Synthesis and Structure−Activity Relationships of 1-Aralkyl-4-Benzylpiperidine and 1-Aralkyl-4-Benzylpiperazine Derivatives as Potent σ Ligands

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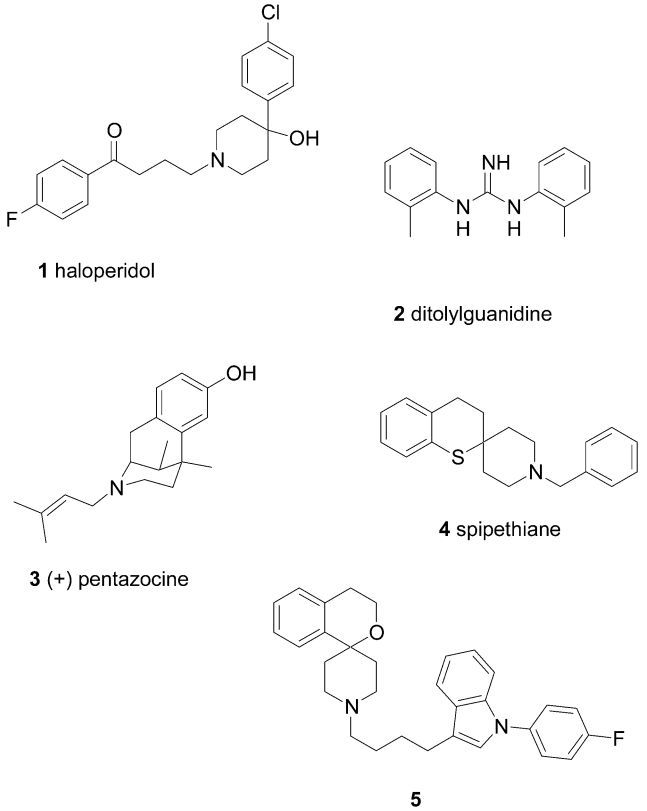
In the attempt to define more accurately structure−affinity relationships for σ_1 and σ_2 ligands, we synthesized and tested on σ subtype receptors a series of aralkyl derivatives of 4-benzylpiperidine, in which the effect of modifications on the aralkyl moiety was studied in a systematic way. The affinity of the compounds here described varied to a great extent, with a σ_2/σ_1 selectivity ranging from 0.1 to 9. Thus, to confirm the ability of the piperazine derivative to bind to σ_1 receptors in a different way than piperidines, we synthesized and tested a series of piperazine compounds; the comparison of their affinity with that of the corresponding piperidines strongly supports the possibility of a different binding mode. While the compounds here described are on the whole selective for σ vs serotonin 5-HT_{1A} and dopamine D_2 receptors, **9aa**, **9ba** and **9ab** possess a remarkable affinity for both σ and 5-HT_{1A} receptors, with K_i in the nanomolar range, and are selective with respect to D_2 receptors. They displayed also a partial agonist profile in a human 5-HT_{1A} [^{35}S]GTPγS binding assay, suggesting their potential use as atypical antipsychotic agents.

Introduction

Sigma receptors were first described by Martin as a subtype of opioid recptors;[1] now it is known that these receptors are a distinct class, divided into two subclasses, σ_1 and σ_2.[2] Several functions for σ_1 receptors have been discovered; among these there are modulatory roles on K^+ and Ca^{2+} channels and on dopaminergic, NMDA, serotoninergic, muscarinic neurotransmission, while σ_2 receptors are involved in regulation of cell proliferation and maintenance of cell viability, and may contribute to the acute motor side effects of antipsychotic drugs;[2,3] however, this last activity was attributed to σ_1 sites too.[4]

Several classes of structurally unrelated compounds interact with σ receptors.[5,6] Both subtypes have high to moderate affinity for typical neuroleptics, with haloperidol (**1**, Chart 1) exhibiting the highest affinity for both sites.[2] Other high-affinity σ ligands are ditolylguanidine (**2**), (+) pentazocine (**3**), and compound **4**, a very potent and selective ligand for σ_1 receptors (σ_1 K_i = 0.5 nM; σ_2 K_i = 416 nM).[7] While several selective, high-affinity σ_1 ligands are available, σ_2 ligands are not characterized to the same extent. Spiro[2]benzopyran-1,4′-piperidine **5** (Chart 1) is the most potent and σ_2 selective ligand described so far (σ_1, IC_{50} = 53 nM; σ_2, IC_{50} = 0.90 nM).[8] There is great interest in the development of selective σ_2 ligands: it appears that this receptor subtype is involved in regulation of cell proliferation and maintenance of cell viability, suggesting a therapeutic use as novel antineoplastic agents. Moreover, σ_2 receptor antagonists have been demonstrated to limit the motor extrapyramidal side effects caused by typical antipsychotic agents.[2,3]

Chart 1



1 haloperidol

2 ditolylguanidine

3 (+) pentazocine

4 spipethiane

5

Substituted 4-benzylpiperidine and 1-benzylpiperazine derivatives were shown to be high-affinity σ ligands (K_i in the nanomolar range) with a slight preference for

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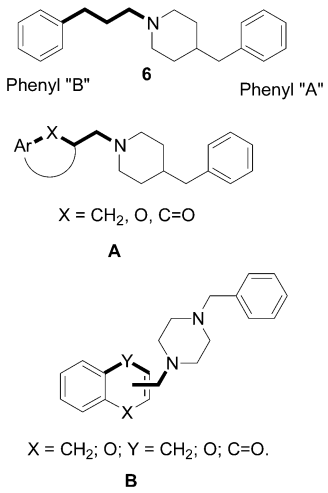
Chart 2



σ_1 over σ_2 receptors.[9−11] One of the proposed pharmacophores for σ_1 binding includes an amine site, flanked by two hydrophobic domains, the primary hydrophobic site that bind phenyl "B" and a secondary binding site that bind phenyl "A" (see compound **6**, Chart 2). This pharmacophoric model has been proposed for phenylalkylamines as σ_1 ligands,[10,11] which is able to accommodate both phenylalkylpiperidines and phenylalkylpiperazines. Where two N atoms occur in a compound, such as in piperazine derivatives, the N atom attached to the longer chain is more important than the other for the binding to this receptor subtype.[11]

Recently, a great number of arylalkylbenzylpiperazine derivatives have been synthesized. Some compounds showed a remarkable affinity and a strong selectivity toward serotonin 5-HT_{1A} and dopamine D_2 receptors, but the selectivity among σ receptor subtypes was not studied, despite the affinity in the nanomolar range of several of the reported compounds, and no clear SAR was established.[13,14]

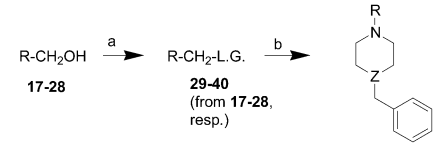
Thus, in the attempt to define structure−affinity relationships for 4-benzylpiperidine derivatives as σ_1 and σ_2 ligands, we synthesized and tested on σ subtype receptors our lead compound **6** (Chart 2), which demonstrated good affinity for both σ_1 and σ_2 receptor subtypes (σ_1 K_i = 0.4 nM; σ_2 K_i = 3.3 nM).[11] Then we synthesized and tested the less symmetrical compounds of general formula A (Chart 2), in the attempt to optimize the interactions with the "phenyl B" site and to avoid the binding in the flipped mode. In these compounds the chain of three atoms connecting the nitrogen of the piperidine and the aromatic ring of the lead **6** was mantained and the effect of modifications was studied in a systematic way. Moreover, a methoxy group was inserted on the available positions of the aromatic moiety (Ar in the General formula A, Chart 2) in order to test the space around it; this substitution could be of great interest, since a strong effect depending on the position of a methoxy group was observed in a series of (tetralinopropyl)piperazines σ ligands.[15]

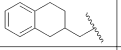
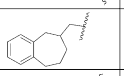
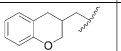
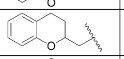
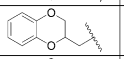
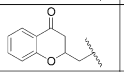
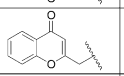
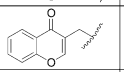
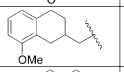
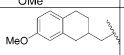
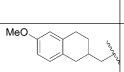
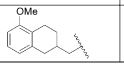
In addition, we synthesized and tested a series of piperazine derivatives of general formula B (Chart 2), to compare their affinity with that of the corresponding piperidines.

The compounds were then tested for binding at σ_2 and also to serotonin 5-HT_{1A} and dopamine D_2 receptors, to determine their selectivity. This aspect is particularly important, since piperidine/piperazine derivatives are known to bind at σ and also at 5-HT_{1A} and D_2 receptors.[9,16]

All the chiral compounds were tested as racemates.

Scheme 1[a]



Comp.	R	Comp.	Yield %	L.G.[c]	Z	Comp.	Yield %
17		**29**	26	Ts	CH N	**7ab** **7bb**	82 83
18		**30**	90	Ts	CH	**7ac**	75
19		**31**	86	Ts	CH N	**8aa** **8ba**	99 66
20		**32**	98	Ts	CH N	**9aa** **9ba**	94 99
21		**33**	91	Ts	CH N	**9ab** **9bb**	94 99
22		**34**	64	Ts	CH N	**9ac** **9bc**	24 31
23		**35**	73	-Cl	CH N	**9ad** **9bd**	81 81
24		**36**	96	-Cl	CH N	**10ac** **10bc**	93 87
25		**37**	88	Ts	CH	**13**	69
26		**38**	79	Ts	CH	**14**	72
27		**39**	77	Ts	CH	**15**	55
28		**40**	95	Ts	CH	**16**	67

[a] (a) $SOCl_2$ (for **23** and **24**) or TsCl (for **17**−**22** and **25**−**28**); (b) 4-benzylpiperidine or 1-benzylpiperazine; (c) L.G. Leaving group.

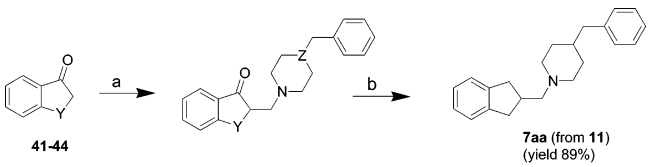
Chemistry

Compounds **9ad, 9bd, 10ac**, and **10bc** were prepared by the reaction of the appropriate alkyl chloride **35** and **36** with 4-benzylpiperidine or 1-benzylpiperazine (Scheme 1); the other target compounds reported in Scheme 1 (**7ab**−**7ac**, **8aa**, **8ba**, **9aa**, **9ba**, **9ab**, **9bb**, **9ac**, **9bc**, **13**−**16**) were prepared in an analogous way, but in this case the alcohols **17**−**22** and **25**−**28** were converted into the corresponding tosylates (Scheme 1).

Compounds **10aa**, **10ab, 10ba**, **10bb**, **11**, and **12** were prepared by the Mannich reaction (Scheme 2); reduction of **11** by means of H_2, Pd/C afforded **7aa** (Scheme 2).

Compounds **8ab** and **8bb** were prepared according to Scheme 3. The β-benzoylpropionic acid **45** was converted into Mannich bases **46** and **47**. The subsequent reduction of the carbonyl group adjacent to the aromatic ring was performed with hydrogenolysis in acidic media, to afford **48** and the debenzylated product **49**. Compounds **48** and **49** were then cyclized with polyphosphoric acid to afford **8ab** and **50**; this last compound was then converted into the final product **8bb** by the treatment with 1 equiv of benzyl bromide.

Scheme 2[a]



Comp.	Y	Z	Comp.	Yield %
41	-CH_2CH_2-	CH	**10aa**	42
		N	**10ba**	22
42	-OCH_2-	CH	**10ab**	3
		N	**10bb**	25
43	-CH_2-	CH	**11**	66
44	-$CH_2CH_2CH_2$-	CH	**12**	77

[a] (a) Formaldehyde, 4-benzylpiperidine or 1-benzylpiperazine; (b) H_2, Pd/C.

Results and Discussion

The synthesized 4-benzylpiperidine derivatives (Table 1) were evaluated for their affinity at both σ_1 and σ_2 receptors as well as for 5-HT_{1A} and D_2 receptors. Most of the compounds exhibited high affinity (in the nanomolar range) at σ receptor subtypes and possess practically no affinity at both 5-HT_{1A} and D_2 receptors.

Compound **6**, here considered as a lead, showed a good σ affinity in our experimental assays, even if the selectivity σ_2/σ_1 is slightly different than that reported in the literature (σ_1 $K_i = 0.4$ nM, σ_2 $K_i = 3.3$ nM, ref 11; σ_1 $K_i = 1.40$ nM, σ_2 $K_i = 0.49$ nM, Table 1).

The affinities of the compounds showed that, compared to the lead **6**, the rigidification of the propyl chain within a five-membered ring (compound **7aa**) or within a six-membered ring (compound **7ab**) showed no influence on σ_1 affinity; however, it should be noted that the presence of a five-membered ring lowers the affinity 25 times for σ_2 receptor subtype, an effect not present in the compound carrying a six-membered ring. On the other hand, the presence of a seven-membered ring (compound **7ac**) showed lower affinity on both σ_1 and σ_2 receptors than the other two compounds.

On the basis of the observed affinities, it was decided to modify the tetrahydronaphthalene moiety of **7ab**, first modifying the ring and then modifying the propyl chain connecting the aromatic ring and the nitrogen.

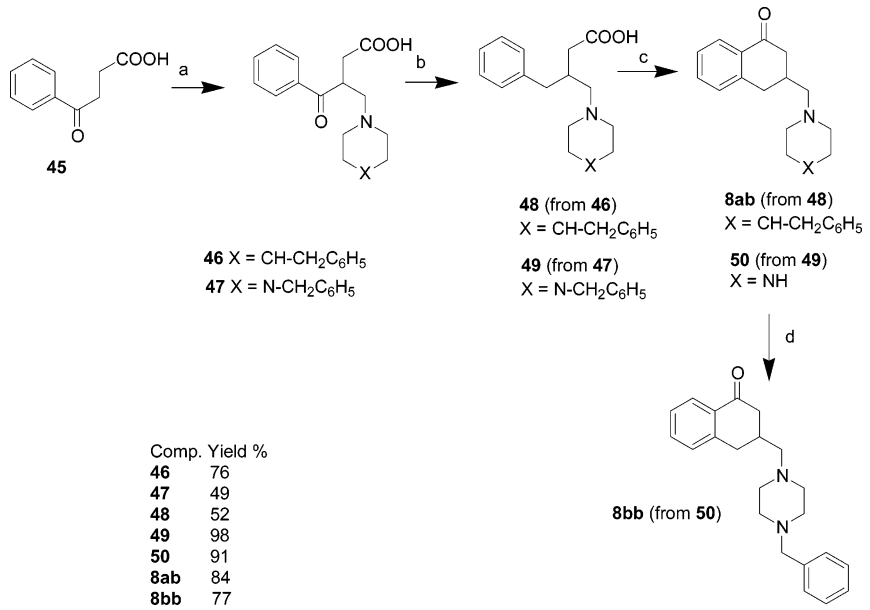
The presence of an oxygen atom or of a carbonyl (compounds **8aa**, **8ab**) in place of X does not influence the affinity of **7ab**.

The presence of an oxygen in place of the CH_2 of the propyl chain at the position adjacent to the aromatic ring (compounds **9aa**, **9ab**) lowers the affinity; in fact, **7ab** and **8aa** are 1 order of magnitude better ligands than the corresponding oxygen isosters; however, the affinity is strongly influenced by other substituents. In fact, while compound **9ac** showed an affinity similar to its CH_2 counterpart **8ab**, compound **9ad**, which possesses a carbonyl and the double bond, showed a marked decrease in affinity for both σ_1 and σ_2 receptor subtypes.

The presence of a carbonyl group on the propyl chain at the position adjacent to the aromatic ring is negative especially for the affinity to σ_1 receptors (compounds **10aa**, **10ab**, and **10ac**). From the data obtained for compounds **11** and **12**, it seems that a carbonyl adjacent to the aromatic ring is tolerated only if it is inserted in a ring greater than cyclopentane; evidence of this hypothesis is the lack of affinity for both σ_1 and σ_2 of compound **11**, while **12** maintains a discrete affinity (Table 1). The compounds **10ab**, **10ac**, and, in particular, **10aa** were selective among σ receptor subtypes (σ_2/σ_1 of 0.5, 0.2, and 0.1, respectively).

Moreover, a methoxy group was inserted on the benzene ring of the tetrahydronaphthalene moiety of the piperidine compound (**7ab**) in order to test the space around it. The effect exerted by the methoxy group is in accordance with that reported in ref 15, where a detrimental presence of a 5- and 7-methoxy substituent was observed, even if this effect is influenced by the length of the chain connecting the aromatic moiety to the basic nitrogen. In this case, the negative effect was limited to the presence of a 5-methoxy group (compound **13**). The 8-substituted derivative (compound **16**) showed also a decrease in affinity, but this position was not examined earlier. The presence of a methoxy group

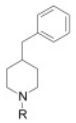
Scheme 3[a]

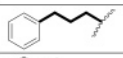
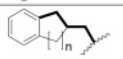
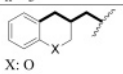
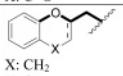
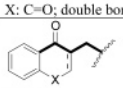
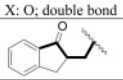
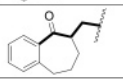
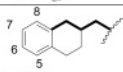


Comp.	Yield %
46	76
47	49
48	52
49	98
50	91
8ab	84
8bb	77

[a] (a) Formaldehyde, 4-benzylpiperidine or 1-benzylpiperazine; (b) H_2, Pd/C; (c) polyphosphoric acid; (d) benzyl bromide.

Table 1. Binding of 1-Aralkyl-4-benzylpiperidine Derivatives



Comp.	R	σ_1 Ki (nM)[a,b]	σ_2 Ki (nM)[a,c]	σ_2/σ_1[d]	5-HT$_{1A}$ Ki (nM)[a,e]	D_2 Ki (nM)[a,f]	D_2 % Inh. at 10 μM[f]
6		1.40	0.49	0.4	>1000	>1000	
7aa	 n = 1	1.40	12.2	9	>1000	575	
7ab	n = 2	1.50	2.52	2	172	1145	74.7
7ac	n = 3	28.7	47.3	2	>1000	>1000	
8aa	 X: O	2.50	5.98	2	226	2493	72.2
8ab	X: C=O	1.40	4.63	3	>1000	N.D.[g]	9.61
9aa	 X: CH_2	11.6	4.80	0.4	11.5	924	97.5
9ab	X: O	16.2	28.4	2	17.6	447	92.5
9ac	X: C=O	1.40	7.90	6	297	N.D.	33.4
9ad	X: C=O; double bond	115	285	2	>1000	N.D.	21.7
10aa	 X: CH_2	24.0	3.38	0.1	260	1093	84.5
10ab	X: O	700	370	0.5	120	N.D.	60.5
10ac	X: O; double bond	100	21.5	0.2	>1000	N.D.	45.0
11		1606	>1000		>1000	>1000	
12		36.4	89.3	2	>1000	>1000	
13	 5-OCH_3	83.4	9.9	0.1	>1000	>1000	
14	6-OCH_3	11.9	30.6	3	>1000	>1000	
15	7-OCH_3	9.4	11.0	1	54.2	>1000	
16	8-OCH_3	36.3	75.0	2	57.2	866	
DTG		69.0	21.0				
haloperidol		2.20	16.0			2.69	
spiperone						0.42	
(+) butaclamol						3.61	
risperidone						6.65	
WAY-100635					0.6		

[a] K_i values agreed to ±20%. [b] Binding assays were performed using 3.0 nM [^{3}H]pentazocine. [c] Binding assays were performed using 3.0 nM [^{3}H]ditolylguanidine. [d] K_i ratio. [e] Binding assays were performed using 1.2 nM [^{3}H]-8-OH-DPAT. [f] Binding assays were performed using 0.2 nM [^{3}H]spiperone. [g] N.D., not determined.

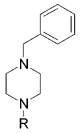
influences also the selectivity σ_2/σ_1 (changing from 0.1 for compound **13** to 3, 1, and 2 for compounds **14**, **15**, and **16**, respectively); thus its presence at position 5 of the tetrahydronaphthalene moiety favors σ_2 affinity.

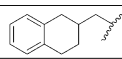
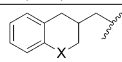
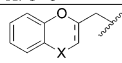
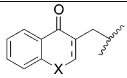
Then, the corresponding benzylpiperazines were studied (Table 2). On the whole, it should be noted that, in agreement with that reported in previous works,[11,13] piperazine derivatives bind to σ receptor subtypes with stronger affinity with respect to the corresponding piperidine derivatives and are better ligands for σ_1 than σ_2 receptors (see Tables 1 and 2).

Moreover, while among piperidine derivatives the nature of the heterocyclic substituent influences markedly the affinity (**10ab**, σ_1, K_i = 700 nM; σ_2, K_i 370 nM), the corresponding piperazine derivative is a high-affinity σ ligand (**10bb**, σ_1 K_i of 18.0, σ_2 K_i of 32.8 nM); the same effect is shared by compounds **9ad** and **10ac** (compare with **9bd** and **10bc**, respectively).

The selectivity vs σ_2 receptor subtype is not influenced by the nature of the propyl chain adjacent to the aromatic ring here considered but depends on the nature of the ring in which the propyl chain is inserted.

Table 2. Binding of 1-aralkyl-4-benzylpiperazine Derivatives



Comp.	R	σ_1 Ki (nM)[a,b]	σ_2 Ki (nM)[a,c]	σ_2/σ_1[d]	5-HT$_{1A}$ Ki (nM)[a,e]	D_2 Ki (nM)[a,f]	D_2 % Inh. at 10 μM[f]
7bb		0.80	1.70	2	185	N.D.[g]	69.0
8ba	 X: O	0.30	1.48	5	>1000	N.D.	29.0
8bb	X: C=O	0.30	1.59	5	>1000	N.D.	48.7
9ba	 X: CH_2	0.30	3.02	10	20.7	N.D.	64.7
9bb	X: O	15.4	25.6	2	69.4	N.D.	44.0
9bc	X: C=O	1.20	4.75	4	>1000	N.D.	3.8
9bd	X: C=O; double bond	2.66	5.35	2	>1000	N.D.	7.6
10ba	 X: CH_2	0.40	1.40	3	445	N.D.	63.2
10bb	X: O	18.0	32.8	2	N.D.	N.D.	N.D.
10bc	X: O; double bond	3.80	14.1	4	>1000	N.D.	3.9
DTG		69.0	21.0				
haloperidol		2.20	16.0			2.69	
spiperone						0.42	
(+) butaclamol						3.61	
risperidone						6.65	
WAY-100635					0.6		

[a] K_i values agreed to ±20%. [b] Binding assays were performed using 3.0 nM [^{3}H]pentazocine. [c] Binding assays were performed using 3.0 nM [^{3}H]ditolylguanidine. [d] K_i ratio. [e] Binding assays were performed using 1.2 nM [^{3}H]-8-OH-DPAT. [f] Binding assays were performed using 0.2 nM [^{3}H]spiperone. [g] N.D., not determined.

Table 3. Potency (pD_2) and Relative Effectiveness Values in the [^{35}S]GTPγS Binding Assay at 5-HT$_{1A}$ Human Cloned Receptors of Selected Compounds

	[^{35}S]GTPγS binding	
compd	pD_2	% E_{max}[a]
9aa	7.89	61.9
9ba	6.29	41.0
9ab	8.20	41.7

[a] E_{max}: maximal stimulation achieved expressed as a percentage of the maximal 5-HT response.

On the whole, the compounds here described showed a marked selectivity vs serotonin 5-HT$_{1A}$ and dopamine D_2 receptors. Only compounds **9aa**, **9ba**, and **9ab** (Tables 1 and 2) behave as high-affinity ligands for 5-HT$_{1A}$ receptors; these compounds were then tested for the evaluation of their potency (pD_2) and relative intrinsic activity in the [^{35}S]GTPγS binding assay (Table 3). The piperidine derivatives **9ab** and **9aa** were found to be potent, partial agonists at the h5-HT$_{1A}$ receptor, with a pD_2 of 8.20 (E_{max} 41.7%) and 7.89 (E_{max} 61.9%), respectively. The affinity for 5-HT$_{1A}$ could be particularly interesting, since this receptor subtype has been, as a therapeutic target for the development of improved antipsychotic drugs:[6,17] several clinically effective antipsychotics bind in vitro, with moderate to high affinity, proposed to cloned human 5-HT$_{1A}$ receptors.[18,19] Moreover, OPC-14523 (affinities for σ_1, σ_2, and

5-HT_{1A} receptors (IC_{50}, nM), 47, 56, and 2.3, respectively)[20] exerts an acute antidepressant-like action, and this pharmacological activity is achieved by the combined stimulation of σ and 5-HT_{1A} receptors.[20] In vivo assays will then be conducted in order to determine their potential as new therapeutic agents for the treatment of psychiatric disorders.

Conclusions

The modifications of the propyl chain of the lead compound **6** led to the 1-aralkyl-4-benzylpiperidines. These compounds showed a great variation in affinity for σ_1 receptors, ranging from compounds practically devoid of affinity to compounds with an affinity in the nanomolar range. Thus, useful information about the structural characteristics for an optimal interaction with the "phenyl B" site was obtained. The compounds showed also selectivity among σ receptor subtypes, with a σ_2/σ_1 selectivity ranging from 0.1 (compounds **10aa** and **13**) to 9. These results will be the basis of further modifications in order to obtain more selective compounds. The comparison of the affinities of these compounds with those of the corresponding 1-aralkyl-4-benzylpiperazines strongly supports the possibility of a different binding mode of the piperazine derivatives to σ receptors, as suggested by earlier literature. While the compounds here described are on the whole selective for σ vs serotonin 5-HT_{1A} and dopamine D_2 receptors, **9aa**, **9ba**, and **9ab** possess a remarkable affinity for both σ and 5-HT_{1A} receptors, with K_i in the nanomolar range. They displayed also a partial agonist profile in a human 5-HT_{1A} [^{35}S]GTPγS binding assay, suggesting their potential use as atypical antipsychotic agents.

Experimental Section

Chemistry. Melting points were determined on a Buchi 510 capillary melting point apparatus and are uncorrected. Elemental analyses were conducted at the Microanalysis Laboratory of the Dipartimento di Scienze Farmaceutiche, Modena University, and the results were within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported in ppm relative to TMS. Only representative ^{1}H NMR spectra were reported; see also the Supporting Informations. $CDCl_3$ was used as a solvent, unless otherwise noted. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck, 70−230 mesh) was used for column chromatography. The following were synthesized as previously reported: 2-[(4-benzylpiperidinyl)methyl]-chromone **9ad**[13] and 2-[(4-benzylpiperazinyl)methyl]chromone **9bd,**[13] 2-hydroxymethyl-1,2,3,4-tetrahydronaphthalene **17**,[21] 3-hydroxymethylchromane **19**,[22] 2-hydroxymethylchromane **20**,[23] 2-hydroxymethylchromanone **22**,[24] 3-(hydroxymethyl)-chromone **24**[25] and 3-(chloromethyl)chromone **36**,[25] and 6-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethanol **26**.[26] 5-Methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethanol **25**, 7-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl-methanol **27**, 8-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethanol **28**, and 6,7,8,9-tetrahydrobenzocycloheptan-6-ylmethanol **18** were synthesized in an analogous way, starting from the corresponding commercially available tetralones or benzosuberone, by reaction with ethyl formate in the presence of tBuOK and subsequent reduction with borane. The starting material for **28**, 8-methoxytetralone was not commercially available and was synthesized starting from 8-naphthosultone as previously reported.[27−29]

Synthesis of 3-[(4-Benzylpiperidinyl- (10ac) and -piperazinyl)methyl]chromone (10bc). General Procedure. A solution of 3-chloromethylchromone **36** (2.58 mmol) and 4-benzylpiperidine or 1-benzylpiperazine (25.8 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 15 min and then refluxed for 1 h. After cooling, EtOAc (20 mL) and a saturated solution of K_2CO_3 up to alkaline pH were added to the reaction mixture. The mixture was extracted with EtOAc (3 × 20 mL), the organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was then purified by column chromatography (CH_3OH/EtOAc 0.75/9.25).

3-[(4-Benzylpiperidinyl)methyl]chromone 10ac: ^{1}H NMR δ 8.22 (1H, m), 8.00 (1H, s), 7.63 (1H, m), 7.33 (7H, m), 3.45 (2H, s), 2.98 (2H, m), 2.56 (2H, d, J = 6.33), 2.08 (2H, m), 1.55 (5H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 227−228 °C (dec); IR (cm^{-1}) (Nujol) 1634. Anal. ($C_{22}H_{23}NO_2$·HCl) C, H, N.

3-[(4-Benzylpiperazinyl)methyl]chromone 10bc: ^{1}H NMR δ 8.26 (1H, m), 7.98 (1H, t, J = 1.0), 7.68 (1H, m), 7.35 (7H, m), 3.55 (2H, s), 3.54 (2H, d, J = 1.0), 2.56 (8H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 275−278 °C (dec); IR (cm^{-1}) (Nujol) 1647. Anal. ($C_{21}H_{22}N_2O_2$·2HCl) C, H, N.

Synthesis of Tosylates 29−34 and 37−40. General Procedure. To a solution of the hydroxymethyl derivatives **17−22** and **25−28** (3 mmol) in anhydrous pyridine (4 mL) at 0 °C and with stirring was added *p*-toluensulfonyl chloride (3 mmol). After 15 min, the cooling bath was removed and the reaction mixture was left for 15 h at room temperature with stirring. At the end of the reaction, the mixture was cooled, acidified with 2 N HCl, and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were dried (Na_2SO_4), the solvent removed under reduced pressure, and the residue purified by means of column chromatography (CH_2Cl_2 100%).

Synthesis of 2-[(4-Benzylpiperidinyl- (7ab, 7ac, 8aa, 9aa−9ac, 13−16) and -piperazinyl)] Derivatives (7bb, 8ba, 9ba, 9bb, 9bc). General Procedure. A solution of the tosylate **29−34** and **37−40** (1.5 mmol) and the corresponding 4-benzylpiperidine or 1-benzylpiperazine (4.5 mmol) in toluene (10 mL) was refluxed for 12 h. In the case of compounds **9ac** and **9bc**, CH_2Cl_2 was used instead of toluene; in the case of **9ab** and **9bb**, anhydrous THF was used. Then the solution was cooled, treated with a saturated solution of K_2CO_3 until alkaline pH was reached, and extracted with CH_2Cl_2 (4 × 20 mL). The organic phases were dried (Na_2SO_4), the solvent was removed by reduced pressure, and the residue thus obtained was purified by means of column chromatography (CH_2Cl_2/CH_3OH 9.5/0.5).

1-[2-(1,2,3,4-Tetrahydronaphthylmethyl)]-4-benzylpiperidine 7ab: ^{1}H NMR δ 7.21 (9H, m), 2.95 (4H, m), 2.60 (2H, d, J = 6.40), 2.43 (1H, m), 2.38 (2H, d, J = 6.95), 2.00 (4H, m), 1.45 (6H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 217−220 °C. Anal. ($C_{23}H_{29}N$·HCl) C, H, N.

4-Benzyl-1-(6,7,8,9-tetrahydrobenzocycloheptan-6-ylmethyl)piperidine 7ac. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 217−218 °C. Anal. ($C_{24}H_{31}N$·HCl) C, H, N.

1-[2-(1,2,3,4-Tetrahydronaphthylmethyl)]-4-benzylpiperazine 7bb: ^{1}H NMR δ 7.40 (5H, m), 7.06 (4H, m), 3.60 (2H, s), 2.90 (3H, m), 2.50 (8H, m), 2.41 (2H, d, J = 7.06), 2.06 (2H, m), 1.35 (2H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 280 °C (lit.[14] mp 191 °C). Anal. ($C_{22}H_{28}N_2$·2HCl) C, H, N.

3-[(4′-Benzylpiperidinyl)methyl]chromane 8aa. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl

was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 212−215 °C (dec). Anal. ($C_{22}H_{27}NO\cdot HCl$) C, H, N.

3-[(4′-Benzylpiperazinyl)methyl]chromane 8ba. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 280−282 °C (dec). Anal. ($C_{21}H_{26}N_2O\cdot 2HCl$) C, H, N.

2-[(4′-Benzylpiperidinyl)methyl]chromane 9aa. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 195−197 °C (dec). Anal. ($C_{22}H_{27}NO\cdot HCl$) C, H, N.

2-[(4′-Benzylpiperazinyl)methyl]chromane 9ba. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 288−290 °C (dec), (lit.[14] mp >300 °C). Anal. ($C_{21}H_{26}N_2O\cdot 2HCl$) C, H, N.

4-Benzyl-1-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-piperidine 9ab. The compound (2.65 mmol) was transformed into the corresponding oxalate salt by treatment with oxalic acid (5.3 mmol) in anhydrous diethyl ether (50 mL) and was purified by crystallization (ethanol): mp 172−175 °C. Anal. ($C_{21}H_{25}NO_2\cdot(COOH)_2$) C, H, N.

1-Benzyl-4-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-piperazine 9bb. The compound (2.65 mmol) was transformed into the corresponding oxalate salt by treatment with oxalic acid (5.3 mmol) in anhydrous diethyl ether (50 mL) and purified by crystallization (CH_3OH): mp 237−239 °C. Anal. ($C_{20}H_{24}N_2O_2\cdot 2(COOH)_2$) C, H, N.

2-[(4′-Benzylpiperidinyl)methyl]chromanone 9ac. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 200−203 °C (dec); IR (cm^{-1}) (Nujol) 1684. Anal. ($C_{22}H_{25}NO_2\cdot HCl$) C, H, N.

2-[(4′-Benzylpiperazinyl)methyl]chromanone 9bc. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 258−261 °C (dec); IR (cm^{-1}) (Nujol) 1693. Anal. ($C_{21}H_{24}N_2O_2\cdot 2HCl$) C, H, N.

4-Benzyl-1-(5-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethyl)piperidine 13. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 224−226 °C. Anal. ($C_{24}H_{31}NO\cdot HCl$) C, H, N.

4-Benzyl-1-(6-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethyl)piperidine 14. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyil ether: mp 237−240 °C. Anal. ($C_{24}H_{31}NO\cdot HCl$) C, H, N.

4-Benzyl-1-(7-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethyl)piperidine 15. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 211−214 °C. Anal. ($C_{24}H_{31}NO\cdot HCl$) C, H, N.

4-Benzyl-1-(8-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylmethyl)piperidine 16. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 207−209 °C. Anal. ($C_{24}H_{31}NO\cdot HCl$) C, H, N.

Synthesis of Mannich Bases 10aa, 10ab, 10ba, 10bb, 11, 12. General Procedure. A suspension of the carbonyl compound **41**−**44** (12.9 mmol), 4-benzylpiperidine, or 1-benzylpiperazine (12.9 mmol) and paraformaldehyde (39.3 mmol) in absolute EtOH saturated with HCl (4.0 mL) was refluxed for 7 h. After cooling, the solvent was removed under reduced pressure, and the residue was treated with water, acidified (concentrated HCl), and extracted with diethyl ether (5 × 15 mL). The aqueous phase was then basified (2 N NaOH) and extracted with diethyl ether (3 × 30 mL). The organic phases thus obtained were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue thus obtained was purified by means of column chromatography (CH_2Cl_2/CH_3OH 9.5/0.5). Representative ^1H NMR spectra were reported.

2-(4-benzylpiperidin-1-ylmethyl)-1-tetralone 10aa: ^1H NMR δ 8.05 (1H, m), 7.41 (1H, m), 7.20 (7H, m), 2.81 (6H, m), 2.58 (2H, d, J = 6.42), 2.40 (2H, m), 2.00 (3H, m), 1.40 (5H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 163−165 °C; IR (cm^{-1}) (Nujol) 1682. Anal. ($C_{23}H_{27}NO\cdot HCl$) C, H, N.

3-[(4′-Benzylpiperidinyl)methyl]chromanone 10ab. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 171−172 °C (dec); IR (cm^{-1}) (Nujol) 1682. Anal. ($C_{22}H_{25}NO_2\cdot HCl$) C, H, N.

2-(4-Benzylpiperazin-1-ylmethyl)-1-tetralone 10ba: ^1H NMR δ 8.06 (1H, m), 7.50 (1H, m), 7.31 (7H, m), 3.51 (2H, s), 3.00 (3H, m), 2.55 (11H, m), 1.96 (1H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 258−260 °C (dec); IR (cm^{-1}) (Nujol) 1682. Anal. ($C_{22}H_{26}N_2O\cdot 2HCl$) C, H, N.

3-(4-Benzylpiperazin-1-ylmethyl)chroman-4-one 10bb. The compound (0.45 mmol) was transformed into the corresponding oxalate salt by treatment with oxalic acid (0.9 mmol) in anhydrous diethyl ether (50 mL). The salt was then washed several times with diethyl ether: mp 198−200 °C. Anal. ($C_{21}H_{24}N_2O_2\cdot 2(COOH)_2$) C, H, N.

(4-Benzyl-1-oxo-indan-2-yl)methylpiperidine 11. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH: mp 155−156 °C (dec); IR (cm^{-1}) (Nujol) 1682. Anal. ($C_{22}H_{25}NO\cdot HCl$) C, H, N.

6-(4-Benzylpiperidin-1-ylmethyl)-6,7,8,9-tetrahydrobenzocycloheptan-5-one 12. The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 153−155 °C; IR (cm^{-1}) (Nujol) 1682. Anal. ($C_{24}H_{29}NO\cdot HCl$) C, H, N.

(4-Benzylindan-2-yl)methylpiperidine 7aa. A solution of **11** (0.60 g, 1.9 mmol) in ethanol (60 mL) and few drops of concentrated HCl was hydrogenated in the presence of Pd/C (5%, 90 mg) at 75 °C at 2 atm for 5 h. After the reaction mixture was filtered, the solvent was removed under reduced pressure and the residue was treated with a saturated solution of Na_2CO_3 and extracted with CH_2Cl_2 (3 × 30 mL). The organic phases were then dried (Na_2SO_4), and the solvent was removed under reduced pressure. The oily product thus obtained was converted into the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 209−211 °C. Anal. ($C_{22}H_{27}N\cdot HCl$) C, H, N

3-(4-Benzylpiperidin-1-ylmethyl)-3-benzoylpropionic Acid 46. A suspension of β-benzoylpropionic acid **45** (5.00 g, 28.1 mmol), formaldehyde (35%, 2.4 mL), and 4-benzylpiperidine (4.9 mL, 27.9 mmol)) in CH_3OH was heated with stirring at 70 °C for 15 min, and then it was left at room temperature for 5 days. A solid was formed; it was triturated with acetone (50 mL), and the residue was collected and washed with acetone: mp 137−139 °C; IR (cm^{-1}) (Nujol) 2188, 1691, 1659.

3-(4-Benzylpiperidin-1-ylmethyl)-4-phenylbutyric Acid 48. To a solution of **46** (3.00 g, 8.2 mmol) in methanol (80 mL) containing an excess of dry HCl was added Pd/C (5%, 0.3 g),

and the mixture was hydrogenated at 2.5 atm at room temperature. After 5 h, the catalyst was removed by filtration and the solvent was removed under reduced pressure. The residue thus obtained was purified by crystallization (EtOAc): mp 130−132 °C.

3-(4-Benzylpiperidin-1-ylmethyl)-1-tetralone 8ab. To polyphosphoric acid (30.0 g), mantained at 60 °C with stirring, was added **13c** (1.00 g, 2.8 mmol). The temperature was maintained at 60 °C for 15 min, raised to 120 °C, and maintained at that temperature for 15 min. After cooling, the reaction mixture was poured into ice−water, and then NaOH was added until alkaline pH was reached. The mixture was then extracted with diethyl ether (3 × 20 mL), the organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure: ^{1}H NMR δ 8.01 (1H, m), 7.51 (1H, m), 7.32 (7H, m), 3.20 (1H, m), 2.81 (4H, m), 2.60 (2H, d, J = 6.22), 2.40 (4H, m), 1.95 (2H, m), 1.50 (5H, m). The product thus obtained was converted into the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 240−242 °C. Anal. ($C_{23}H_{27}NO$·HCl) C, H, N.

3-(4-Benzylpiperazin-1-ylmethyl)-3-benzoylpropionic Acid 47. A suspension of β-benzoylpropionic acid **45** (5.00 g, 28.6 mmol), formaldehyde (35%, 2.4 mL), and 1-benzylpiperazine (4.97 mL) in CH_3OH (3 mL) was heated at 65 °C 15 min, and then it was left at room temperature for 48 h. The reaction mixture was then treated with acetone (5 mL) and the solid thus formed was collected, washed with acetone, and crystallized from EtOH: mp 155−158 °C.

3-(Piperazin-1-ylmethyl)-4-phenylbutyric Acid 49. A solution of the acid **47** (1.87 g, 5.11 mmol) in acetic acid (60 mL) was hydrogenated at 2.5 atm at 75 °C in the presence of Pd/C (5%, 0.150 g) for 3 h; after filtration of the catalyst, the solvent was removed under reduced pressure. The residue thus obtained was triturated with diethyl ether: IR (cm^{-1}) (Nujol) 3337, 1686, 1600. The solid thus obtained was used for the next step without further purification.

3-(1-Piperazinylmethyl)-1-tetralone 50. A mixture of **49** (1.30 g, 4.96 mmol) in polyphosphoric acid (40.0 g) was heated at 120 °C for 15 min; after cooling, the reaction mixture was diluted into ice/water (300 mL), treated with 5 N NaOH until alkaline pH, and then extracted with diethyl ether (3 × 40 mL). The organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure.

3-(4-Benzylpiperazin-1-ylmethyl)-1-tetralone 8bb. To a suspension of **50** (1.0 g, 4.1 mmol) and K_2CO_3 (0.56 g, 4.1 mmol) in anhydrous acetone (10 mL) at 0 °C and under stirring was added benzyl bromide (0.49 mL, 4.1 mmol). The reaction mixture was maintained at room temperature for 12 h, and then salts were removed by filtration, and the solvent was removed under reduced pressure. The residue thus obtained was purified by means of column chromatography (CH_2Cl_2/CH_3OH 9.75/0.25): ^{1}H NMR δ 8.01 (1H, m), 7.50 (1H, m), 7.36 (7H, m), 3.60 (2H, s), 3.18 (1H, m), 2.80 (2H, m), 2.50 (12H, m). The compound was converted to the HCl salt by dissolving it in anhydrous ether, and then a saturated ethereal solution of HCl was added. The salt thus obtained was recrystallized from CH_3OH/diethyl ether: mp 262−265 °C (dec); IR (cm^{-1}) (Nujol) 1682. Anal. ($C_{22}H_{26}N_2O$·2HCl) C, H, N.

Radioligand Binding Assays. σ_1-Site Binding Assay. σ_1-Site binding assays were carried out on guinea pig brain membranes according to Matsumoto et al.[30] The protein concentration of the suspension was measured by the method of Lowry et al.[31] and generally ranged from 6.5 to 8.5 mg of protein/mL. Binding assays were performed as described by DeHaven et al.[32] Each tube contained 500 mg of membrane protein and was incubated with 3 nM [^{3}H]-(+)-pentazocine (45 Ci/mmol); the value of the apparent dissociation constant (K_d) was 1.2 ± 0.3 nM (n = 3) in 50 mM Tris-HCl (pH 7.4). Test compounds were dissolved in DMSO and then diluted in buffer to a total volume of 1 mL. Test compounds were added to give a concentration in the range of 10^{-5}−10^{-11} M. Nonspecific binding was assessed in the presence of 10 μM haloperidol. The reaction was performed for 150 min at 37 °C and terminated by filtering the solution through a Whatman GF/B glass fiber filter which had been presoaked for 1 h in a 0.5% poly(ethyilenimine) solution. Filters were washed twice with 4 mL of ice-cold buffer.

σ_2-Site Binding Assays. σ_2-Site binding assays were carried out on guinea pig brain membranes, prepared as described by Mach et al.[33] The membranes were incubated with 3 nM [^{3}H]DTG [1,3-di-2-tolylguanidine] (31 Ci/mM; K_d = 9.9 ± 0.8 nM; n = 3) in the presence of 400 nM (+)-SKF10,047 to block σ_1 sites. Incubation was carried out in 50 mM Tris-HCl (pH 8.0) for 120 min at room temperature. Each assay was terminated by the addition of ice-cold 10 mM Tris-HCl, pH 8.0, followed by filtration through a Whatman GF/B glass fiber filter which had been presoaked for 1 h in a 0.5% poly-(ethylenimine) solution. Filters were washed twice with 4 mL of ice-cold buffer. Nonspecific binding was evaluated in the presence of 5 mM DTG. Inhibition constants (*Ki* values) for the tested compounds were calculated using the EBDA-LIGAND program[34] purchased from Elsevier/Biosoft.

D_2 Receptors Binding Assays. Binding experiments were performed on rat striatal membranes according to Creese and co-workers[35] with minor modifications. Rats were killed by decapitation, the brain was quickly removed, and the corpora striata was dissected. The corpora striata (1.0 g) was homogenized with a Brinkman polytron (setting 5 for 3 × 15 s) in 25 mL of 50 mM Tris buffer, pH 7.4. The surnatant was discarded, and the pellet was washed once. The final pellet was stored at −80 °C until used. Each tube received, in a final volume of 3 mL of incubation buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5.7 mM ascorbic acid, pH 7.4), rat striatal membranes suspension and 0.2 nM [^{3}H]spiperone. For competitive inhibition experiments, various concentrations of drugs studied were incubated. Nonspecific binding was defined using 1 μM haloperidol. Samples were incubated at 37 °C for 20 min and then filtered on Whatman GF/B glass microfiber filters.

Human Cloned 5-HT_{1A} Serotonergic Receptors Binding Assays. Genomic clone G-21 coding for the human 5-HT_{1A} serotoninergic receptor is stably transfected in a human cell line (HeLa).[36] 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) and 7% 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 5 mM Tris and 5 mM EDTA buffer (pH 7.4). Homogenates were centrifuged at 40 000g for 20 min, and pellets were resuspended in a small volume of ice-cold 5 mM Tris and 5 mM EDTA buffer (pH 7.4) and immediately frozen and stored at −70 °C until use. On the day of the experiment, cell membranes were resuspended in binding buffer (50 mM Tris (pH 7.4), 2.5 mM $MgCl_2$, and 10 mM pargiline).[37] Membranes were incubated in a final volume of 1 mL for 30 min at 30 °C with 1.2 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 buffer and rapid filtration through 0.2% polyethyleneimine-pretreated Schleicher & Schuell GF52 filters.

Stimulation of [^{35}S]GTPγS Binding at Cloned 5-HT_{1A} Receptors. The effects of the different compounds tested on [^{35}S]GTPγS binding were evaluated according to the method of Stanton and Beer[38] with minor modifications. On the day of the experiment, cell membranes from HeLa cells transfected with human cloned 5-HT_{1A} receptors were resuspended in buffer containing 20 mM HEPES, 3 mM $MgCl_2$, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 μM GDP and decreasing concentrations (from 100 μM to 0.1 nM) of test drugs or 5-HT (reference curve) for 20 min at 30 °C in a final volume of about 0.5 mL. Samples were then transferred to ice, with [^{35}S]GTPγS (200−250 pM) added, and then incubated for 30 min further at 30 °C. Nonspecific binding was determined in the presence of 10 μM GTPγS. The incubation was stopped by the addition of ice-cold HEPES buffer and

rapid filtration on Schleicher and 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 with an efficiency greater than 90%.

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Supporting Information Available: Microanalysis data relative to the target compounds and ^{1}H NMR spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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