−1293.

(8) Hieble, J. P.; Ruffolo, R. R., Jr. Recent advances in the identification of α_1- and α_2-adrenoceptor subtypes: therapeutic implications. *Exp. Opin. Invest. Drugs* **1997**, *6*, 367−387.

(9) Hibert, M. F.; Gittos, M. W.; Middlemiss, D. N.; Mir, A. K. Fozard, J. R. Graphics computer-aided receptor mapping as a predictive tool for drug design: development of potent, selective, and stereospecific ligands for the 5-HT$_{1A}$ receptor. *J. Med. Chem.* **1988**, *31*, 1087−1093.

(10) Melchiorre, C.; Minarini, A.; Tumiatti, V.; Giraldo, E.; Schiavi, G. B.; Turconi, M. 8-[4-[(1,4-Benzodioxan-2-ylmethyl)amino]-butyl]-8-azaspiro[4,5]decane-7,9-dione-related compounds: synthesis and interactions with 5-HT$_{1A}$ receptors and α_1-adrenoreceptors. *Med. Chem. Res.* **1993**, *4*, 140−145.

(11) McCarthy, J. R.; Zimmerman, M. B.; Trepanier, D. L.; LeTourneau, M. E.; Wiedeman, P. E.; Whitten, J. P.; Broersma, R. J.; Shea, P. J.; Wiech, N. L.; Huffman, J. C. 6,7-Dihydro-5-[[(*cis*-2-hydroxy-*trans*-3-phenoxy-cyclopentyl)amino]methyl]-2-methylbenzo[*b*]thiophen-4(5*H*)-one: a novel α_1-adrenergic receptor antagonist and renal vasodilator. *J. Med. Chem.* **1985**, *28*, 1142−1145.

(12) Eltze, M.; Boer, R.; Sanders, K. H.; Kolossa, N. Vasodilatation elicited by 5-HT$_{1A}$ receptor agonists in constant-pressure-perfused rat kidney is mediated by blockade of α_{1A}-adrenoreceptors. *Eur. J. Pharmacol.* **1991**, *202*, 33−44.

(13) Pigini, M.; Brasili, L.; Giannella, M.; Giardinà, D.; Gulini, U.; Quaglia, W.; Melchiorre, C. Structure−activity relationships in 1,4-benzodioxan-related compounds. Investigation on the role of the dehydrodioxane-ring on α_1-adrenoreceptor blocking activity. *J. Med. Chem.* **1988**, *31*, 2300−2304.

(14) Nelson, W. L.; Powell, M. L.; Dyer, D. C. Absolute configuration of glycerol derivatives. 7. Enantiomers of 2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxane (WB 4101), a potent competitive α-adrenergic antagonist. *J. Med. Chem.* **1979**, *22*, 1125−1127.

(15) Andrisano, V.; Marucci, G.; Melchiorre, C.; Tumiatti, V. Stereoselectivity at α-adrenoreceptor subtypes: observations with the enantiomers of WB 4101 separated through their amides of N-tosyl-(S)-proline. *Chirality* **1992**, *4*, 16−20.

(16) McCarthy, J. R.; Wiedeman, P. E.; Schuster, A. J.; Whitten, J. P.; Barbuch, R. J.; Huffman, J. C. Stereospecific syntheses of the four diastereomeric 2-amino-5-phenoxycyclopentanols. *J. Org. Chem.* **1985**, *50*, 3095−3103.

(17) Campbell, S. F.; Davey, M. J.; Hardstone, J. D.; Lewis, B. N.; Palmer, M. J. 2,4-Diamino-6,7-dimethoxyquinazolines. 1. 2-[4-(1,4-Benzodioxan-2-ylcarbonyl)piperazin-1-yl] derivatives as α_1-adrenoceptor antagonists and antihypertensive agents. *J. Med. Chem.* **1987**, *30*, 49−57.

(18) Khouili, M.; Guillaumet, G.; Coudert, G.; Pujol, M. D. Synthesis and α,β-adrenergic blocking potency of 2,3-dihydro-1,4-benzodioxin derivatives. II. *Farmaco* **1996**, *51*, 185−188.

(19) Braun, H.; Felber, H.; Krebe, G.; Ritter, A.; Schmidtchen, F. P.; Schneider, A. Asymmetric synthesis of primary amines from alkenes and chiral chloronitroso sugar derivatives. *Tetrahedron* **1991**, *47*, 3313−3328.

(20) Ko, F. N.; Guh, J. H.; Yu, S. M.; Hou, Y. S.; Wu, Y. C.; Teng, C. M. (−)-Discretamine, a selective α_{1D}-adrenoreceptor antagonist, isolated from *Fissistigma glaucescens*. *Br. J. Pharmacol.* **1994**, *112*, 1174−1180.

(21) Stanton, J. A.; Beer, M. S. Characterization of a cloned human 5-HT$_{1A}$ receptors cell line using [^{35}S]GTPγS binding. *Eur. J. Pharmacol.* **1995**, *6*, 79−86.

(22) Testa, R.; Taddei, C.; Poggesi, E.; Destefani, C.; Cotecchia, S.; Hieble, J. P.; Sulpizio, A. C.; Naselsky, D.; Bergsma, D.; Ellis, S.; Swift, A.; Ganguly, S.; Ruffolo, R. R.; Leonardi, A. Rec 15/2739 (SB 216469): a novel prostate selective α_1-adrenoceptor antagonist. *Pharmacol. Commun.* **1995**, *6*, 79−86.
(23) Diop, L.; Dausse, J.-P.; Meyer, P. Specific binding of [^{3}H]-rauwolscine to α_2-adrenoceptors in rat cerebral cortex: comparison between crude and synaptosomal plasma membranes. *J. Neurochem.* **1983**, *41*, 710−715.
(24) Meltzer, Y. H.; Matsubara, S.; Lee, J.-C. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pK_i values. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238−246.
(25) Craig, A. S.; Kenneth, J. K. In vivo regulation of the serotonin-2 receptor in rat brain. *Life Sci.* **1986**, *38*, 117−127.
(26) Fargin, A.; Raymond, J. R.; Regan, J. W.; Cotecchia, S.; Lefkowitz, R. J.; Caron, M. G. Effector coupling mechanisms of the cloned 5-HT$_{1A}$ receptor. *J. Biol. Chem.* **1989**, *284*, 14848−14852.
(27) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT$_{1A}$ receptor. *Nature* **1988**, *335*, 358−360.
(28) For example, see: Melchiorre, C.; Belleau, B. *Adrenoceptors and Catecholamine Action*, Part A; Kunos, G., Ed.; Wiley: New York, 1981; pp 131−179.
(29) Melchiorre, C.; Bolognesi, M. L.; Budriesi, R.; Chiarini, A.; Giardinà, D.; Minarini, A.; Quaglia, W.; Leonardi, A. Search for selective antagonists at α_1-adrenoreceptors: neutral or negative antagonism? *Farmaco* **1998**, *53*, 278−286.
(30) Noguera, M. A.; Ivorra, I. D.; D′Ocon, P. Functional evidence of inverse agonism in vascular smooth muscle. *Br. J. Pharmacol.* **1996**, *119*, 158−164.
(31) Koo, J.; Avakian, S.; Martin, G. Derivatives of 1,4-benzodioxan. I. 1,4-Benzodioxan-2-carboxamide. *J. Am. Chem. Soc.* **1955**, *77*, 5373−5375.
(32) Vince, R.; Daluge, S. Puromycic analogues. Studies on ribosomal binding with diastereomeric carbocyclic puromycin analogues. *J. Med. Chem.* **1974**, *17*, 578−583.
(33) Andrisano, V.; Gotti, R.; Cavrini, V.; Tumiatti, V.; Felix, G.; Wainer, I. W. Capillary electrophoretic and high-performance liquid chromatographic studies of the enantioselective separation of α_1-adrenoreceptor antagonists. *J. Chromatogr. A* **1998**, *803*, 189−195.
(34) Tallarida, R. J.; Murray, R. B. *Manual of pharmacologic calculations with computer programs*; Springer-Verlag: New York, 1991; Version 2.
(35) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **1959**, *14*, 48−58.
(36) De Lean, A.; Munson, P. J.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose−response curves. *Am. J. Physiol.* **1978**, *235*, E97−E102.
(37) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

JM991065J