Structure−Activity Relationships in 1,4-Benzodioxan-Related Compounds. 6.[1] Role of the Dioxane Unit on Selectivity for α_1-Adrenoreceptor Subtypes

Wilma Quaglia,[†] Maria Pigini,[†] Alessandro Piergentili,[†] Mario Giannella,[†] Gabriella Marucci,[†] Elena Poggesi,[‡] Amedeo Leonardi,[‡] and Carlo Melchiorre*[,§]

Department of Chemical Sciences, University of Camerino, Via S. Agostino 1, 62032 Camerino (MC), Italy, Research and Development Division, Recordati S.p.A., Via Civitali 1, 20148 Milano, Italy, and Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

Received March 2, 1999

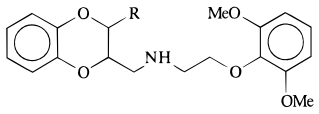
WB 4101-related benzodioxans **3**−**9** were synthesized, and their biological profiles at α_1-adrenoreceptor subtypes and 5-HT_{1A} serotoninergic receptors were assessed by binding assays in CHO and HeLa cells membranes expressing the human cloned receptors. Furthermore, receptor selectivity of selected benzodioxan derivatives was further determined in functional experiments in isolated rat vas deferens (α_{1A}) and aorta (α_{1D}) and guinea pig spleen (α_{1B}), in additional receptor binding assays in rat cortex membranes containing α_2-adrenoreceptors and 5-HT_2 serotoninergic receptors, and in rat striatum membranes containing D_2 dopaminergic receptors. An analysis of the results of receptor binding experiments for benzodioxan-modified derivatives **3**−**9** showed high affinity and selectivity toward the α_{1a}-adrenoreceptor subtype for compounds **3**−**5** and **7** and a reversed selectivity profile for **9**, which was a selective α_{1d} antagonist. Furthermore, the majority of structural modifications performed on the prototype **1** (WB 4101) led to a marked decrease in the affinity for 5-HT_{1A} serotoninergic receptors, which may have relevance in the design of selective α_{1A}-adrenoreceptor antagonists. The exception to these findings was the chromene derivative **8**, which exhibited a 5-HT_{1A} partial agonist profile.

Introduction

In recent years much effort has been directed toward characterization of receptor systems, which are comprised usually of multiple subtypes. α_1-Adrenoreceptors do not represent an exception to the rule as they can be divided into at least three subtypes, namely α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}), with upper and lower case subscripts being used to designate the native or recombinant receptor, respectively.[2−4] However, some functional experiments indicate that an additional α_1-adrenoreceptor subtype may exist, which was named α_{1L}-adrenoreceptor.[5] Efforts to clone this receptor subtype have been unsuccessful so far.[6] It has been suggested that α_{1L}-adrenoreceptors may represent a different affinity state of α_{1A}-adrenoreceptors.[7]

The existence of multiple α_1-adrenoreceptor subtypes is a challenge to medicinal chemists to realize useful drugs, which target only one receptor while not affecting others. As a result, several relatively selective ligands for α_1-adrenoreceptors are now available.[8] Whereas it has been claimed that α_{1A}-adrenoreceptor antagonists can be useful in the treatment of benign prostatic hyperplasia,[9] a potential therapeutic use for either α_{1B} or α_{1D} subtype antagonists has not been defined yet.

Benzodioxans represent one of the oldest and best-known classes of α-adrenoreceptor antagonists whose chemical structure incorporates a 1,4-benzodioxan-2-yl moiety as the main feature.[10] WB 4101 {*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine, **1**} is the prototype of α_1-adrenoreceptor antagonists bearing a benzodioxan moiety. Several investigations were devoted to improving both affinity and selectivity of **1**.[11−13] As a result, a variety of analogues have been studied involving modification of the benzodioxan ring, the amine function, or the (2,6-dimethoxyphenoxy)ethyl moiety. Among these structural modifications performed on **1**, the insertion of a phenyl ring at the 3-position having a trans relationship with the 2-side chain afforded phendioxan {*trans*-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-3-phenyl-1,4-benzodioxin-2-methanamine, **2**},[14] which retained high affinity for α_1-adrenoreceptors while displaying only a markedly reduced affinity for α_2-adrenoreceptors. As an overall result, the presence of a 3-phenyl unit as in **2**



1 (WB 4101): R = H

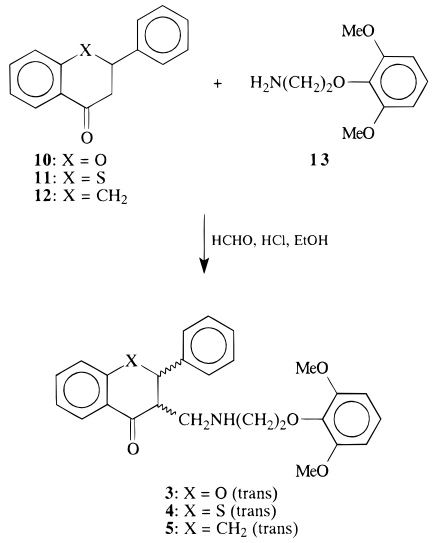
2 (phendioxan): R = Ph (trans)

determined a significant improvement in selectivity toward α_1-adrenoreceptors compared to the prototype **1**. It should be emphasized, however, that the majority of **1** analogues, including **2** and related compounds, were not assayed to evaluate the affinity for the three α_1-adrenoreceptor subtypes. Most of these compounds were tested only on the epididymal portion of the isolated rat vas deferens,[11−15] which proved to be a preparation containing a mixture of α_{1A}- and α_{1L}-adrenoreceptors.[16] Thus, the affinity for the three α_1-adrenoreceptors was not determined, which prevents drawing a relevant conclusion on the structural features that determine

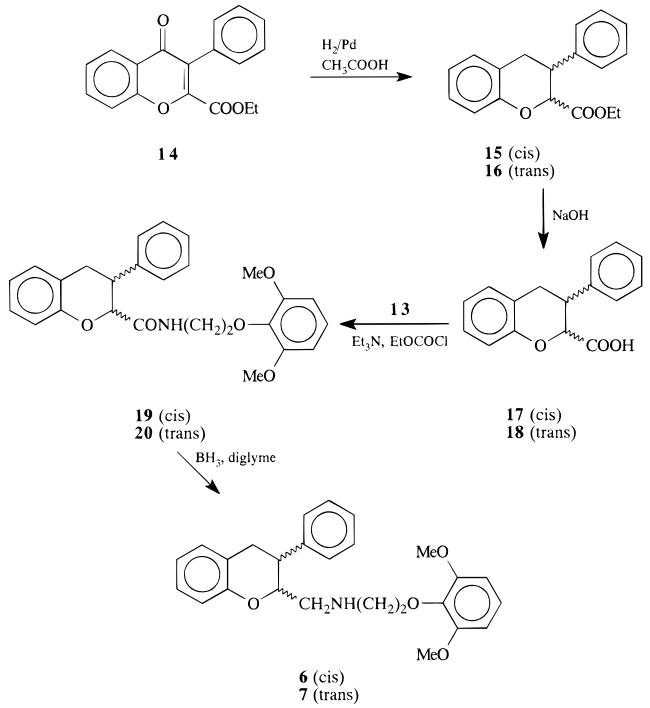
[†] University of Camerino.
[‡] Recordati S.p.A.
[§] University of Bologna.

10.1021/jm9910324 CCC: $18.00
Published on Web 07/13/1999

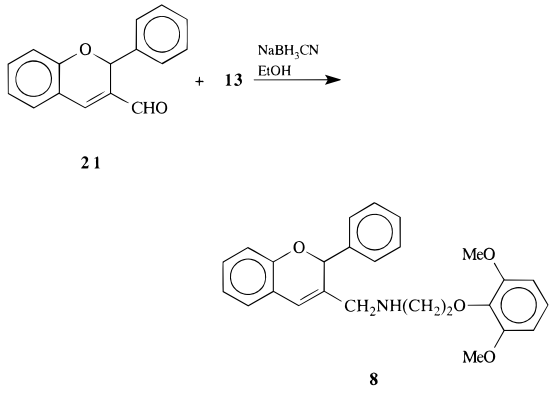
Scheme 1



Scheme 2



Scheme 3



selectivity for one α_1-adrenoreceptor subtype relative to the others. Mephendioxan, a *p*-tolyl analogue of phendioxan, represents an exception as its enantiomers were assayed at the three cloned α_1-adrenoreceptor subtypes. It turned out that, in binding experiments, (−)-(2*S*,3*S*)-mephendioxan is a selective antagonist for α_{1a}-adrenoreceptors.[17]

In the present study, our aim was to investigate further the role of the dioxane unit and in particular of the oxygens at positions 1 and 4 of **2** in the interaction with the three different α_1-adrenoreceptors. Furthermore, we wanted to verify whether benzodioxan-bearing derivatives might allow us to understand the structural requirements that differentiate the binding sites of α_1-adrenoreceptors and 5-HT_{1A} serotoninergic receptors. It is known that benzodioxans are effective ligands of 5-HT_{1A} serotoninergic receptors as well.[18] To this end, we describe here the synthesis and the pharmacological profile of compounds **3**−**8** related to both **1** and **2**. Since we demonstrated that opening of the dioxane ring of **1** did not result in a loss of α_1-adrenoreceptor blocking activity,[11] we included in this study compound **9** to verify whether the increased flexibility might allow a better interaction with α_1-adrenoreceptor subtypes leading hopefully to selectivity.

Chemistry

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

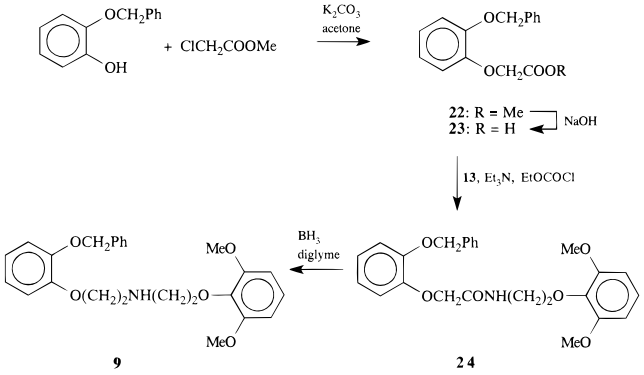
Compounds **3**,[14] **4**, and **5** were synthesized by a Mannich reaction of the corresponding ketones **10**, **11**,[19] and **12**[20] with amine **13**[21] and paraformaldehyde (Scheme 1). This reaction afforded only one of the two possible isomers. The stereochemical relationship between the amino side chain and the phenyl ring in **4** and **5** was deduced from the coupling constant of hydrogens at the corresponding positions. A trans relationship was assigned to these compounds since their coupling constants (J = 11.64 Hz for **4** and J = 11.90 Hz for **5**) were similar to the coupling constant observed previously for **3** (J = 11.29 Hz),[14] which has a trans relationship.

Isomers **6** and **7** were synthesized as shown in Scheme 2. Catalytic hydrogenation of ester **14**[22] afforded a cis/trans mixture (ratio 65:35) of the corresponding saturated esters **15** and **16**, which were separated by column chromatography. The stereochemical relationship between the ester function at position 2 and the 3-phenyl in **15** and **16** was deduced from the coupling constants of the hydrogen atoms at the same positions. Thus, a cis relationship was assigned to **15** since the coupling constant (J = 3.60 Hz) was lower than that found for the corresponding trans isomer **16** (J = 8.10 Hz). Basic hydrolysis of **15** and **16** afforded acids **17** and **18**, respectively. These acids, in chloroform, were amidated in the presence of Et_3N and EtOCOCl with amine **13**[21] to give corresponding amides **19** and **20**. Reduction of **19** and **20** with borane−methyl sulfide complex in dry diglyme gave the corresponding amines **6** and **7**.

Compound **8** was synthesized by reductive amination of aldehyde **21**[23] with amine **13**[21] (Scheme 3).

The open analogue **9** was obtained following a similar synthetic pathway described for amines **6** and **7** as shown in Scheme 4. Thus, ester **22**, which was synthesized from 2-(benzyloxy)phenol and methyl chloroacetate, was hydrolyzed to the corresponding acid **23**. The

Scheme 4



latter compound was transformed into amide **24**, which was reduced with borane to give the desired compound **9**.

Biology

Binding Experiments. The pharmacological profile of compounds **3**−**9** was evaluated by receptor binding assays using WB 4101 (**1**), 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY-7378), and 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT) as standard compounds. [^{3}H]Prazosin was used to label cloned human α_1-adrenoreceptors expressed in Chinese hamster ovary (CHO) cells.[24] Furthermore, [^{3}H]rauwolscine, [^{3}H]spiperone, and [^{3}H]-ketanserin were used to label α_2-adrenoreceptors in rat cortex,[25] D_2 receptors in rat striatum,[26] and 5-HT_{2A} receptors in rat cortex,[27] respectively, whereas [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin was the radioligand used to label cloned human 5-HT_{1A} receptors expressed in HeLa cells.[28,29]

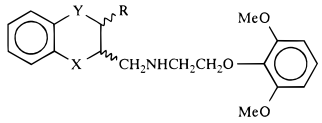
Functional Studies. Receptor subtype selectivity of selected WB 4101-related benzodioxans was further determined at α_1-adrenoreceptors on different isolated tissues using WB 4101 (**1**) and BMY-7378 as standard compounds. α_1-Adrenoreceptor subtype blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of prostatic vas deferens (α_{1A})[30] or thoracic aorta (α_{1D})[31] and by antagonism of (−)-phenylephrine-induced contraction of guinea pig spleen (α_{1B}).[31] Finally, the agonist efficacy of **8** 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[32] using DPAT, 5-hydroxytryptamine, and 5-carboxamidotryptamine as reference compounds.

Results and Discussion

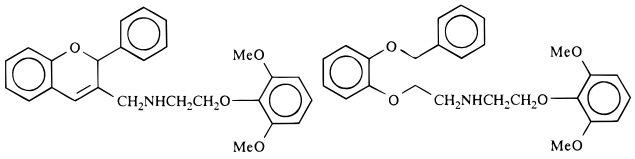
The main goal of this project was to synthesize benzodioxan-like-bearing derivatives displaying an improved α_1-adrenoreceptor subtype selectivity relative to the prototype WB 4101 (**1**). Furthermore, it was also our aim to determine whether structural modifications of **1** would discriminate between the binding sites of 5-HT_{1A} serotoninergic receptors and α_1-adrenoreceptors while retaining hopefully the affinity for the latter ones.

The receptor affinities, expressed as pK_i values, at human cloned α_1-adrenoreceptor subtypes and 5-HT_{1A} serotoninergic receptors and at native α_2-adrenoreceptors, D_2 dopaminergic receptors, and 5-HT_2 serotoninergic receptors of compounds used in the present study are shown in Table 1. To make relevant considerations on structure−activity relationships, prototype **1** and the reference compounds BMY-7378, a selective α_{1d}-adrenoreceptor antagonist displaying also an even higher affinity for 5-HT_{1A} receptors, and DPAT, a selective 5-HT_{1A} agonist, were included for comparison. By taking as a starting point the prototype **1**, it is possible to observe how affinity and selectivity for α_1-adrenoreceptor subtypes can be markedly affected by inserting in the prototype structure a phenyl ring at position 3 and (a) by replacing the oxygen atom at position 1 by a carbonyl group, as in **3**, (b) by replacing the oxygen atoms at position 1 by a carbonyl function and at position 4 by a sulfur atom, as in **4**, or a methylene group, as in **5**, (c) by replacing the oxygen atom at position 4 by a methylene group, affording **6** and **7**, (d) by replacing the oxymethylene moiety by a vinyl group, as in **8**, and (e) by opening the dioxane ring through cleavage of the C2−C3 bond, as in **9**.

Table 1. Affinity Constants, Expressed as pK_i (−log K_i), of **1**, **3**−**9**, 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]



1: R = H, X = Y = O
3: R = Ph, X = CO, Y = O (trans)
4: R = Ph, X = CO, Y = S (trans)
5: R = Ph, X = CO, Y = CH_2 (trans)
6: R = Ph, X = O, Y = CH_2 (cis)
7: R = Ph, X = O, Y = CH_2 (trans)



8 **9**

	pK_i (nM), cloned receptors (human brain)				pK_i (nM), native receptors (rat brain)		
compd	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	9.22	6.84	7.93	6.73	<6	<6	6.20
4	7.42	<6	6.37	7.36	−[d]	−	−
5	8.06	6.11	6.88	6.83	6.45	<6	6.63
6	6.78	6.51	6.86	6.76	−	−	−
7	8.49	6.58	7.28	6.84	<6	<6	<6
8	7.14	6.84	6.95	8.08	−	−	−
9	9.33	9.27	10.17	7.93	−	−	−
BMY[b]	6.42	6.15	8.89	9.43	−	−	−
DPAT[c]	<6	<6	<6	8.47	6.08	<6	<6

[a] Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng−Prusoff equation.[35] 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] BMY, BMY-7378. [c] DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin. [d] Not determined.

Table 2. Agonist Efficacy ([^{35}S]GTP Binding) of **8** on 5-HT$_{1A}$ Serotoninergic Receptors in Comparison to DPAT, 5-HT, and 5-CT[a]

	binding [^{35}S]GTP	
compd	pD_2	% max
8	6.44	40
DPAT	7.60	100
5-HT	7.30	100
5-CT	8.45	96

[a] DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine.

An analysis of the results shown in Table 1 reveals that all compounds, with the exception of **3** and **9**, were weaker ligands at α_1-adrenoreceptor subtypes relative to **1**. Furthermore, compounds **3**−**8** displayed a selectivity profile at α_1-adrenoreceptors, which deserves comment. The insertion of a phenyl ring in a trans relationship with the 2-side chain and the replacement of an oxygen atom with a carbonyl group, leading to **3**, resulted in a marked drop in affinity at both α_{1b}- and α_{1d}-adrenoreceptors (14- and 23-fold, respectively), while not affecting the affinity at the α_{1a} subtype. Furthermore, this structural modification caused a marked drop in affinity for the other receptor systems investigated, namely 89-, >68-, and >8-fold for 5-HT$_{1A}$, α_2, and D_2 receptors, respectively, relative to **1**. As an overall result, **3** resulted as a potent and selective antagonist for the α_{1a}-adrenoreceptor subtype while displaying a weak, if any, affinity for the other receptor systems.

Surprisingly enough, replacement of the oxygen at position 1 of **3** by a sulfur atom or a methylene group, affording **4** and **5**, respectively, caused a significant decrease in affinity for the three α_1-adrenoreceptor subtypes. This result does not parallel that found by replacing the oxygen atom at position 4 of **1** by either a sulfur or a methylene because the resulting analogues were as active as the prototype.[12,13] However, compound **7**, which bears an oxygen atom at position 1 instead of a carbonyl group as in **5**, was only slightly less potent than both **1** and **3** at α_{1a}-adrenoreceptors. The decrease in affinity observed for **8** at all α_1-adrenoreceptor subtypes in comparison to **1** and at α_{1a}-adrenoreceptors in comparison to **3**, **5**, and **7** clearly suggests that the presence of a double bond alters the spatial orientation of the molecule in such a way that the binding with the adrenoreceptor is made difficult. Alternatively, the low affinity displayed by **8** for α_1-adrenoreceptors might indicate that the oxygen at position 1 of **1** plays a role in the binding process owing to its nonbonded electrons. However, interestingly enough, unsaturated analogue **8** retained high affinity for 5-HT$_{1A}$ receptors and turned out to be a partial agonist with an efficacy (pD_2 = 6.44), that was only about 10-fold lower than that of full agonists such as DPAT and 5-hydroxytryptamine as shown in Table 2.

Opening the dioxane ring gave **9**, which was a very potent ligand at α_1-adrenoreceptors while retaining also high affinity for 5-HT$_{1A}$ receptors, although the affinity for the latter was 22−174-fold lower than that at the former ones. This structural modification also resulted in an inversion of the selectivity profile as **9** was more potent at α_{1d}-adrenoreceptors than at both α_{1a} and α_{1b} subtypes.

A most intriguing finding of the present investigation was the observation that, whatever the kind of the oxygen-bearing ring, the presence of a phenyl ring at position 3 in a trans relationship with the side chain at position 2 imparts selective affinity for the α_{1a} subtype not only with regard to both α_{1b} and α_{1d} subtypes but also with 5-HT$_{1A}$, 5-HT$_2$, α_2, and D_2 receptors as well. This result may have relevance for the development of new WB 4101-related compounds having high affinity and high specificity for α_{1a}-adrenoreceptors. Another interesting finding was the observation that opening the dioxane ring, as in **9**, markedly increased the affinity toward α_{1d}-adrenoreceptors. Consequently, open analogue **9** may represent a lead for the design of ligands selective for this receptor subtype.

Table 3. Antagonist Affinities, Expressed as Apparent pK_b, of **1**−**3**, **7**, **9**, and BMY-7378 at α_1-Adrenoreceptors on Isolated Rat Prostatic Vas Deferens (α_{1A}) and Thoracic Aorta (α_{1D}) and Guinea Pig Spleen (α_{1B})[a]

	pK_b		
compd	α_{1A}	α_{1B}	α_{1D}
1	9.36 ± 0.04	8.21 ± 0.02	8.60 ± 0.02
2	8.18 ± 0.08	5.55 ± 0.06	7.51 ± 0.04
3	7.88 ± 0.06	6.05 ± 0.23	6.98 ± 0.26
7	7.36 ± 0.04	5.54 ± 0.04	7.46 ± 0.05
9	8.39 ± 0.18	7.87 ± 0.01	9.37 ± 0.15
BMY-7378	6.98 ± 0.09	7.49 ± 0.09	8.38 ± 0.07

[a] Apparent pK_b values ± SE were calculated according to Arunlakshana and Schild[33] 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.

Compounds **3**, **7**, and **9** were selected for further pharmacological investigation in functional experiments, and the results are shown in Table 3 in comparison with those obtained with prototypes **1** and **2** and the reference compound BMY-7378. Although lower, the antagonist affinity displayed by **3** and **9** had a trend similar to that found in binding assays. On the contrary, these data did not confirm the selectivity for the α_{1a} subtype found for **7** in the binding assays.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and ^{1}H NMR spectra were recorded on Perkin-Elmer 297 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), br t (broad triplet), dt (double triplet), q (quartet), or m (multiplet). Although the IR spectral data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The elemental compositions of the compounds agreed to within ±0.4% of the calculated values. When the elemental analyses are 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.

***trans*-2-Phenyl-3-[[[2-(2,6-dimethoxyphenoxy)ethyl]-amino]methyl]thiochroman-4-one Hydrochloride (4).** A solution of **11**[19] (0.51 g, 2.12 mmol), **13**[21] hydrochloride (0.35 g, 1.50 mmol), paraformaldehyde (0.2 g, 6.66 mmol), and concentrated HCl (0.05 mL) in absolute EtOH (2 mL) was heated at reflux for 6 h. The resulting mixture was left at 0 °C overnight. The solid was filtered and crystallized from 2-PrOH: 0.4 g (55% yield); mp 150−152 °C; ^{1}H NMR (DMSO)

δ 2.91 (m, 1, 3-H), 3.11−3.53 (m, 4, CH_2NCH_2), 3.75 (s, 6, OCH_3), 3.98−4.24 (m, 2, CH_2O), 5.15 (d, J = 11.64 Hz, 1, 2-H), 6.67−8.12 (m, 12, ArH), 8.52 (br s, 1, NH, exchangeable with D_2O), 9.25 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{26}H_{27}NO_4S\cdot HCl\cdot 0.5H_2O$) C, H, N, S.

***trans*-3-Phenyl-2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1-tetralone Hydrochloride (5).** This was synthesized from **12**[20] (0.43 g, 1.93 mmol) following the procedure described for **4**. After evaporation of the solvent, the residue was dissolved in 2 N NaOH and extracted with chloroform. Removal of dried solvent gave an oil, which was purified by column chromatography. Eluting with cyclohexane−acetone (7:3) afforded **5** as the free base: ^{1}H NMR ($CDCl_3$) δ 2.45 (br s, 1, NH, exchangeable with D_2O), 2.57−2.81 (m, 3, $CHNCH_2$), 2.94 (m, 2, CHN and 2-H), 3.06−3.35 (m, 2, 4-H), 3.5 (dt, J = 11.90 and 3.96 Hz, 1, 3-H), 3.79 (s, 6, OCH_3), 3.99 (t, 2, CH_2O), 6.50−8.11 (m, 12, ArH).

The free base was transformed into the hydrochloride salt, which was crystallized from 2-PrOH/Et_2O: 0.3 g (48% yield); mp 161−163 °C; ^{1}H NMR (DMSO) δ 2.67−3.54 (m, 7, CH_2NCH_2, 2-H, 3-H, and 4-H), 3.75 (s, 6, OCH_3), 4.04 (m, 2, CH_2O), 6.68−8.07 (m, 12, ArH), 8.53 (br s, 1, NH, exchangeable with D_2O), 9.59 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{27}H_{29}NO_4\cdot HCl$) C, H, N.

***cis*- and *trans*-3-Phenylchroman-2-carboxylic Acid Ethyl Esters (15 and 16).** Ester **14**[22] (1.1 g, 3.74 mmol) in AcOH (9.35 mL) was hydrogenated over 10% Pd on charcoal (0.074 g) for 10 h at 70 °C and 50 psi of pressure. Following catalyst removal, the solution was diluted with water and extracted with Et_2O. The extracts were washed with 2 N NaOH and water. Removal of dried solvent gave an oil, which was purified by column chromatography eluting with cyclohexanes−EtOAc (98:2). The trans isomer **16** eluted first: 0.22 g (21% yield); R_f 0.21; ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3, CH_3), 3.10 (m, 2, 4-H), 3.42 (m, 1, 3-H), 4.01 (q, 2, CH_2), 4.7 (d, J = 8.10 Hz, 1, 2-H), 6.87−7.40 (m, 9, ArH).

The second fraction was the cis isomer **15**: 0.41 g (39% yield); mp 99−101 °C; R_f 0.16; ^{1}H NMR ($CDCl_3$) δ 1.09 (t, 3, CH_3), 3.04−3.38 (two dd, 2, 4-H), 3.72 (m, 1, 3-H), 4.09 (m, 2, CH_2), 4.89 (d, J = 3.60 Hz, 1, 2-H), 6.90−7.35 (m, 9, ArH).

***trans*-3-Phenylchroman-2-carboxylic Acid (18).** A mixture of **16** (0.22 g, 0.78 mmol) and 2 N NaOH (3.1 mL) was stirred at room temperature for 40 h. The mixture was extracted with $CHCl_3$, and the aqueous layer was acidified with concentrated HCl. Extraction with $CHCl_3$, followed by washing, drying, and evaporation of the extracts, gave **18**: 0.18 g (91% yield); mp 172−173 °C; ^{1}H NMR ($CDCl_3$) δ 3.10 (m, 2, 4-H), 3.52 (q, 1, 3-H), 4.82 (d, J = 6.7 Hz, 1, 2-H), 6.35 (br s, 1, COOH, exchangeable with D_2O), 6.90−7.40 (m, 9, ArH).

***cis*-3-Phenylchroman-2-carboxylic Acid (17).** This was synthesized from **15** (0.41 g, 1.45 mmol) following the procedure described for **18**: 0.3 g (81% yield); mp 193−195 °C; ^{1}H NMR ($CDCl_3$) δ 3.09 (dd, 1, 4-H), 3.45 (dd, 1, 4-H), 3.79 (m, 1, 3-H), 4.50 (br s, 1, COOH, exchangeable with D_2O), 4.90 (d, J = 3.01 Hz, 1, 2-H), 6.93−7.34 (m, 9, ArH).

***trans*-3-Phenylchroman-2-carboxylic Acid [2-(2,6-Dimethoxyphenoxy)ethyl]amide (20).** Ethyl chlorocarbonate (0.079 g, 0.708 mmol) was added dropwise to a stirred and cooled (0 °C) solution of **18** (0.18 g, 0.708 mmol) and Et_3N (0.072 g, 0.708 mmol) in $CHCl_3$ (10 mL), followed after 30 min by the addition of a solution of **13**[21] (0.14 g, 0.708 mmol) in $CHCl_3$ (5 mL). The resulting reaction mixture was stirred for 4 h at room temperature and then washed with 2 N HCl, 2 N NaOH, and finally water. Removal of dried solvent gave an oil, which was purified by column chromatography. Eluting with cyclohexanes−EtOAc (8:2) gave **20** as an oil: 0.26 g (85% yield); R_f 0.11; ^{1}H NMR ($CDCl_3$) δ 3.07 (t, 2, NCH_2), 3.48 (m, 2, 4-H), 3.61 (q, 1, 3-H), 3.80 (s, 6, OCH_3), 4.01 (t, 2, CH_2O), 4.73 (d, J = 6.02 Hz, 1, 2-H), 6.55−7.32 (m, 12, ArH), 7.50 (br t, 1, NH, exchangeable with D_2O).

***cis*-3-Phenylchroman-2-carboxylic Acid [2-(2,6-Dimethoxyphenoxy)ethyl]amide (19).** This was synthesized from **17** (0.3 g, 1.18 mmol) following the procedure described for **20**. Removal of dried solvent gave an oil, which was triturated with Et_2O to give **19**: 0.37 g (72% yield); mp 123−125 °C; R_f 0.09; ^{1}H NMR ($CDCl_3$) δ 3.0−3.70 (m, 5, NCH_2, 3-H and 4-H), 3.71 (s, 6, OCH_3), 3.84−4.12 (m, 2, CH_2O), 4.73 (d, J = 2.44 Hz, 1, 2-H), 6.50−7.27 (m, 12, ArH), 7.43 (br t, 1, NH, exchangeable with D_2O).

***trans*-3-Phenyl-2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]chromane Oxalate (7).** A solution of 10 M $BH_3\cdot CH_3SCH_3$ (0.06 mL) in dry diglyme (1 mL) was added dropwise at room temperature to a solution of **20** (0.26 g, 0.60 mmol) in dry diglyme (12 mL) with stirring under a stream of dry nitrogen with exclusion of moisture. When the addition was completed, the reaction mixture was heated at 120 °C for 8 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 4 h. Removal of the solvent under reduced pressure gave a residue, which was dissolved in water. The aqueous solution was basified with NaOH pellets and extracted with $CHCl_3$. Removal of dried solvent gave a residue, which was purified by column chromatography. Eluting with cyclohexanes−EtOAc−EtOH (8:1.5:0.5) afforded **7** as the free base: 0.18 g (72% yield); R_f 0.29; ^{1}H NMR ($CDCl_3$) δ 1.64 (br s, 1, NH, exchangeable with D_2O), 2.52−3.13 (m, 7, CH_2NCH_2, 3-H and 4-H), 3.82 (s, 6, OCH_3), 4.09 (m, 2, CH_2O), 4.36 (m, 1, 2-H), 6.52−7.40 (m, 12, ArH).

The free base was transformed into the oxalate salt and crystallized from EtOH/Et_2O. The melting point was indefinite; fusion started at 103 °C and was complete at 159−161 °C. Anal. ($C_{26}H_{29}NO_4\cdot H_2C_2O_4$) C, H, N.

***cis*-3-Phenyl-2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]chromane Oxalate (6).** This was synthesized from **19** (0.37 g, 0.85 mmol) following the procedure described for **7**. Eluting with cyclohexanes−EtOAc−EtOH (8:1.2:0.8) afforded **6** as the free base: 0.17 g (48% yield); R_f 0.20; ^{1}H NMR ($CDCl_3$) δ 2.60−3.48 (m, 8, CH_2NCH_2, 3-H, 4-H, and NH, exchangeable with D_2O), 3.80 (s, 6, OCH_3), 4.11 (m, 2, CH_2O), 4.52 (m, 1, 2-H), 6.52−7.34 (m, 12, ArH).

The free base was transformed into the oxalate salt and crystallized from 2-PrOH. The melting point was indefinite; fusion started at 120 °C and was complete at 154−156 °C. Anal. ($C_{26}H_{29}NO_4\cdot H_2C_2O_4$) C, H, N.

2-Phenyl-3-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-3-chromene Oxalate (8). A 2.03 M solution of HCl gas in EtOH (1.67 mL) was added to a solution of **13**[21] hydrochloride (2.0 g, 10.14 mmol) and **21**[23] (0.4 g, 1.69 mmol) in EtOH (13 mL), followed by the addition of $NaBH_3CN$ (0.09 g, 1.35 mmol) and molecular sieves (4 Å). The mixture was stirred at room temperature for 15 h, then acidified at pH 1 with 2 N HCl, filtered, and evaporated. The residue was taken up with water and basified with 6 N KOH and the mixture extracted with Et_2O. After drying, the solvent was evaporated and the residue purified by column chromatography. Eluting with EtOAc gave **8** as the free base: 0.35 g (50% yield); ^{1}H NMR ($CDCl_3$) δ 2.10 (br s, 1, NH, exchangeable with D_2O), 2.98 (m, 2, NCH_2), 3.30 (br s, 2, CH_2N), 3.82 (s, 6, OCH_3), 4.17 (m, 2, CH_2O), 5.95 (br s, 1, CH═), 6.54−7.46 (m, 13, ArH and 2-H).

The free base was transformed into the oxalate salt, which was crystallized from EtOH: mp 162−163 °C; ^{1}H NMR (DMSO) δ 3.22 (m, 2, CH_2N), 3.47−3.89 (m, 2, NCH_2), 3.75 (s, 6, OCH_3), 4.11 (t, 2, CH_2O), 6.09 (s, 1, CH═), 6.64−7.60 (m, 13, ArH and 2-H), 9.20 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{26}H_{27}NO_4\cdot H_2C_2O_4$) C, H, N.

2-(Benzyloxy)phenoxyacetic Acid Methyl Ester (22). A mixture of 2-(benzyloxy)phenol (3 g, 14.98 mmol), methyl chloroacetate (1.63 g, 14.98 mmol), and K_2CO_3 (2.07 g, 14.98 mmol) in dry acetone (100 mL) was refluxed for 8 h. After cooling, the solid was filtered and the solvent was evaporated. The residue was dissolved in Et_2O and washed with 2 N NaOH. Removal of dried solvent gave **22** as an oil: 1.91 g (47% yield); ^{1}H NMR (DMSO) δ 3.70 (s, 3, OCH_3), 4.82 (s, 2, OCH_2CO), 5.12 (s, 2, OCH_2Ph), 6.85−7.60 (m, 9, ArH).

2-(Benzyloxy)phenoxyacetic Acid (23). A mixture of **22** (1.91 g, 7.01 mmol) and 2 N NaOH (10 mL) was stirred at 70 °C for 30 min. The cooled mixture was extracted with $CHCl_3$, and the aqueous layer was acidified with concentrated HCl. Extraction with $CHCl_3$, followed by washing, drying, and evaporation of the extracts, gave a solid, which was crystallized from cyclohexane: 1.5 g (83% yield); mp 90−91 °C; ^{1}H NMR ($CDCl_3$) δ 4.69 (s, 2, OCH_2CO), 5.16 (s, 2, OCH_2Ph), 6.90−7.49 (m, 10, ArH and COOH, exchangeable with D_2O).

2-(Benzyloxy)phenoxyacetic Acid [2-(2,6-Dimethoxyphenoxy)ethyl]amide (24). This was synthesized from **23** (0.66 g, 2.56 mmol) following the procedure described for **20**. The oil residue was purified by column chromatography. Eluting with cyclohexanes−EtOAc (75:25) gave **24**: 0.87 g (78% yield); ^{1}H NMR ($CDCl_3$) δ 3.58 (q, 2, NCH_2), 3.76 (s, 6, OCH_3), 4.08 (t, 2, CH_2O), 4.57 (s, 2, OCH_2CO), 5.09 (s, 2, OCH_2-Ph), 6.50−7.44 (m, 12, ArH), 7.90 (br t, 1, NH, exchangeable with D_2O).

***N*-[2-[2-(Benzyloxy)phenoxy]ethyl]-*N*-[2-(2,6-dimethoxyphenoxy)ethyl]amine Oxalate (9).** This was synthesized from **24** (0.85 g, 1.94 mmol) following the procedure described for **7**. The oil residue was purified by column chromatography. Eluting with a dried mixture of EtOAc−33% NH_4OH (200:0.6) gave **9** as the free base: 0.3 g (37% yield); ^{1}H NMR ($CDCl_3$) δ 2.62 (br s, 1, NH, exchangeable with D_2O), 2.98 (t, 2, CH_2N), 3.11 (t, 2, CH_2N), 3.80 (s, 6, OCH_3), 4.09−4.26 (dt, 4, OCH_2 and CH_2O), 5.10 (s, 2, OCH_2Ph), 6.51−7.48 (m, 12, ArH).

The free base was transformed into the oxalate salt, which was crystallized from 2-PrOH. The melting point was indefinite; fusion started at 120 °C and was complete at 140−142 °C. Anal. ($C_{25}H_{29}NO_5 \cdot H_2C_2O_4$) C, H, N.

Biology. Functional Antagonism in Isolated Tissues. Male Sprague−Dawley rats and guinea pigs (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.

Rat Vas Deferens Prostatic Portion. This tissue (from rats of 200−230 g) was used to assess α_{1A}-adrenergic antagonism.[30] Prostatic portions of 2-cm length were mounted under 300−350-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. Cocaine hydrochloride (0.1 μM) was added to the Tyrode to prevent the neuronal uptake of (−)-noradrenaline. The preparations were equilibrated for 60 min with washing every 20 min. After the equilibration period, tissues were primed two times by addition of 10 μM noradrenaline. After another washing and equilibration period of 60 min, a noradrenaline concentration−response curve was constructed (basal response). 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.

Guinea Pig Spleen. This tissue (from guinea pigs of 250−300 g) was employed to determine α_{1B}-adrenoreceptor antagonist potency.[31] 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, 120.0; KCl, 5.5; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 20.0; NaH_2PO_4, 1.2; glucose, 11.7. Cocaine hydrochloride (0.1 μM) and normetaepinephrine hydrochloride (1 μM) were added to prevent neuronal and extraneuronal uptake of (−)-noradrenaline. The spleen strips were placed under 1-g resting tension and equilibrated for 1 h. The cumulative concentration−response curves to (−)-noradrenaline were measured isometrically and obtained at 45-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 constructed.

Rat Aorta. This tissue (from rats of 250−300 g) was used to assess α_{1D}-adrenoreceptor antagonist potency.[31] 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. Cocaine hydrochloride (0.1 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 (100 μM)-induced relaxation to preparations contracted with (−)-noradrenaline (1 μM) 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 a 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. 1. Native Receptors. Binding studies on native α_2-adrenoreceptors, 5-HT_{2A} serotoninergic receptors, and D_2 dopaminergic receptors were carried out in membranes of rat cerebral cortex (α_2 and 5-HT_{2A})[25,27] and striatum (D_2).[26] Male Sprague−Dawley rats (200−300 g; Charles River, Italy) were killed by cervical dislocation, and different tissues were excised immediately frozen, and stored at −70 °C until use. For α_2 and 5-HT_{2A} membrane preparations, cerebral cortex were homogenized (2 × 20 s) in 50 volumes of cold Tris-HCl buffer, pH 7.4, using a Politron 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} serotoninergic 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 Politron 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, and 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} serotoninergic receptors), and 1 μM (+)-butaclamol (D_2 dopaminergic 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.

2. 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.[24] 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 is stably transfected in a human cell line (HeLa).[28] 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 40000g × 20 min, and pellets were resuspended in a small volume of ice-cold Tris 5 (mM) and EDTA 5 (mM) buffer (pH 7.4), 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.[29] 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$_{1A}$ Serotoninergic Receptors. The effects of compound **8** tested with [^{35}S]GTPγS binding were evaluated according to the method of Stanton and Beer[32] with minor modifications. On the experimental day, cell membranes from HeLa cells transfected with human cloned 5-HT$_{1A}$ serotoninergic receptors, prepared as above-described, were resunspended 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 efficiency >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. pK_b values were calculated according to Arunlakshana and Schild[33] 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.[34] 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:[35] $K_i = IC_{50}/(1 + L/K_d)$, 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 the 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 curve-fitting program Allifit.[34] 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 grants from the University of Camerino, University of Bologna, and MURST.

References

(1) For parts 4 and 5, see refs 15 and 17, respectively.
(2) (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.
(3) 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.
(4) 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.
(5) For a review, see: Docherty, J. R. Subtypes of Functional α_1- and α_2-Adrenoreceptors. *Eur. J. Pharmacol.* **1998**, *361*, 1−15.
(6) 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.
(7) (a) 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. (b) Ford, A. P. D. W.; Daniels, D. V.; Chang, D. J.; Gever, J. R.; Jasper, J. R.; Lesnick, J. D.; Clarke, D. E. Pharmacological Pleiotropism of the Human Recombinant α_{1A}-Adrenoceptor: Implications for α_1-Adrenoceptor Classification. *Br. J. Pharmacol.* **1997**, *121*, 1127−1135.
(8) (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.
(9) Matyus, P.; Horvath, K. α-Adrenergic Approach in the Medical Management of Benign Prostatic Hyperplasia. *Med. Res. Rev.* **1997**, *17*, 523−535.
(10) For example, see: Melchiorre, C.; Belleau, B. *Adrenoceptors and Catecholamine Action*, Part A; Kunos, G., Ed.; Wiley: New York, 1981; pp 131−179.
(11) (a) Melchiorre, C.; Giardinà, D.; Gallucci, P.; Brasili, L. Structural Requirements for Competitive α-Adrenoreceptor Occupancy by Cyclic and Open Analogues of WB 4101. *J. Pharm. Pharmacol.* **1982**, *34*, 683−644. (b) Giardinà, D.; Angeli, P.; Brasili, L.; Gulini, U.; Melchiorre, C.; Strappaghetti, G. Synthesis and α-Blocking Activity of Some Cyclic and Opened Analogues of WB 4101. *Eur. J. Med. Chem.* **1984**, *19*, 411−414.
(12) Melchiorre, C.; Brasili, L.; Giardinà, D.; Pigini, M.; Strappaghetti, G. 2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzoxathian: a New Antagonist with High Potency and Selectivity Toward α_1-Adrenoreceptors. *J. Med. Chem.* **1984**, *27*, 1535−1536.
(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) Quaglia, W.; Pigini, M.; Giannella, M.; Melchiorre, C. 3-Phenyl Analogues of 2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxan (WB 4101) as Highly Selective α_1-Adrenoreceptor Antagonists. *J. Med. Chem.* **1990**, *33*, 2946−2948.
(15) Quaglia, W.; Pigini, M.; Tayebati, S. K.; Piergentili, A.; Giannella, M.; Marucci, G.; Melchiorre, C. Structure−Activity Relationships in 1,4-Benzodioxan-Related Compounds. 4. Effect of Aryl and Alkyl Substituents at Position 3 on α-Adrenoreceptor Blocking Activity. *J. Med. Chem.* **1993**, *36*, 1520−1528.
(16) Ohmura, T.; Oshita, M.; Kigoshi, S.; Muramatsu, I. Identification of α_1-Adrenoreceptor Subtypes in the Rat Vas Deferens: Binding and Functional Studies. *Br. J. Pharmacol.* **1992**, *107*, 697−704.
(17) Quaglia, W.; Pigini, M.; Tayebati, S. K.; Piergentili, A.; Giannella, M.; Leonardi, A.; Taddei, C.; Melchiorre, C. Synthesis, Absolute Configuration, and Biological Profile of the Enantiomers of *trans*-[2-(2,6-Dimethoxyphenoxy)ethyl][(3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine (Mephendioxan), a Potent Competitive α_{1A}-Adrenoreceptor Antagonist. *J. Med. Chem.* **1996**, *39*, 2253−2258.
(18) 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.
(19) Hortmann, A. G.; Harris, R. L.; Miles, J. A. Thiabenzenes. IV. Synthesis and Ylidic Properties of 1-Methyl-3,5-diphenylthiabenzene and 1-Aryl-2-methyl-2-thianaphthalenes. *J. Am. Chem. Soc.* **1974**, *96*, 6119−6132 and references therein.
(20) Vebrel, J.; Carrié, R. Synthèse de Méthoxycarbonylindènes, Dihydro-1,2 naphthalènes et Benzocycloheptène. Obtention des Indanones-1, des Tétralones-1 et de la Benzosubérone Correspondantes. *Bull. Soc. Chim.* **1982**, *3*−*4*, 116−124.
(21) Woolley, D. W. Probable Evolutionary Relations of Serotonin and Indole-Acetic Acid, and Some Practical Consequences Therefrom. *Nature* (*London*) **1957**, *10*, 630−633.
(22) Baker, W.; Chadderton, J.; Harborne, J. B.; Ollis, W. D. A New Synthesis of *iso*Flavones. Part I. *J. Chem. Soc.* **1953**, 1852−1860.
(23) René, L.; René, R. Sur la Synthèse de Δ^3-Chromènes Substitués par un Groupement Électro-Attractif, Comme Analogues Pharmacochimiques de Benzofurannes. *Eur. J. Med. Chem.*−*Chim. Ther.* **1975**, *10*, 72−78.
(24) 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.
(25) 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.
(26) 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.
(27) Craig, A. S.; Kenneth, J. K. In Vivo Regulation of the Serotonin-2 Receptor in Rat Brain. *Life Sci.* **1986**, *38*, 117−127.
(28) 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.
(29) 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.
(30) 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.
(31) 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.
(32) 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.
(33) Arunlakshana, O.; Schild, H. O. Some Quantitative Uses of Drug Antagonists. *Br. J. Pharmacol.* **1959**, *14*, 48−58.
(34) 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.
(35) 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.

JM9910324