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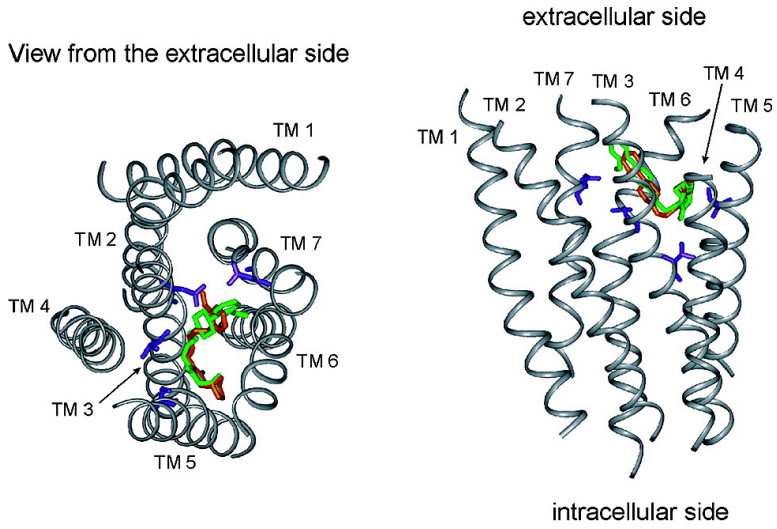

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New 5-Hydroxytryptamine$_{1A}$ Receptor Ligands Containing a Norbornene Nucleus: Synthesis and in Vitro Pharmacological Evaluation

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New arylpiperazine derivatives were prepared to identify highly selective and potent ligands for the 5-hydroxytryptamine 1A (5-HT$_{1A}$) receptor as potential pharmacological tools in studies of central nervous system (CNS) disorders. The combination of structural elements (heterocyclic nucleus, oxyalkyl chain, and arylpiperazine) known to introduce 5-HT$_{1A}$ receptor affinity and the proper selection of substituents led to compounds with higher receptor specificity and affinity. In binding studies, several molecules showed affinity in the nanomolar and subnanomolar ranges at 5-HT$_{1A}$ and moderate to no affinity for other relevant receptors (5-HT$_{2A}$, 5-HT$_{2C}$, D$_1$, D$_2$, α_1, and α_2). The 4-[3-[4-(*o*-methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**3b**), with K_i = 0.021 nM, was the most active and selective derivative for the 5-HT$_{1A}$ receptor with respect to other serotonin receptors, whereas the most selective derivative for dopaminergic and adrenergic receptors was a CF_3-substituted arylpiperazine (**2e**). As a general trend, compounds with a piperazinylpropoxy chain (**3b**−**g**) showed a preferential affinity for the 5-HT$_{1A}$ receptor, suggesting that the alkyl chain length represents a critical structural feature in determining 5-HT$_{1A}$ receptor affinity and selectivity, as confirmed by the molecular modeling invoked for explaining the differential binding affinities of the new arylpiperazines.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is one of the most important targets for medicinal chemistry as it is implicated in numerous physiological and pathophysiological processes.[1−4] Discovery of ligands for 5-HT receptors (5-HTRs) is an area of intense research because of the potential for new therapeutic drugs. In particular, the 5-HT$_{1A}$ receptor, found in high concentration in the limbic system, where it is thought to play a role in emotional processes, is a major target for neurobiological research and drug development. Its activation leads to a number of physiological changes that can be easily quantified.[5−7] Agonists and partial agonists have been proven to be effective in treating anxiety and depression.[8−11]

5-HT$_{1A}$ belongs to the G protein coupled receptors (GPCRs),[12] and the members of this family possess similar amino acid compositions. In particular, the 5-HT$_{1A}$ transmembrane amino acid sequence presents 45% homology with the respective part of the α_1-adrenergic receptor.[13]

Several agents are already known for their high affinity toward these receptors, and from a chemical point of view, they can be subdivided into different classes. The most studied group is that of long-chain arylpiperazines (LCAPs).[7,14] Their general chemical structure contains an alkyl chain (two to four methylene units) attached to the N-4 atom of the piperazine moiety on a terminal amide or imide fragment. The influence of each part of the LCAP structures on the 5-HT$_{1A}$ receptor affinity, intrinsic activity, and selectivity has been the subject of many structure−affinity relationship (SAR) studies. In particular, much effort has been devoted to understanding the role of the terminal part in the ligand−receptor interaction and, consequently, a great number of many different fragments were used.[15] However, a limitation of many 5-HT$_{1A}$ receptor ligands in their potential use as drugs or pharmacological tools is their undesired high affinity for other receptor subtypes. The dopaminergic D$_2$ receptor and α_1-adrenoceptor are two examples of receptor sites to which several 5-HT$_{1A}$ ligands bind with high affinity.

Our group has undertaken a research program aimed at developing new 5-HT$_{1A}$ agents[16−21] with high affinity and selectivity over other serotoninergic, dopaminergic, and adrenergic receptors. In continuation of our research program, we have analyzed a new set of arylpiperazine-*N*-alkyl derivatives with a novel exo-*N*-oxy-5-norbornene-2,3-dicarboximide fragment as the terminal part of the LCAP, which in addition contains an oxygen atom in the spacer (Figure 1). To explore its influence on the serotoninergic activity, this original bicyclic nucleus was linked to some of the most thoroughly studied aryl-substituted piperazines (Ph-*o*-OCH_3, Ph-*o*-Cl, Ph-*m*-Cl, and Ph-*m*-CF_3 both pyrimidyl and pyridyl) via two or three methylene spacing units. All the new compounds were tested for their affinity for 5-HT$_{1A}$,

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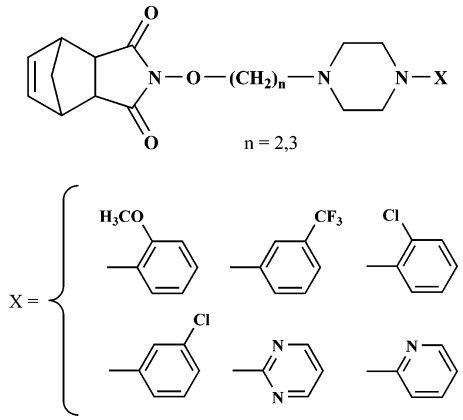


Figure 1. General structures.

5-HT_{2A}, and 5-HT_{2C} receptors. Moreover, the multireceptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. Parallel to the pharmacological evaluation, molecular modeling studies were carried out for explaining the differential binding affinities of the new class of arylpiperazines.

Chemistry

The general strategy for the synthesis of the target compounds (Table 1) is summarized in Scheme 1. The general procedure is as follows: alkylation of the starting heterocycle endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide with 1-bromo-2-chloroethane or 1-bromo-3-chloropropane in the presence of NaOH in absolute ethanol gave the corresponding chloro-alkyl norbornene derivatives **2a** and **3a**. Subsequent condensation of compounds **2a** and **3a** with the desired 4-X-substituted piperazines, performed in CH_3CN in the presence of K_2CO_3 and NaI under reflux, provided the final compounds **2b**−**g** ($n = 2$) and **3b**−**g** ($n = 3$), respectively. Purification of each final product was obtained by chromatography on a silica gel column and further by crystallization from the appropriate solvent. All the new compounds gave satisfactory elemental analyses and were characterized by ^{1}H NMR and mass spectrometry (LCQ-MS Thermoquest-Ion trap). ^{1}H NMR and MS data for all final compounds were consistent with the proposed structures.

Pharmacology

The newly synthesized compounds were tested for in vitro affinity for serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors by radioligand binding assays. The more active compounds on serotonin receptors have been selected and evaluated for their affinity for dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. All the compounds were dissolved in ethanol or in 5% DMSO. The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptor, [^{3}H]-8-OH-DPAT, rat brain cortex; (b) serotonin 5-HT_{2A} receptor, [^{3}H]ketanserin, rat brain cortex; (c) serotonin 5-HT_{2C} receptor, [^{3}H]mesulergine, rat brain cortex; (d) dopamine D_1 receptor, [^{3}H]SCH-23390, rat striatum; (e) dopamine D_2 receptor, [^{3}H]spiperone, rat striatum; (f) adrenergic α_1 receptor, [^{3}H]prazosin, rat brain cortex; and (g) adrenergic α_2 receptor, [^{3}H]yohimbine, rat brain cortex.

Nonspecific binding was determined as described in the Experimental Section, and specific binding was determined as the difference between total and nonspecific binding. Blank experiments were carried out to determine the effect of 5% DMSO on the binding, and no effects were observed. Competition experiments were analyzed with the "Easy Fit" program[22] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}). Six concentrations of test compounds were used in these experiments, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prusoff,[23] from the following equation: $K_i = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the hot ligand used and K_D its receptor dissociation constant (K_D values, obtained by Scatchard analysis,[24] were calculated for each labeled ligand).

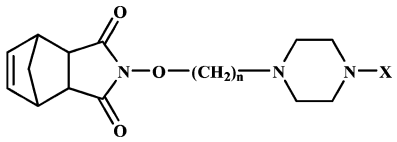
Results and Discussion

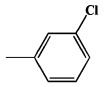
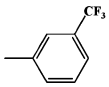
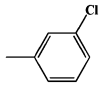
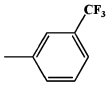
Twelve new arylpiperazine derivatives were synthesized and evaluated for activity and selectivity. Introduction of a novel heterotricyclic nucleus as the terminal part, slight modifications concerning the alkoxy spacer chain length (three to four units), and the introduction of various aromatic rings into the N-4 piperazine atom are depicted in Figure 1 and were performed in an effort to obtain compounds with high affinity and selectivity for 5-HT_{1A} over other serotoninergic receptors as well as dopaminergic and adrenergic receptors. As anticipated on the basis of our previous investigations, several of these compounds were potent 5-HT_{1A} receptor ligands. In fact, they showed nanomolar or even subnanomolar 5-HT_{1A} receptor affinities (Table 1). Besides the outstanding 5-HT_{1A} receptor affinity of compound **3b** (K_i = 0.021 nM), most K_i values were clustered in a relatively narrow range from 2.14 nM (**2e**) to 49.5 nM (**3f**). Only compounds **2f** and **2g** where less active, with K_i values of 384 and 382 nM, respectively.

The two series, **2b**−**g** and **3b**−**g**, differ in the length of the connecting chain between the exocyclic oxygen atom and the piperazine ring. As a general trend, compounds with a piperazinylpropyl chain linked to the exocyclic oxygen atom (**3b**−**g**) showed good and preferential affinity for the 5-HT_{1A}R, relative to compounds in which the spacer is one atom shorter (**2b**−**g**). Therefore, the influence of the alkyl chain length observed for our compounds is also in accordance with the recently reported structure−affinity relationships of a new model of arylpiperazines.[25]

Concerning the influence of the substituent on the N-4 atom of the piperazine moiety, the 3-trifluoromethylphenyl group (for the **2b**−**g** series) and the 2-methoxyphenyl group (for the **3b**−**g** series) conferred the highest affinity for the 5-HT_{1A} receptor. The presence of a chlorine on the phenyl ring, in particular for the **3b**−**g** series, led also to compounds which exhibited high affinity for the 5-HT_{1A} receptor (**3c**, K_i = 4.13 nM; **3d**, K_i = 3.36 nM). However, the position of the substituent on the phenyl ring seems to have little influence on the 5-HT_{1A}R affinity. The pyridine or pyrimidine moiety seems especially unfavorable when associated to a shorter chain spacer ($n = 2$, **2f** and **2g**), while the same aromatic nucleus associated to a longer chain ($n = 3$,

Table 1. Affinities of Compounds **2b**−**g** and **3b**−**g** for 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ Receptors[a]



Compd	Substituents		Receptor affinity Ki ± SD (nM)		
	X	n	5-HT$_{1A}$ [^{3}H]8OH-DPAT	5-HT$_{2A}$ [^{3}H]Ketanserin	5-HT$_{2C}$ [^{3}H]Mesulergine
2b		2	45±2.61	>10^4	>10^4
2c		2	7.12±0.21	>10^4	>10^4
2d		2	27.1±6.31	2700 ± 210	>10^4
2e		2	2.14±0.83	4010 ± 260	>10^4
2f		2	384±75.2	>10^4	no affinity
2g		2	382±73.9	3510 ± 228	>10^4
3b		3	0.021±0.01	>10^4	>10^4
3c		3	4.13±1.89	3900 ± 846	423 ± 69.7
3d		3	3.36±1.62	547 ± 126.4	733 ± 98.7
3e		3	5.13±0.11	2680 ± 495	>10^4
3f		3	49.5±1.37	118 ± 57.6	>10^4
3g		3	30.3±10.9	>10^4	>10^4

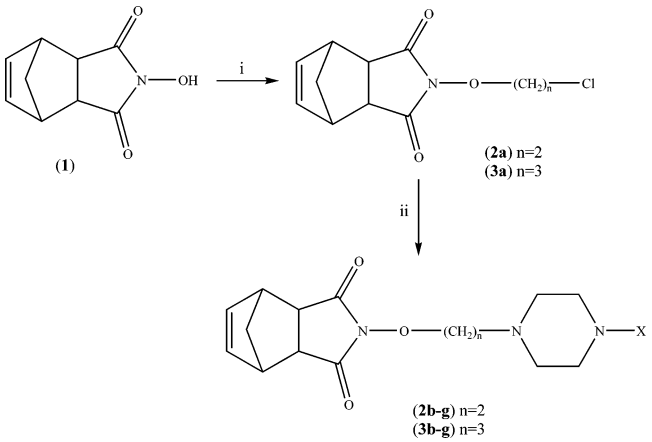
[a] For the purpose of comparison, 8-OH-DPAT, Ketanserine, and Mesulergine bind 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors with values of 0.80, 0.85, and 1.90 nM, respectively, under these assay conditions.

3f and **3g**) resulted in a smaller decrease in binding compared to the other N-4 substituents at the piperazine ring.

The 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor affinities of the tested compounds were always lower than those observed for 5-HT$_{1A}$ receptors and ranged from 118 nM (**3f**) to >10^4 nM (**2b**, **2c**, **2f**, **3b**, and **3g**) for 5-HT$_{2A}$ and from 423 nM (**3c**) to >10^4 nM (**2b**−**g**, **3b**, **3e**, **3f**, and **3g**) for 5-HT$_{2C}$ receptors. The most 5-HT$_{1A}$ active compound, 4-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione **(3b**, K_i = 0.021 nM), was also the most selective derivative with respect to the serotonin receptors 5-HT$_{2A}$ and 5-HT$_{2C}$.

Additionally, the affinity of the most active compounds (**2c**, **2e**, **3b**, **3c**, and **3e**) on several other receptors (dopaminergic D$_1$ and D$_2$ and adrenergic α_1 and α_2 receptors) was examined in order to verify the selectivity of these compounds. The results are summarized in Table 2. All the compounds proved highly selective against dopaminergic receptors with K_i values of above 10^4 nM except for compound **3e**, which exhib-

Scheme 1[a]



[a] Reagents and conditions: (i) $Br(CH_2)_nCl$, NaOH, absolute EtOH, 70 °C, 24 h; (ii) 4-X-substituted piperazine, K_2CO_3, NaI, CH_3CN, reflux, 24 h.

Table 2. Affinities of Compounds **2c**, **2e**, **3b**, **3c**, **3d**, and **3e** for D_1, D_2, α_1, and α_2 Receptors

	receptor affinity $K_i \pm$ SD (nM)			
compd	D_1 [^{3}H]SCH-23390	D_2 [^{3}H]spiperone	α_1 [^{3}H]prazosin	α_2 [^{3}H]yohimbine
2c	$>10^4$	$>10^4$	747	691
2e	$>10^4$	$>10^4$	$>10^4$	$>10^4$
3b	$>10^4$	$>10^4$	75.3	3650
3c	no affinity	no affinity	128	3140
3d	$>10^4$	no affinity	141	1710
3e	4510	670	358	4680

ited K_i values of 670 nM on the D_2 receptor. Regarding α_1 and α_2 adrenergic receptors, only compounds **3b** and **3c** showed quite moderate affinities (75.3 and 128 nM, respectively). The high selectivity toward α_1 receptors, exhibited by compound **2e**, is very interesting considering that the amino acid sequence of the transmembrane part of 5-HT_{1A} is highly homologous to that of the adrenergic α_1 receptor.

These results emphasize the selectivity within the discussed scaffold and suggest that the alkyl chain length represents a critical structural feature in determining 5-HT_{1A} receptor affinity and selectivity. Compounds **2b**−**g**, in fact, showed lower 5-HT_{1A} receptor affinity than their analogues in the series **3b**−**g**. To rationalize the differential binding affinities of the new arylpiperazines, molecular modeling experiments were carried out.

Molecular Modeling

As a receptor structure, the model of Lopez-Rodriguez et al.[26] was used. It agrees very well with the model of Strzelczyk et al.[27] for the transmembrane helices but lacks the extra- and intracellular loops. We observed that our compounds seem completely buried in the receptor part that is represented by the former model[26] (Figure 2) and, therefore, avoided the computationally expensive consideration of the helix-connecting loops of the latter model.[27] As the membrane environment is not included in all modeling studies of serotonin receptors, additional constraints have to be employed for stabilizing the structure. However, no restraints were imposed on receptor residues close to the ligand to allow for an induced fit, where the receptor rearranges to accommodate and properly bind the ligand. Receptor residues with at least one atom closer than 5 Å to any ligand atom defined this binding site. Initial placement of the ligands was achieved by imposing restraints based on experimental data from mutation and (Q)SAR studies. We introduced three distance constraints to allow hydrogen bonds between the protonated amine of the piperazine ring and Asp-116 (3.32 according to the nomenclature of Ballesteros and Weinstein[28]) and between the carbonyl oxygens of the pyrrole moiety and Ser-199 (5.42) on one side and Thr-121 (3.37) on the other side. After conformational searching at 500 K followed by simulated annealing, these artificial restraints were removed, and a 1 ns molecular dynamics (MD) simulation at 300 K was performed without constraints to the ligand (see the Experimental Section). It should be noted that most previous modeling studies of serotonin receptor/ligand complexes employed artificial constraints between the receptor and the ligand throughout the calculations. Contrarily, we only used the constraints for the initial placement of the ligand and discarded these artificial restraints for the productive simulation at 300 K. All twelve ligands remained in the binding pocket during all of the unconstrained simulations with only some motions of the piperazine moiety. The difference in linker length

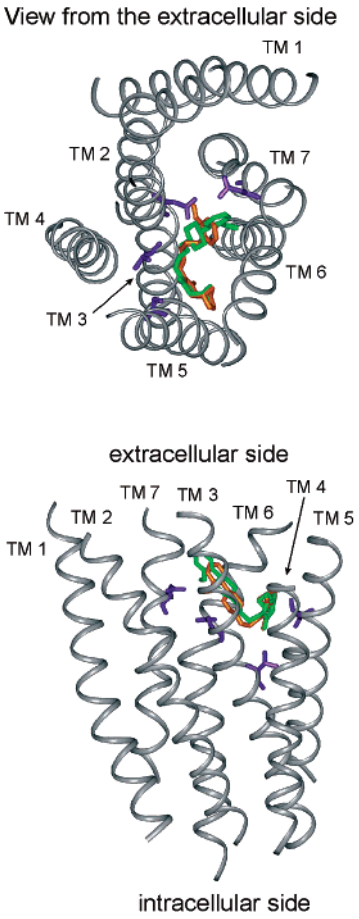


Figure 2. Two views of the 5-HT_{1A}/**3b** complex model. The transmembrane helices of 5-HT_{1A} are depicted as gray ribbons, and the **3b** ligand is depicted as green sticks. Asp-116 (3.32), Ser-199 (5.42), Thr-121 (3.37), and Asn-386 (7.39) are rendered as violet sticks. Of the 5-HT_{1A}/**2b** complex model, only the **2b** ligand is shown in orange, as the receptor structures of both models are almost indistinguishable on this scale.

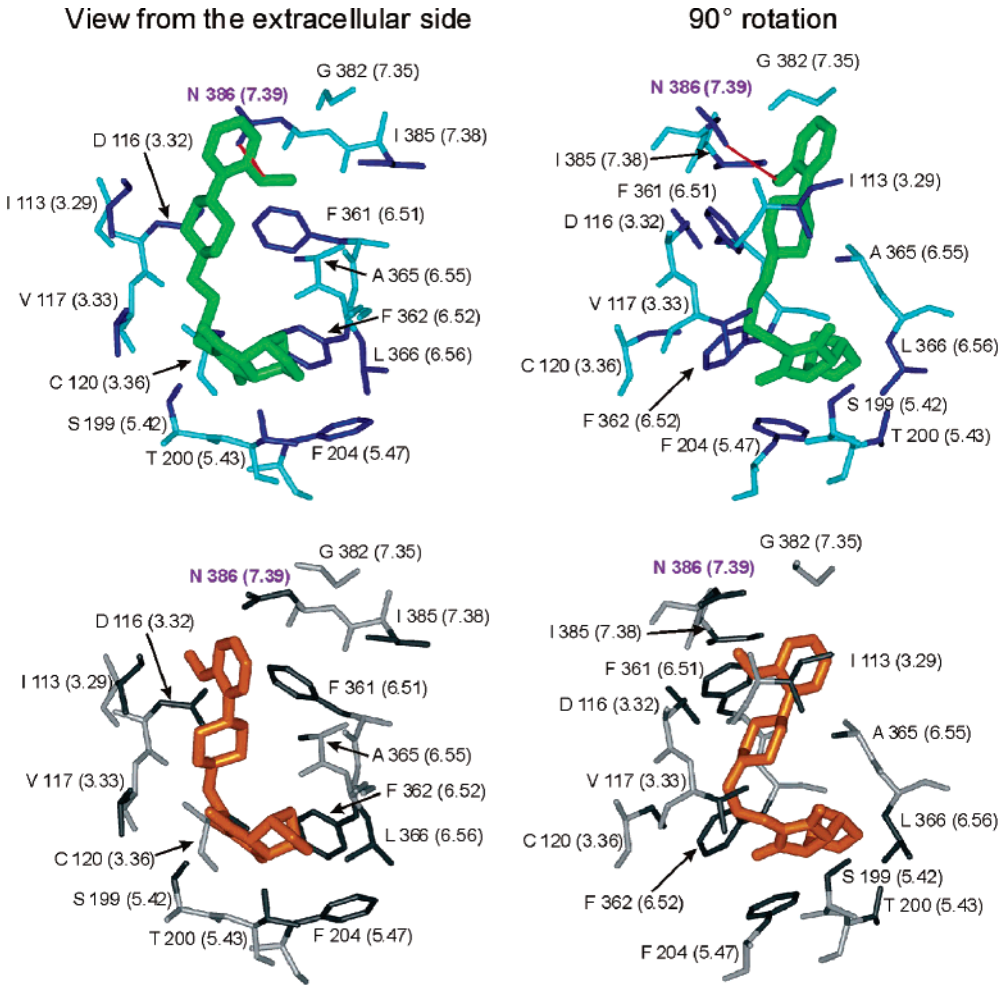


Figure 3. Detailed view of the binding site. Top: The 5-HT$_{1A}$/**3b** complex is shown in blue (receptor) and green (**3b**). Only 5-HT$_{1A}$ receptor residues that are closer than 3 Å to the **3b** ligand are displayed. Bottom: The 5-HT$_{1A}$/**2b** complex is colored gray (receptor) and orange (**2b**). The receptor backbone is depicted in light gray or light blue while the side chains are in dark colors. The red line marks the hydrogen bond between Asn-386 (7.39) and **3b**.

between compounds **2b**−**g** and **3b**−**g** resulted in a differential positioning of the substituted piperazine while the norbornene part remained fixed relative to the receptor, reflecting its superior properties as an anchoring group.

Figures 2 and 3 display the conformations of **3b** and **2b** in complex with the 5-HT$_{1A}$ model as observed at the end of the MD calculations. The exceptionally high affinity of **3b** compared to all other compounds with a propyl chain between the piperazine and oxyimido moieties ($n = 3$) despite their similarity is most probably explained by an additional hydrogen bond between the methoxy substituent in the ortho position and the side chain of Asn-386 (7.39) (Figure 3, top). Reducing the length of the alkyl chain by one methylene group (giving **2b**) pulls the phenyl ring further into the receptor, leading to unfavorable steric interactions of the methoxy group with the surrounding receptor residues and loss of the hydrogen bond to Asn-386 (7.39) (Figure 3, bottom). Therefore, in the case of the shorter alkyl chain (**2b**), the 2-methoxyphenyl substituent results in lower affinity compared to the other substituents tested, while for the longer alkyl chain (**3b**) the highest affinity is achieved. Additional simulations were performed with an analogue of **3b** where the oxypropyl linker was replaced by a butyl chain to investigate the role of the oxygen atom of the oxypropyl moiety. It was found that the structure of the complex between the 5-HT$_{1A}$ model and the butyl variant is almost identical to the one observed for **3a** also preserving the hydrogen bond to Asn-386 (7.39). However, the slight rearrangement of the linker due to the different covalent geometries of carbon and oxygen leads to small but unfavorable changes in steric as well as electrostatic interactions. For our ligands, the oxypropyl chain seems to be the more favorable linker between the arylpiperazine and norbornene moieties.

In contrast to all other substituents, the 3-trifluoromethyl-phenyl derivative exhibits a slightly higher affinity for the shorter chain compared to the longer chain probably due to the very hydrophobic trifluoromethyl group. Although the 5-HT$_{1A}$ ligands are quite deeply buried inside the receptor (see Figure 2, right), the arylpiperazine part remains partially accessible to the extracellular aqueous solvent (see Figure 2, left, and Figure 3, left). Our model does not allow a quantitative calculation of solvent accessible area or solvation energies, but a qualitative comparison clearly indicates that solvent accessibility is lower for the shorter alkyl spacer, thus explaining the higher affinity of **2e** compared to **3e**. Contrarily, the more hydrophilic pyridine and pyrimidine moieties are less unfavorable with the longer chain, as they experience no unfavorable interactions with the aqueous solvent.

Conclusion

We have synthesized a series of arylpiperazine 5-HT_{1A} ligands (**2b**−**g** and **3b**−**g**) containing a novel heterotricyclic fragment. All the new compounds showed high in vitro affinity toward 5-HT_{1A} receptors. For some arylpiperazine substituents, very selective 5-HT_{1A} ligands were obtained. Compound **3b** was the most potent (K_i = 0.021 nM) and selective derivative for the 5-HT_{1A} receptor with respect to the other serotonin receptors; the most selective derivative for dopaminergic and adrenergic receptors was a CF_3-substituted arylpiperazine (**2e**). The binding data presented in this study identified the new fused norbornene nucleus as an optimal structural element to enhance 5-HT_{1A} receptor binding, although the oxyalkyl chain length and the nature of the substituent on the N-4 piperazine ring play an important role in determining affinity and selectivity. In accordance with the biological results, unconstrained molecular dynamics simulations showed a very stable orientation and position of the norbornene part, emphasizing its favorable properties as an anchoring group. Additionally, the major features of the experimental binding potencies for the different substituents could be rationalized: the exceptionally high affinity of compound **3b** is probably due to its unique ability to form a hydrogen bond between the methoxy substituent and Asn-386 of the receptor. For the other derivatives, solvent accessibility and hydrophobic interactions with the receptor are decisive.

Experimental Section

Chemistry. All reagents and substituted piperazines were commercial products purchased from Aldrich. Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AMX-500 instrument. Unless otherwise stated, all spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm using Me_4Si as an internal standard. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), m (multiplet), and mm (multiplet of multiplet). Mass spectra of the final products were performed on a LCQ Thermoquest-Ion trap mass spectrometer. Where analyses are indicated only by the symbols of the elements, results obtained are within ±0.4% of the theoretical values. All reactions were followed by thin-layer chromatography (TLC), carried out on Merck silica gel 60 F_{254} plates with a fluorescent indicator, and the plates were visualized with UV light (254 nm). Preparative chromatographic purifications were performed using a silica gel column (Kieselgel 60). Solutions were dried over Na_2SO_4 and concentrated with a Buchi rotary evaporator at low pressure.

4-(2-Chloroethoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2a). A solution of absolute ethanol (50 mL) and 0.72 g (0.018 mol) of sodium hydroxide was reacted with 3.22 g (0.018 mol) of commercially available endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide and 2.58 g (0.018 mol) of 1-bromo-2-chloroethane at 70 °C for 24 h. Afterward, the mixture was cooled to room temperature and concentrated to dryness, and the residue was diluted in water (40 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (diethyl ether/ethanol 9:1 (v/v)). The combined and evaporated product fractions were crystallized from diethyl ether/hexane, yielding 3.5 g (80%) of white solid. Mp: 56−58 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.50 (d, 1H, J = 9.1); 1.76 (dt, 1H, J = 9.1); 3.18 (dd, 2H, J = 1.4, 2.8); 3.42 (s, 2H); 3.58 (t, 2H, J = 6.4); 4.09 (t, 2H, J = 5.9); 6.16 (t, 2H, J = 1.8).

4-(3-Chloropropoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3a). A solution of absolute ethanol (50 mL) and 0.72 g (0.018 mol) of sodium hydroxide was reacted with 3.22 g (0.018 mol) of commercially available endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide and 2.83 g (0.018 mol) of 1-bromo-3-chloropropane at 70 °C for 24 h. Afterward, the mixture was cooled to room temperature and concentrated to dryness, and the residue was diluted in water (40 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (diethyl ether/ethanol 9:1 (v/v)). The combined and evaporated product fractions were crystallized from diethyl ether/hexane, yielding 4.30 g (94%) of the desired product as a white solid. Mp: 59−61 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.50 (d, 1H, J = 9.1); 1.76 (dt, 1H, J = 9.1); 2.16 (q, 2H, J = 6.2); 3.18 (dd, 2H, J = 1.4, 2.8); 3.42 (s, 2H); 3.58 (t, 2H, J = 6.4); 4.09 (t, 2H, J = 5.9); 6.16 (t, 2H, J = 1.8).

General Procedure for the Condensation of 4-Substituted Arylpiperazines with Derivatives 2a and 3a. A mixture of 4-(2-chloroethoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2a**) or 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**3a**) (0.006 mol) and NaI (0.009 mol) was stirred under reflux for 30 min. Then, the appropriate 4-substituted arylpiperazine (0.03 mol) and anhydrous K_2CO_3 (0.009 mol) were added. The reaction mixture was stirred under reflux for 24 h. After cooling, the mixture was filtered and concentrated to dryness, and the residue was dissolved in water (50 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The crude mixtures were purified by silica gel column chromatography using diethyl ether/methanol 8:2 (v/v) as the eluent. The crude products were recrystallized from diethyl ether.

4-[2-[4-(*o*-Methoxyphenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2b) was synthesized from **2a** and *o*-methoxyphenylpiperazine. Yield: 70%. Mp: 96−98 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.48 (d, 1H, J = 8.9); 1.75 (d, 1H, J = 8.9); 2.72 (bs, 4H, $2CH_2$ pip); 2.74 (t, 2H, N^1-CH_2, J = 7.3); 3.07 (bs, 4H, $2CH_2$ pip); 3.17 (s, 2H); 3.42 (s, 2H); 3.84 (s, 3H, $-OCH_3$); 4.11 (t, 2H, O-CH_2, J = 6.5); 6.16 (s, 2H); 6.84−7.00 (mm, 4H, ArH). ESI-MS: 397.9 $[M + H]^+$. Anal. ($C_{22}H_{27}N_3O_4$), C, H, N.

4-[2-[4-(*o*-Chlorophenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2c) was synthesized from **2a** and *o*-chlorophenylpiperazine. Yield: 46%. Mp: 111−113 °C. ESI-MS: 402.0 $[M + H]^+$; 424.0 $[M + Na]^+$; 440.0 $[M + K]^+$. Anal. ($C_{21}H_{24}ClN_3O_3$), C, H, N.

4-[2-[4-(*m*-Chlorophenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2d) was synthesized from **2a** and *m*-chlorophenylpiperazine. Yield: 31%. Mp: 122−124 °C. ESI-MS: 402.0 $[M + H]^+$; 424.0 $[M + Na]^+$. Anal. ($C_{21}H_{24}ClN_3O_3$), C, H, N.

4-[2-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]-ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2e) was synthesized from **2a** and *m*-trifluoromethylphenylpiperazine. Yield: 54%. Mp: 113−115 °C. ESI-MS: 435.9 $[M + H]^+$; 458.0 $[M + Na]^+$; 473.9 $[M + K]^+$. Anal. ($C_{22}H_{24}F_3N_3O_3$), C, H, N.

4-[2-[4-(Pyrimidyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (2f) was synthesized from **2a** and 1-(2-pyrimidyl)piperazine. Yield: 41%. Mp: 132−134 °C. ESI-MS: 370.0 $[M + H]^+$; 392.1 $[M + Na]^+$; 408.0 $[M + K]^+$. Anal. ($C_{19}H_{23}N_5O_3$), C, H, N.

4-[2-[4-(Pyridyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (2g) was synthesized from **2a** and 1-(2-pyridyl)piperazine. Yield: 37%. Mp: 117−119 °C. ESI-MS: 369.1 $[M + H]^+$; 391.0 $[M + Na]^+$; 407.0 $[M + K]^+$. Anal. ($C_{20}H_{24}N_4O_3$), C, H, N.

4-[3-[4-(*o*-Methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3b) was synthesized from **3a** and *o*-methoxyphenylpiperazine. Yield: 45%. Mp: 121−123 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.49 (d, 1H,

J = 8.9); 1.75 (d, 1H, J = 8.9); 1.85 (q, 2H, $-CH_2-$, J = 6.6, 7.3); 2.55 (t, 2H, N^1-CH_2, J = 7.3); 2.64 (bs, 4H, $2CH_2$ pip); 3.07 (bs, 4H, $2CH_2$ pip); 3.18 (s, 2H); 3.42 (s, 2H); 3.85 (s, 3H, $-OCH_3$); 4.02 (t, 2H, O-CH_2, J = 6.5); 6.16 (s, 2H); 6.84−7.00 (mm, 4H, ArH). ESI-MS: 412.0 $[M + H]^+$; 434.1$[M + Na]^+$; 450.1$[M + K]^+$. Anal. ($C_{23}H_{29}N_3O_4$), C, H, N.

4-[3-[4-(*o*-Chlorophenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3c) was synthesized from **3a** and *o*-chlorophenylpiperazine. Yield: 52%. Mp: 98−100 °C. ESI-MS: 415.9 $[M + H]^+$. Anal. ($C_{22}H_{26}ClN_3O_3$), C, H, N.

4-[3-[4-(*m*-Chlorophenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3d) was synthesized from **3a** and *m*-chlorophenylpiperazine. Yield: 30%. Mp: 110−112 °C. ESI-MS: 416.2 $[M + H]^+$; 438.0 $[M + Na]^+$. Anal. ($C_{22}H_{26}ClN_3O_3$), C, H, N.

4-[3-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3e) was synthesized from **3a** and *m*-trifluoromethylphenylpiperazine. Yield: 60%. Mp: 117−119°C. ESI-MS: 450.1 $[M + H]^+$. Anal. ($C_{23}H_{26}F_3N_3O_3$), C, H, N.

4-[3-[4-(Pyrimidyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3f) was synthesized from **3a** and 1-(2-pyrimidyl)piperazine. Yield: 32%. Mp: 78−81 °C. ESI-MS: 383.9 $[M + H]^+$; 406.0 $[M + Na]^+$; 422.0 $[M + K]^+$. Anal. ($C_{20}H_{25}N_5O_3$), C, H, N.

4-[3-[4-(Pyridyl)piperazin-1-yl]propoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (3 g) was synthesized from **3a** and 1-(2-pyridyl)piperazine. Yield: 55%. Mp: 96−98 °C. ESI-MS: 383.0 $[M + H]^+$. Anal. ($C_{21}H_{26}N_4O_3$), C, H, N.

Molecular Modeling. All calculations were performed with the DISCOVER program (Accelrys, San Diego, CA) on Silicon Graphics O2 R5000 computers (SGI, Mountain View, CA). The force field CVFF with a time step of 1 fs and a cutoff of 12 Å for the nonbonded interactions was employed for all calculations. A distance-dependent dielectric constant $\epsilon = 4r$ (Å) was used for mimicking the dielectric properties inside the protein molecule. For the structure of the 5-HT$_{1A}$ receptor, we choose the model of Lopez-Rodriguez et al.[26] because it agrees very well with the model of Strzelczyk et al.[27] for the transmembrane helices. Both of these models, like most recent GPCR models, are based on the X-ray structure of bovine rhodopsin[29] (PDB entry 1F88). Because the simulation system completely lacks the membrane environment, artificial constraints had to be introduced to prevent the receptor structure from disintegrating. Restraining backbone atoms of the receptor model to their initial positions with a force constant of 1000 kcal Å^{-2} led to root mean squared (rmsd) values for these atoms of ∼0.5 Å at a simulated temperature of 500 K (∼1.2 Å for all heavy atoms).

The ligand (**3b** or **2b**) was manually placed inside the receptor close to the putative binding position. In the first part of the calculations, additional constraints were used for achieving the correct binding mode by favoring known interactions of arylpiperazines with the 5-HT$_{1A}$ receptor. These additional constraints were based on experimental data from mutation studies and are in agreement with previous modeling studies.[26,27,30−32] Specifically, three hydrogen bonds were enforced via constraints for the O−H distance with the upper limit of 2.5 Å and a corresponding force constant of 10 kcal Å^{-2}. The first hydrogen bond was placed between the side chain carboxylate of Asp-116 (3.32 according to the nomenclature of Ballesteros and Weinstein[28]) and the protonated amine of the piperazine ring. This interaction is generally accepted to exist and has been used in previous modeling studies.[26,27,30−32] A distance restraint between one carbonyl oxygen at the pyrrole ring and the side chain hydroxy group of Ser-199 (5.42) was used to allow hydrogen bonding of the ligand carbonyl to the receptor residue Ser-199 (5.42), as previously observed.[26,30−32] The other carbonyl oxygen of the pyrrole ring is expected to form a hydrogen bond to the hydroxy group of Thr-121 (3.37).[26] The initial conformation of the piperazine ring was chosen according to the X-ray structures of the arylpiperazines of the Cambridge Structural Database (CSD).[33] All of the 22 arylpiperazines of the CSD exhibited the same conformation for the piperazine ring, and this conformation did not change during our calculations. The pyrrole ring can be flipped by 180° relative to the alkyl chain with a corresponding switch of hydrogen bonds. Initial simulations revealed a preference for the conformation depicted in Figures 2 and 3, however, without consequences for the binding mode.

In the first step, the model complex consisting of the transmembrane part of the 5-HT$_{1A}$ receptor and the respective ligand was energy minimized to accommodate the additional constraints between receptor and ligand that were described above. Subsequently, the tethering of the receptor backbone was removed in the vicinity of the ligand to allow for an induced fit. Amino acid residues of the receptor that had at least one atom closer than 5 Å to the ligand (meaning any atom of the ligand) were allowed to move without artificial constraints. Thus, a flexible binding site consisting of Cys-109 (3.25), Phe-112 (3.28), Ile-113 (3.29), Asp-116 (3.32), Val-117 (3.33), Cys-120 (3.36), Thr-121 (3.37), Ser-199 (5.42), Thr-200 (5.43), Ala-203 (5.46), Phe-204 (5.47), Trp-358 (6.48), Phe-361 (6.51), Phe-362 (6.52), and Asn-386 (7.39) was constructed. Molecular dynamics simulated annealing of the complex was started at 10 K (for 10 ps). Coupling to a temperature bath of 300 K (time constant of 10 ps) led to gradual heating during 100 ps. Then, the coupling to the bath was strengthened (time constant of 1 ps for the remaining part of the simulation), and the temperature was increased to 500 K. After 100 ps at 500 K, the temperature was reduced again to 300 K for another 100 ps. Finally, the temperature was lowered to 0 K over 10 ps. Individual conformations were saved every 5 ps. After these preparative steps, the artificial constraints between receptor and ligand were removed, and a second MD run followed. The simulation temperature of 300 K was reached after two slow heating steps (time constant of 10 ps) from 10 to 100 K in 100 ps and from 100 to 300 K in another 100 ps. After 1 ns at 300 K, two slow cooling steps to 100 K in 100 ps and to 0 K in 100 ps completed the simulation.

In Vitro Receptor Binding. All the solvents and reagents used were for analysis (J. T. Baker, Deventer, Holland, and Sigma Chemical Co., St. Louis, MO). Male Sprague−Dawley rats were from Harlan Italy srl, Correzzana, Milan, Italy.

5-HT$_{1A}$ Binding Assay. These radioligand binding assays were performed following a published procedure.[34] Cerebral cortex from male Sprague−Dawley rats (180−220 g) was homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 22 °C) with a Polytron PT10, Brinkmann Instruments (setting 5 for 15 s), and the homogenate was centrifuged at 50 000g for 10 min. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37 °C, and centrifuged at 50 000g for 10 min. The final pellet was resuspended in 80 volumes of the Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. To each assay tube was added the following: 0.1 mL of the drug dilution (0.1 mL of distilled water if no competing drug was added), 0.1 mL of [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]-8-OH-DPAT) (170.0 Ci mmol^{-1}, Perkin-Elmer Life Sciences, Boston, MA) in the same buffer as above to achieve a final assay concentration of 0.1 nM, and 0.8 mL of resuspended membranes. The tubes were incubated for 30 min at 37 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters (Brandel Biomedical Research and Laboratories Inc., Gaithersburg, MD). The filters were washed twice with 5 mL of ice-cold Tris-HCl buffer, and the radioactivity bound to the filters was measured with a liquid scintillation spectrometer (Packard TRI-CARB 2000CA, Packard BioScience srl, Pero, Milan, Italy). Specific [^{3}H]-8-OH-DPAT binding was defined as the difference between binding in the absence and in the presence of 5-HT (10 μM).

5-HT$_{2A}$ and 5-HT$_{2C}$ Binding Assays. These radioligand binding assays were performed as previously reported by Herndon et al.[35] Briefly, frontal cortical regions of male Sprague−Dawley rats (180−220 g) were dissected on ice, homogenized (1:10 w/v) in an ice-cold buffer solution (50 mM

Tris HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4) with a Polytron PT10 (setting 5 for 15 s), and centrifuged at 3000*g* for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37 °C for 15 min, and then centrifuged twice more at 3000*g* for 10 min (with resuspension between centrifugations). The final pellet was resuspended in buffer that also contained 0.1% ascorbate and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0 mL volume containing 5 mg of wet weight of tissue and 0.4 nM [^{3}H] ketanserin hydrochloride (88.0 Ci mmol^{-1}, Perkin-Elmer Life Sciences, Boston, MA) for 5-HT_{2A} receptors and 10 mg of wet weight of tissue and 1 nM [^{3}H]mesulergine (87.0 Ci mmol^{-1}, Amersham Biosciences Europe GmbH) for 5-HT_{2C} receptors. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT_{2A} assay. In the 5-HT_{2C} assays, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block binding to 5-HT_{2A} receptors. Tubes were incubated for 15 min at 37 °C, filtered on Schliecher and Schuell (Keene, NH) glass fiber filters presoaked in polyethylene imine, and washed with 10 mL of ice-cold buffer. Filters were counted at an efficiency of 50%.

Dopaminergic D_1 Binding Assay. The binding assay for dopaminergic D_1 receptors was that described by Billard et al.[36] Corpora striata were homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL of [^{3}H]SCH-23390 (85.0 Ci mmol^{-1}, Perkin-Elmer Life Sciences, Boston, MA), to achieve a final concentration of 0.4 nM, and 900 μL of resuspended membranes (3 mg of fresh tissue). The tubes were incubated for 15 min at 37 °C, and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured with a liquid scintillation counter. Specific [^{3}H]SCH-23390 binding was defined as the difference between binding in the absence and in the presence of 0.1 μM piflutixol.

Dopaminergic D_2 Binding Assay. The procedure used in this radioligand binding assay was reported in detail by Creese et al.[37] Corpora striata were homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL of [^{3}H]spiperone (15.7 Ci mmol^{-1}, Perkin-Elmer Life Sciences, Boston, MA), to achieve a final concentration of 0.4 nM, and 900 μL of resuspended membranes (3 mg of fresh tissue). The tubes were incubated for 15 min at 37 °C, and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]-spiperone binding was defined as the difference between binding in the absence and in the presence of 1 μM (+)-butaclamol.

Adrenergic α_1 Binding Assay. The procedure used in this radioligand binding assay has been reported in detail by Greengrass and Bremner.[38] Brain cortex was homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl (pH 7.4 at 25 °C). Each assay tube contained 50 μL of drug solution, 50 μL of [^{3}H]prazosin (80.5 Ci mmol^{-1}, Perkin-Elmer Life Sciences, Boston, MA), to achieve a final concentration of 0.4 nM, and 900 μL of resuspended membranes (10 mg of fresh tissue). The tubes were incubated for 30 min at 25 °C, and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured with a liquid scintillation counter. Specific [^{3}H]prazosin binding was defined as the difference between binding in the absence and in the presence of 10 μM phentolamine.

Adrenergic α_2 Binding Assay. The procedure used in this radioligand binding assay was reported in detail by Perry and U'Prichard.[39] Brain cortex was homogenized in 30 volumes (w/v) of ice-cold 5 mM Tris-HCl/5mM EDTA buffer (pH 7.3 at 25 °C) using a polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged three times for 10 min at 50 000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl/0.5 mM EDTA (pH 7.5 at 25 °C). Each assay tube contained 50 μL of drug solution, 50 μL of [^{3}H]yohimbine (80.5 Ci mmol^{-1}, Perkin-Elmer Life Sciences, Boston, MA), to achieve a final concentration of 1 nM, and 900 μL of resuspended membranes (10 mg of fresh tissue). The tubes were incubated for 30 min at 25 °C, and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl/0.5 mM EDTA buffer (pH 7.5 at 25 °C). The radioactivity bound to the filters was measured with a liquid scintillation counter. Specific [^{3}H]yohimbine binding was defined as the difference between binding in the absence and in the presence of 10 μM phentolamine.

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Supporting Information Available: Spectroscopic data of final compounds **2c**−**g** and **3c**−**g** and results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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