trans-4-[4-(Methoxyphenyl)cyclohexyl]-1-arylpiperazines: A New Class of Potent and Selective 5-HT$_{1A}$ Receptor Ligands as Conformationally Constrained Analogues of 4-[3-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]-1-arylpiperazines

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The present paper concerns the influence of conformational parameters on the recognition by rat 5-HT$_{1A}$ receptors of derivatives 4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]-1-(2-pyridinyl)piperazine (**1a**) and 3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*N*-[2-(2-pyridyloxy)ethyl]propanamine (**3b**), two highly potent and selective 5-HT$_{1A}$ receptor ligands. Fifteen corresponding flexible and rigid analogues were prepared following several synthetic routes and were tested in binding assays with radioligands at 5-HT$_{1A}$, D$_2$, and α$_1$ receptors from rat brain membranes. Among the new derivatives emerged *trans*-4-[4-(3-methoxyphenyl)-cyclohexyl]-1-(2-pyridinyl)piperazine (*trans*-**8a**) and *trans*-*N*-[4-(3-methoxyphenyl)cyclohexyl]-2-(2-pyridyloxy)ethylamine (*trans*-**8b**). These compounds can be considered as conformationally constrained analogues of compounds **1a** and **3a**, respectively. In fact, compounds *trans*-**8a** and *trans*-**8b** showed a marked enhancement in 5-HT$_{1A}$ receptor affinity when compared to the corresponding cis isomers. Because compound *trans*-**8a** was a potent and selective 5-HT$_{1A}$ ligand (K_i, nM: 5-HT$_{1A}$ = 0.028, D$_2$ = 2194, α$_1$ = 767), it was chosen as a lead to prepare other analogues that were tested at 5-HT$_{1A}$, D$_2$, and α$_1$ receptors from rat brain membranes, showing high affinity at the 5-HT$_{1A}$ and selectivity vs D$_2$ and α$_1$ receptors. Selected compounds were tested for their affinity at the human cloned 5-HT$_{1A}$, α$_{1a}$, α$_{1b}$, α$_{1d}$ receptor subtypes. They were also submitted to the [^{35}S]GTPγS binding assay stimulating the 5-HT$_{1A}$ receptor-mediated G-protein activation, therefore behaving as full or as partial agonists. Finally, the ability of iv administration of *trans*-**8a** to induce fore-paw treading in rats was evaluated in comparison with 8-OH-DPAT. Although the affinity (K_i) and in vitro activity (pD′$_2$) of *trans*-**8a** at the 5-HT$_{1A}$ receptor were higher than those of 8-OH-DPAT, the compound was less potent than the reference standard in inducing the symptom.

Serotonin (5-HT) is involved in various physiological and pathological process by interaction with seven classes of receptors (5-HT$_{1-7}$) containing 14 distinct receptors grouped on the basis of amino acid sequence, pharmacology, and signal transduction pathways.[1,2] The 5-HT$_{1A}$ receptor subtype has been the target of considerable research because of its involvement in psychiatric disorders such as anxiety and depression.[3,4] Earlier studies were focused on the development of 5-HT$_{1A}$ receptor agonists such as buspirone, the first 5-HT$_{1A}$ agent launched in the market. More recently new therapeutic perspectives have been proposed: 5-HT$_{1A}$ agonists may be useful as antidepressants[5,6] and as neuroprotective agents.[7] The full elucidation of the involvement of 5-HT$_{1A}$ receptors in the above-mentioned diseases still awaits the proper pharmacological tools. Although a large number of compounds with high affinity for 5-HT$_{1A}$ receptors have been described in the past, few of them are both selective and highly effica-

cious at the receptor. During the past years many 1-arylpiperazines (Chart 1) have been synthesized, and our research group has been interested in some arylpiperazine derivatives containing the tetralin nucleus (structure **I**) as 5-HT$_{1A}$ receptor ligands. SAFIR studies have allowed us to establish optimal structural features for the interaction with 5-HT$_{1A}$ receptors and to obtain highly potent and selective compounds.[8−12] The fundamental elements were the 1-aryl-4-propylpiperazinyl moiety directly linked to the 5-methoxy-1-tetralinyl nucleus; chirality did not play any role in 5-HT$_{1A}$ receptor affinity.[13] The best 1-aryl group on the piperazine moiety in terms of both affinity and selectivity for the 5-HT$_{1A}$ receptor was 2-pyridinyl, so compounds of structure **1a**[9] (Table 1) represent the most relevant member of that series. Furthermore, we discovered that the 1-(2-pyridinyl)piperazine nucleus can be successfully replaced by a 2-(2-pyridyloxy)ethylamino moiety (compound **1b**).[14]

Although a large number of papers have been published on "long-chain" arylpiperazines as 5-HT$_{1A}$ ligands,

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Chart 1

Scheme 1[a]

[a] Reagents: (A) (i) NBS, (ii) triethylamine; (B) 1-(2-pyridinyl)piperazine; (C) 2-(2-pyridyloxy)ethylamine.
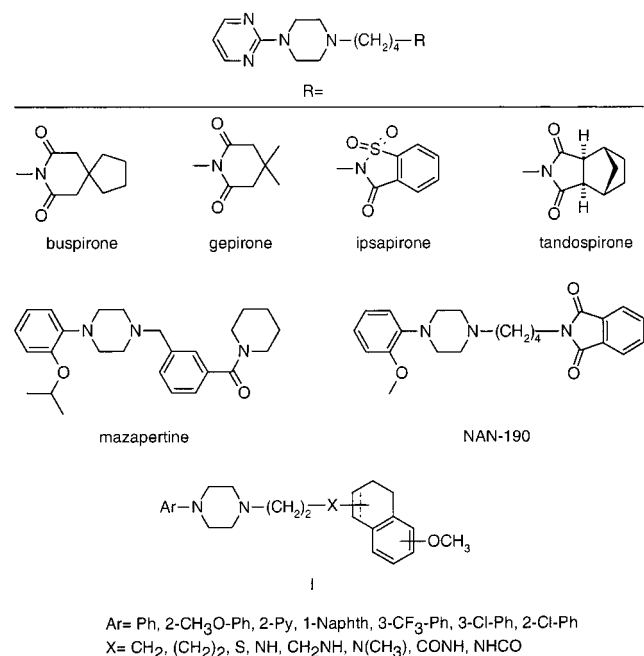
only a few dealt with the conformation of the polymethylene chain of these ligands. Crystallographic data of buspirone,[15] gepirone,[15] and mazapertine[16] and a ^{1}H NMR study on tandospirone in water solution[17] indicated that these compounds adopted conformations between bent and extended that are believed to be responsible for the biological activity at the 5-HT$_{1A}$ receptor. However, these studies do not give information about the conformational changes in the ligand caused by the interaction with 5-HT$_{1A}$ receptors, so the structural requirements of the receptor may force the molecule to bind in a conformation that would not be favored in the nonbonded state. One way to circumvent this uncertainty is to evaluate the pharmacological activity of conformationally rigid analogues. This approach has been used in two related cases: Mokrosz et al.[18] prepared some rigid analogues of NAN-190; Romero and co-workers[19] studied the semirigid analogues of ipsapirone. Both studies indicated that these ligands bind at the 5-HT$_{1A}$ receptor in an extended linear conformation.

In the present paper we address the influence of conformational parameters on the recognition of derivatives **1a** and **1b** by the 5-HT$_{1A}$ receptor. We prepared and tested some corresponding more flexible and more rigid analogues; in particular, the 5-HT$_{1A}$ receptor affinity of compound **1a** has been compared with those of two analogues having similar conformational freedom (compounds **2a** and **3a**) and with the flexible analogue **4a** obtained by removal of the saturated ring of the tetralin nucleus. More conformationally constrained analogues were obtained by blocking the spacer within a cyclic structure (compounds **5a**, **6a**, **7a**, **8a**). These modifications have been also extended to the abovementioned 2-(2-pyridyloxy)ethylamino derivative **1b**. All these compounds were tested in vitro for their receptor binding affinity at 5-HT$_{1A}$, D$_2$, and α$_1$ receptors from rat brain membranes: among them emerged compound *trans*-**8a**, which was chosen for further modification that led to compounds where the 2-pyridinyl group was
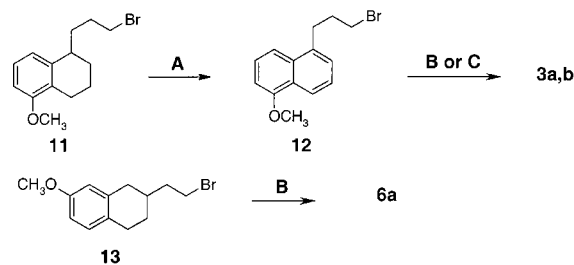
replaced by a phenyl, 2-methoxyphenyl, or 1-naphthalenyl (compounds **8c**−**e**). The role of the methoxy group position on the aromatic ring of compounds related to *cis*-**8a** and *trans*-**8a** was also investigated (derivatives **9a** and **10a**).

Finally, selected compounds were tested for their affinity at the human cloned 5-HT$_{1A}$, α$_{1a}$, α$_{1b}$, α$_{1d}$ receptor subtypes, and they were submitted to the [^{35}S]-GTPγS binding assay for the evaluation of their intrinsic activity on the 5-HT$_{1A}$ receptor. The ClogP values of all compounds were calculated as a predictive parameter for membrane permeation.
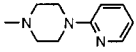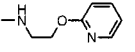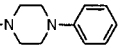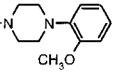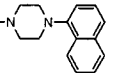
Chemistry

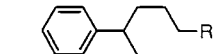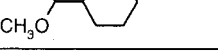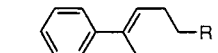The preparation of the target compounds required several synthetic routes.
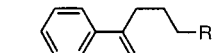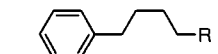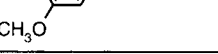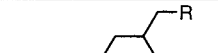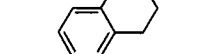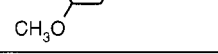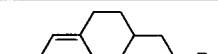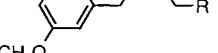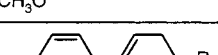
Compounds **3a,b** and **6a** (Scheme 1) were prepared by alkylating 1-(2-pyridinyl)piperazine or 2-(2-pyridyloxy)ethylamine with bromopropyl derivative **12**, obtained from the aromatization of 1-(3-bromopropyl)-5-methoxy-1,2,3,4-tetrahydronaphthalene (**11**)[9] with N-bromosuccinimide (NBS) and triethylamine; bromoethyl derivative **13**[11] and 1-(2-pyridinyl)piperazine reacted to afford compound **6a**.

The synthesis of compounds **4a** and **4b** (Scheme 2) started from stannate **14**,[20] which underwent a cross-coupling reaction[21] with methyl 4-bromocrotonate in the presence of Pd(0), generated from triphenylphosphine-palladium(II) chloride and diisobutylaluminum hydride (DIBAL-H), to give the unsaturated ester **15**. Reduction of the latter with lithium aluminum hydride afforded alcohol **16**, which was reacted with methanesulfonyl chloride to give the key intermediate **17**. The reaction between 1-(2-pyridinyl)piperazine and mesilate **17** gave the final compound **4a**, whereas the reaction of 2-(2-pyridinyloxy)ethylamine with compound **17** gave the corresponding N,N-disubstituted amine. Therefore, to prevent this shortcoming, mesylate **17** was reacted with N-benzyl derivative **19**, prepared from commercially available N-benzylethanolamine (**18**) and 2-chloropyridine under phase-transfer catalysis[22] to give amine **20**. Hydrogenolysis of the latter gave the final compound **4b**.

The preparation of compounds **5a** and **5b** was accomplished as depicted in Scheme 3. Carboxylic acid **21**[23] was refluxed with ethanol in the presence of a catalytic amount of sulfuric acid to give ester **22**, which was reduced to alcohol **23** with LiAlH$_4$. This compound was transformed into its mesylate derivative **24**. The reaction of the latter with 1-(2-pyridinyl)piperazine or 2-(2-pyridyloxy)ethylamine afforded the final compounds **5a** and **5b**.

Table 1. Binding Affinities Determined on Rat Brain Membranes

a: R=   **b**: R=   **c**: R=   **d**: R=   **e**: R=

compound	structure	$K_i \pm$ S.E.M., nM			selectivity vs 5-HT$_{1A}$ (K_i ratio)	
		5-HT$_{1A}$	D$_2$	α_1	D$_2$	α_1
1a[a]		0.48	117	55	244	115
1b[a]		0.54	>850	83	1574	154
2a[a]		0.31	125	60	403	194
2b[a]		0.71	>850	>850	>1200	>1200
3a		0.38 ± 0.07	450 ± 25	833 ± 60	1200	2200
3b		0.19 ± 0.04	378 ± 18	1300 ± 180	1990	6800
4a		23 ± 4	750 ± 30	467 ± 19	33	20
4b		39 ± 5	>850	733 ± 27	22	19
5a		676 ± 28	>850	585 ± 60	1	1
5b		59 ± 6	>850	>850	14	14
6a		0.16 ± 0.08	1730 ± 220	117 ± 24	>10000	730
7a		536 ± 24	1220 ± 120	512 ± 16	2	1
7c		257 ± 17	2190 ± 300	5160 ± 130	9	20
7d		7.1 ± 4.0	2930 ± 120	408 ± 38	419	58
7e		15 ± 3	327 ± 12	986 ± 70	22	66
cis-**8a**		815 ± 20	>850	783 ± 32	1	1
trans-**8a**		0.028 ± 0.003	2190 ± 120	767 ± 50	>10000	>10000
cis-**8b**		373 ± 18	783 ± 32	>850	2	2
trans-**8b**		17 ± 2	586 ± 23	>850	34	50
cis-**8c**		183 ± 22	2380 ± 160	2250 ± 160	13	12
trans-**8c**		0.026 ± 0.007	910 ± 20	730 ± 21	>10000	>10000
cis-**8d**		79.6 ± 6	668 ± 12	1050 ± 110	8	13
trans-**8d**		0.020 ± 0.008	60 ± 8	312 ± 27	3000	>10000
cis-**8e**		74 ± 8	1310 ± 150	5540 ± 250	18	11
trans-**8e**		0.22 ± 0.04	70 ± 8	470 ± 36	318	2136
cis-**9a**		480 ± 15	2120 ± 130	712 ± 35	4	1
trans-**9a**		0.20 ± 0.04	243 ± 15	303 ± 34	1215	1515
cis-**10a**		94 ± 8	787 ± 43	786 ± 29	8	8
trans-**10a**		0.046 ± 0.006	792 ± 61	847 ± 39	>10000	>10000
8-OH-DPAT		2.1 ± 0.4				
buspirone		32 ± 7				
haloperidol			0.8 ± 0.1			
phentolamine				18 ± 3		

[a] See ref 14.

The synthetic route followed to prepare derivatives **7a**, **7c**−**e**, **8a**−**e**, **9a**, and **10a** parallels a method reported for the synthesis of similar compounds[24] (Scheme 4). Ketone **25** was reacted with the appropriate amine in the presence of a catalytic amount of *p*-toluenesulfonic acid to give the intermediate enamines. These were first transformed into their hydrochloride salts, then reduced with NaBH$_3$CN.[25] Deprotec-tion of ketals **26a**−**e** with HCl gave 4-substituted cyclohexanones **27a**−**e**. Reaction of these ketones with the appropriate Grignard reagent gave the inter-mediate alcohols, which were dehydrated to afford alkenes **7a**−**e**, **28a**, and **29a**. Final compounds **8a**−**e**, **9a**, and **10a** were obtained by reduction of the double bond with H$_2$ in the presence of Pd/C. Trans and cis isomers were easily separated by column chromatogra-

Scheme 2[a]
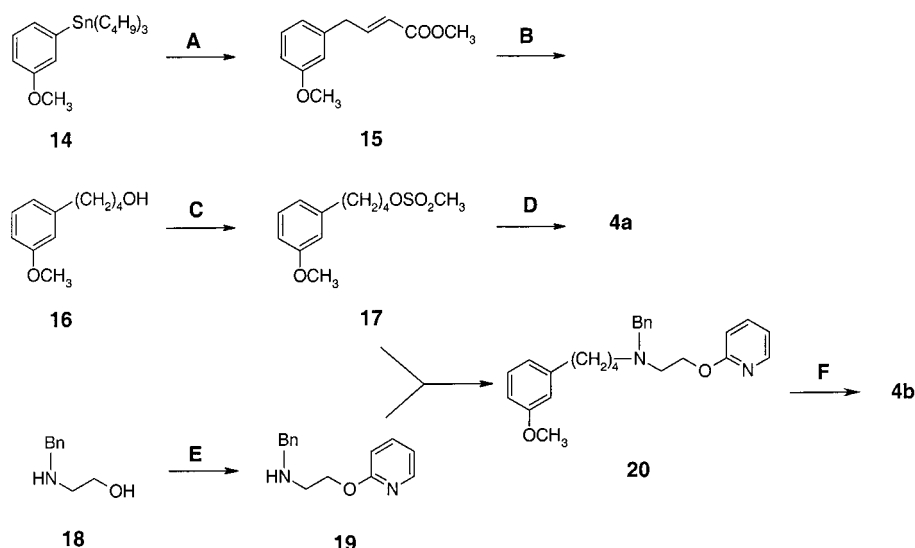
[a] Reagents: (A) (i) DIBAL-H, triphenylphosphinepalladium(II) chloride, (ii) methyl 4-bromocrotonate; (B) LiAlH₄; (C) methanesulfonyl chloride, triethylamine; (D) 1-(2-pyridinyl)piperazine; (E) 2-chloropyridine, KOH, 18-crown-6; (F) H₂, 10% Pd/C.

Scheme 3[a]
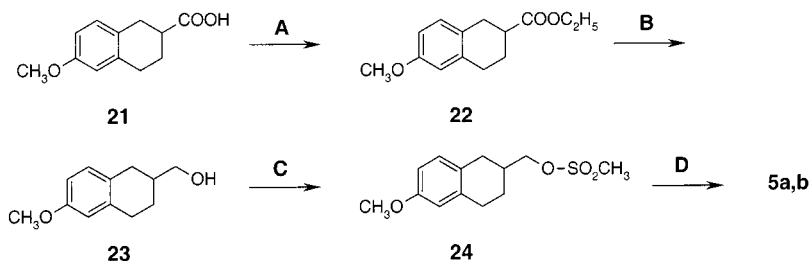
[a] Reagents: (A) 96% H₂SO₄, EtOH; (B) LiAlH₄; (C) methanesulfonyl chloride, triethylamine; (D) 1-(2-pyridinyl)piperazine or 2-(2-pyridinyloxy)ethylamine.

phy. Stereochemical assignment is described in detail in Experimental Section. When necessary, final compounds were transformed into their hydrochloride or hydrogen oxalate salts in the usual manner. Physical properties of target compounds are listed in Table 2.

Results and Discussion

Binding affinities determined on rat brain membranes are listed in Table 1. Considering first the 1-(2-pyridinyl)piperazine derivatives, there were not great differences in affinities at the 5-HT$_{1A}$ receptor for compounds **2a** and **3a** compared with the reference compound **1a**. However, the limited blocking of the alkyl chain affects the selectivity; compounds **2a** and **3a** are more selective than the reference compound **1a** over D$_2$ and α$_1$ receptors. The removal of the saturated ring of tetralin in compound **1a** gave the analogue **4a**; the 5-HT$_{1A}$ receptor affinity value indicates that the flexibility of the molecule is detrimental for a high affinity but still allows the molecule to bind at the receptor. Derivatives **5a** and **6a** represent two different ways to block the alkyl chain in a semirigid conformation. The 5-HT$_{1A}$ receptor affinity of compound **5a** clearly indicated that the blocking of the alkyl chain in a folded manner caused a dramatical loss in affinity. On the other hand, the extended conformation reached by the alkyl chain in compound **6a** seemed to favor a good interaction with the receptor. More complete information in this sense can be achieved by considering the cyclohexane derivatives **7a**, *cis*-**8a**,

and *trans*-**8a**. For all these compounds the alkyl chain is blocked in an extended conformation, but their affinities for the 5-HT$_{1A}$ receptor were markedly different. First of all, *trans*-**8a** is more potent and selective than the reference compound **1a**. Subsequently, *trans*-**8a** is 29 000-fold more potent than the isomer *cis*-**8a** at the 5-HT$_{1A}$ receptor, being strikingly selective over D$_2$ and α$_1$ receptors. The unsaturated derivative **7a** displayed the same binding profile as *cis*-**8a**. These results highlight the stringent requirements for these compounds to interact with the 5-HT$_{1A}$ receptor. The bioactive conformation of the alkyl chain in derivative **1a** seems to be in an extended manner bearing the 1-(2-pyridinyl)piperazine moiety and the aromatic ring of the tetralin nucleus in the same relative position reached by 1-(2-pyridinyl)piperazine and the 3-methoxyphenyl group in *trans*-**8a**.

The same modifications have been effected on the 2-(2-pyridyloxy)ethylamino derivative **1b**. The 5-HT$_{1A}$ receptor affinity of compounds **1**−**5b**, *cis*-**8b**, and *trans*-**8b** displayed the same trend observed for the corresponding 1-(2-pyridinyl)piperazines. However, it can be noted that *trans*-**8b** is less potent than the reference **1b** and the difference in affinity from the affinity of the cis isomer is less marked than that found between the corresponding 1-(2-pyridinyl)piperazine derivatives *cis*-**8a** and *trans*-**8a**. This behavior could be due to the flexibility of the 2-(2-pyridyloxy)ethylamino moiety that

Scheme 4^a
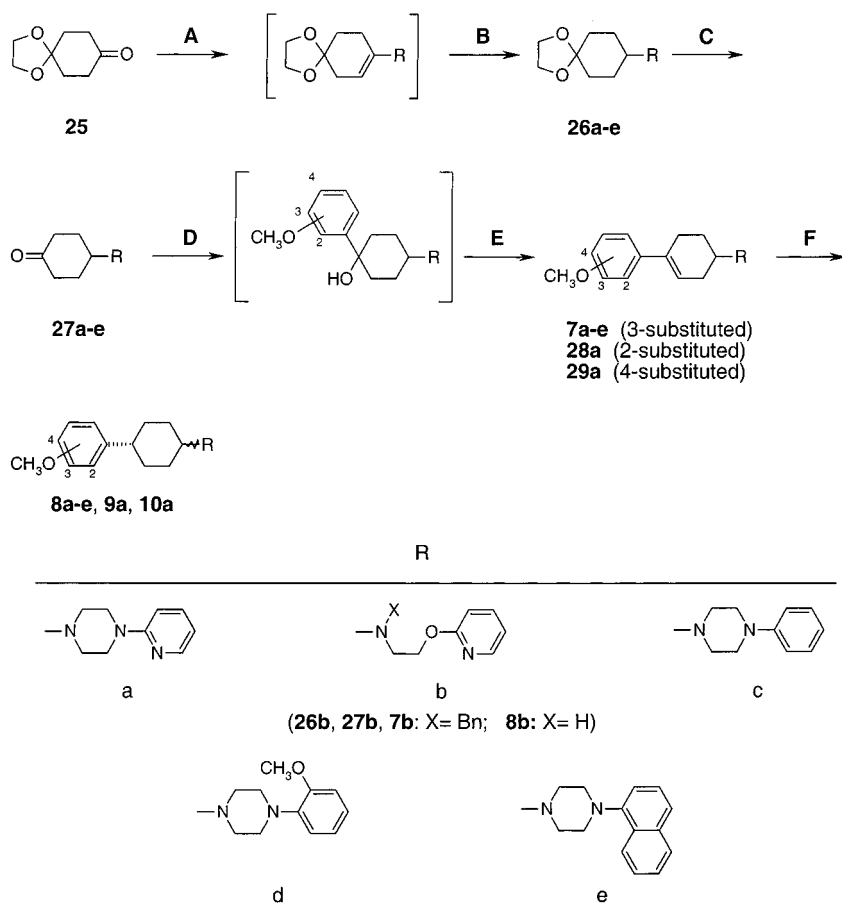
^{a} Reagents: (A) 1-arylpiperazine or amine **19**, *p*-toluenesulfonic acid; (B) (i) HCl (g), (ii) NaBH$_3$CN; (C) 3 N HCl; (D) methoxyphenyl-MgBr; (E) 20% H$_2$SO$_4$; (F) H$_2$, 10% Pd/C.

can assume a unfavorable conformation for the binding at 5-HT$_{1A}$ receptor.

Starting from these results, we considered some modifications on the structure of compound **8a**. In particular, compounds **8c**−**e** were obtained by replacing the 2-pyridinyl group with other aromatic rings, such as phenyl, 2-methoxyphenyl, 1-naphthalenyl, already studied in the tetralinyl series of 5-HT$_{1A}$ ligands; compounds **9a** and **10a** were obtained by shifting the methoxy group of compound **8a** from the 3-position to the 2- and 4-position, respectively.

The 5-HT$_{1A}$ receptor affinity values of these derivatives are in the same range as those of compounds **8a**, revealing the same behavior: the trans isomers **8c**−**e** are always more potent than the corresponding cis, the latter isomers being nearly equipotent to the cyclohexene precursors **7c**−**e**. The presence of a moderate D$_2$ receptor affinity in compounds *trans*-**8d,e** reflected in a lowering in selectivity versus D$_2$ receptor, whereas the selectivity for α$_1$ receptor is always greater than 1000-fold. The phenyl derivative *trans*-**8c** demonstrated the same affinity and selectivity as *trans*-**8a**.

The position of the methoxy group on the aromatic ring of *trans*-**8a** seemed to play a marginal role in the interaction with the 5-HT$_{1A}$ receptor. In fact, the shifting of the methoxy group to the 2-position (*trans*-**9a**) caused only a slight decrease in 5-HT$_{1A}$ receptor affinity, whereas the 4-substituted analogue *trans*-**10a** presented the same binding profile as *trans*-**8a**. Finally, it can be

noted that *cis*-**9a** and *cis*-**10a** are less potent than the corresponding trans isomers, confirming the trend discussed above.

Seven of the compounds studied were selected for further in vitro pharmacological evaluation on the basis of their binding profile: compounds **1a,b**, **3a,b**, and *trans*-**8a,b,d**, displaying quite different structural features, were tested for their affinity at human cloned 5-HT$_{1A}$, α$_{1a}$, α$_{1b}$, and α$_{1d}$ receptors and in the [^{35}S]GTPγS binding at the human cloned 5-HT$_{1A}$ receptor.

All these compounds showed nano- or subnanomolar affinity values at the human 5-HT$_{1A}$ receptor (Table 3) but displayed different selectivity versus the human α$_1$-adrenoceptor subtypes: compounds **1a** and **1b** were endowed with similar affinity for 5-HT$_{1A}$ serotonergic receptor and α$_{1a}$-adrenoceptor subtype; compound **3a** showed the same affinity for 5-HT$_{1A}$ receptor and α-adrenoceptor subtypes; compounds **3b** and *trans*-**8a** showed good selectivity versus the α$_{1a}$, α$_{1b}$, α$_{1d}$ receptors because *trans*-**8a** is more than 100-fold selective over the α-adrenoceptor subtypes.

Compounds **1a,b**, **3a,b**, and *trans*-**8a,b,d** stimulated 5-HT$_{1A}$ receptor-mediated G-protein activation, as measured by [^{35}S]GTPγS binding, behaving as the full agonists 5-HT and 8-OH-DPAT (e.g., *trans*-**8a**, **1a**) or as partial agonists (Table 3). This behavior indicated that the structural differences in this group of compounds did not play any role in their activity.

Table 2. Physical Properties of Target Compounds

compound	formula[a]	mp, °C	recryst solv	ClogP
1a[b]				4.59
1b[b]				4.96
2a[b]				4.28
2b[b]				4.65
3a	$C_{23}H_{27}N_3O \cdot 3HCl$	228–230	MeOH/Et$_2$O	4.17
3b	$C_{21}H_{24}N_2O_2 \cdot (COOH)_2 \cdot {}^1/_2H_2O$	206–208	MeOH	4.54
4a	$C_{20}H_{27}N_3O \cdot 2HCl$	226–228	MeOH/Et$_2$O	3.52
4b	$C_{18}H_{24}N_2O_2 \cdot (COOH)_2 \cdot H_2O$	177–178	MeOH/Et$_2$O	3.89
5a	$C_{21}H_{27}N_3O \cdot 3HCl$	233–234	MeOH/Et$_2$O	4.06
5b	$C_{19}H_{24}N_2O_2 \cdot 2(COOH)_2 \cdot {}^1/_2H_2O$	207–208	MeOH	4.43
6a	$C_{22}H_{29}N_3O \cdot 2HCl \cdot {}^1/_2H_2O$	222	MeOH/Et$_2$O	4.06
7a	$C_{22}H_{27}N_3O$	137–138	CHCl$_3$/n-hexane	3.56
7c	$C_{23}H_{28}N_2O$	135–136	CHCl$_3$/n-hexane	4.51
7d	$C_{24}H_{30}N_2O_2$	127–129	CHCl$_3$/n-hexane	4.53
7e	$C_{27}H_{30}N_2O$	120–121	CHCl$_3$/n-hexane	5.68
cis-**8a**	$C_{22}H_{29}N_3O \cdot 2HCl \cdot H_2O$	239–241	MeOH/Et$_2$O	3.87
trans-**8a**	$C_{22}H_{29}N_3O \cdot 2HCl \cdot {}^1/_3H_2O$	234–235	MeOH/Et$_2$O	3.87
cis-**8b**	$C_{20}H_{26}N_2O_2 \cdot (COOH)_2$	162–164	MeOH/Et$_2$O	4.33
trans-**8b**	$C_{20}H_{26}N_2O_2 \cdot (COOH)_2$	169–171	MeOH/Et$_2$O	4.33
cis-**8c**	$C_{23}H_{30}N_2O$	101–103	CHCl$_3$/n-hexane	4.82
trans-**8c**	$C_{23}H_{30}N_2O \cdot 2HCl$	250–253	MeOH/Et$_2$O	4.82
cis-**8d**	$C_{24}H_{32}N_2O_2$	107–108	CHCl$_3$/n-hexane	4.84
trans-**8d**	$C_{24}H_{32}N_2O_2$	101–102	CHCl$_3$/n-hexane	4.84
cis-**8e**	$C_{27}H_{32}N_2O \cdot HCl$	246–247	MeOH/Et$_2$O	5.99
trans-**8e**	$C_{27}H_{32}N_2O \cdot HCl$	250–252	MeOH/Et$_2$O	5.99
cis-**9a**	$C_{22}H_{29}N_3O$	123–124	CHCl$_3$/n-hexane	3.87
trans-**9a**	$C_{22}H_{29}N_3O$	99–100	CHCl$_3$/n-hexane	3.87
cis-**10a**	$C_{22}H_{29}N_3O$	105–106	CHCl$_3$/n-hexane	3.87
trans-**10a**	$C_{22}H_{29}N_3O$	112–113	CHCl$_3$/n-hexane	3.87
8-OH-DPAT				4.01
buspirone				1.22
haloperidol				3.85
phentolamine				3.81

[a] Analysis for C, H, N; results were within ±0.4% of the theoretical values for the formulas given. [b] See ref 14.

Table 3. Affinity for Human Recombinant 5-HT$_{1A}$ Receptor and α_1-Adrenoceptor Subtypes and Potency (pD$_2$) and Relative Effectiveness Values (E_{max}: Maximal Stimulation Achieved Expressed as a Percentage of the Maximal 5-HT Response) in the [^{35}S]GTPγS Binding Assay at 5-HT$_{1A}$ Human Cloned Receptors of Selected Compounds

compound	binding affinity (K_i, nM)[a]				selectivity vs 5-HT$_{1A}$			[^{35}S]GTPγS binding	
		α_{1a}	α_{1b}	α_{1d}	α_{1a}	α_{1b}	α_{1d}	pD$_2$	% max
1a	0.08	0.33	7.88	5.95	4	98	74	10.22	98.2
1b	0.23	0.55	26.4	12.65	2	115	55	10.24	86.8
3a	3.0	1.62	4.21	12.57	0.5	1	4	7.87	86.3
3b	0.86	14.6	270	83.24	17	314	97	8.2	48
trans-**8a**	0.2	33.0	53.1	118	165	265	590	9.28	95.9
trans-**8b**	3.77	201	332	142	53	88	37	7.46	82
trans-**8d**	0.21	4.98	43.3	4.49	24	206	21	9.91	26.5
8-OH-DPAT	3.44	1757	5975	>1000				7.6	100

[a] Standard error of the mean was less than 10% of the mean.

The lipophilicity of all compounds (Table 2) was estimated by calculations using the ClogP program,[26] and it was used as a guide for selecting compounds for further pharmacological evaluations. ClogP values of compounds **1a**,**b**, **3a**,**b**, and *trans*-**8b** were greater than 4.0, predicting fair blood–brain barrier penetration, whereas *trans*-**8a** and trans-**8d** displayed a ClogP of 3.87, suggesting reasonable blood–brain barrier permeation;[27] for *trans*-**8a** the log P value was experimentally determined to be 3.68 at pH 7.4. Therefore, this evidence made *trans*-**8a** suitable for in vivo testing.

The agonistic interaction of *trans*-**8a** at the 5-HT$_{1A}$ receptor was confirmed in vivo. The intravenous or subcutaneous injection of a full 5-HT$_{1A}$ agonist (namely, 8-OH-DPAT) in rats induces, usually within 1–2 min, a dose-dependent increase of locomotion, fore-paw treading, head weaving, and flat body posture. This "5-HT syndrome" in rats is mediated by postsynaptic 5-HT$_{1A}$ receptors. Among the symptoms evoked, fore-paw tread-

ing appears to be the most closely associated with activation of 5-HT$_{1A}$ postsynaptic receptors.[28]

The ability of iv administration of *trans*-**8a** to induce fore-paw treading in rats was evaluated in comparison with 8-OH-DPAT. Although the affinity (K_i) and the agonist activity (pD$_2$) of *trans*-**8a** at the 5-HT$_{1A}$ receptor were higher than those of 8-OH-DPAT (Table 3), the compound assayed was less potent than the reference standard in inducing the symptom (Table 4). This finding was confirmed after subcutaneous administration of both reference and tested compounds.

In conclusion, this study provides further insight about the conformations of the polymethylene chain of "long-chain" arylpiperazines in the interaction with the 5-HT$_{1A}$ receptor. Moreover, *trans*-4-[4-(methoxyphenyl)-cyclohexyl]-1-arylpiperazines have been identified as a new class of potent 5-HT$_{1A}$ receptor ligands, highly selective over the D$_2$ and α_1 receptors. Two members of this class (*trans*-**8a** and trans-**8d**) were found to display

agonistic properties on the human cloned 5-HT$_{1A}$ receptor measured by the [^{35}S]GTPγS binding assay.

Experimental Section

Chemistry. Column chromatography was performed with 1:30 ICN silica gel 60A (63–200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on a Carlo Erba model 1106 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded either on a Varian EM-390 at 90 MHz where indicated (TMS as internal standard) or on a Bruker AM 300 WB instrument, with CDCl$_3$ as solvent. All values are reported in ppm (δ). 2-D NMR experiments (COSY and HETCOR) were performed on a Varian NMR 300 Mercury-VX (300 MHz) instrument. Recording of mass spectra was done on an HP6990-5973 MSD gas chromatograph/mass spectrometer; only significant *m/z* peaks, with their percentage of relative intensity in parentheses, are reported. All spectra were in accordance with the assigned structures. HPLC analyses were carried out using a Perkin-Elmer series 200 LC pump. UV absorbance was monitored with a Perkin-Elmer 785A UV/vis detector. 1-(1-Naphthalenyl)piperazine was synthesized according to a published procedure.[29]

1-(3-Bromopropyl)-5-methoxynaphthalene (12). *N*-Bromosuccinimide (4.66 g, 26.2 mmol) and benzoylperoxide (0.073 g, 0.3 mmol) were added to a solution of 1-(3-bromopropyl)-5-methoxy-1,2,3,4-tetrahydronaphthalene (**11**) (2.97 g, 10.5 mmol) in CCl$_4$. The mixture was refluxed for 15 h and then was cooled, and the obtained suspension was filtered. The filtrate was concentrated under reduced pressure, and the residue was refluxed for 1 h in ethanol/triethylamine (1:1, v/v, 50 mL). Then the solvent was evaporated in vacuo and the residue was taken up with CH$_2$Cl$_2$ and washed with 3 N HCl. The organic phase was separated, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The crude residue was chromatographed (petroleum ether/CHCl$_3$, 4:1, as eluent) to give compound **12** as a pale-yellow oil (0.95 g, 32% yield). ^{1}H NMR (90 MHz): δ 2.05–2.45 (m, 2H, C*H$_2$*CH$_2$Br), 3.05–3.60 (m, 4H, ArC*H$_2$*, CH$_2$-Br), 3.95 (s, 3H, CH$_3$), 6.75–8.35 (m, 6H, aromatic). GC/MS: *m/z* 280 (M$^+$ + 2, 56), 279 (M$^+$ + 1, 10), 278 (M$^+$, 58), 199 (69), 171 (100), 128 (35).

Methyl 4-(3-methoxyphenyl)-2-butenoate (15). Diisobutylaluminum hydride (1.0 M in toluene, 0.3 mL, 0.3 mmol) was added by syringe to a suspension of bis(triphenylphosphine)-palladium(II) chloride (0.093 g, 0.14 mmol) in anhydrous THF (30 mL). The mixture was stirred for 5 min at room temperature under N$_2$, then a solution of methyl 4-bromocrotonate (mixture of isomers, 3.13 g, 17.5 mmol) and (3-methoxyphenyl)-tributyl stannate (**14**) (6.95 g, 17.5 mmol) in the same solvent (15 mL) was added dropwise. The mixture was refluxed overnight, then the solvent was removed under reduced pressure. The crude residue was chromatographed (petroleum ether/AcOEt, 9:1, as eluent) to give ester **15** (mixture of isomers) as a colorless oil (2.06 g, 57% yield). GC/MS: *m/z* 207 (M$^+$ + 1, 5), 206 (M$^+$, 35), 147 (47), 146 (100), 91 (40).

4-(3-Methoxyphenyl)-1-butanol (16). A solution of ester **15** (2.04 g, 9.9 mmol) in anhydrous THF was added dropwise to a cooled suspension of LiAlH$_4$ (0.75 g, 19.8 mmol) in the same solvent. The resulting suspension was stirred for 3 h at room temperature. Then the mixture was cooled at 0 °C, and a few drops of H$_2$O were added to destroy the excess hydride. The mixture was filtered, and the solvent was evaporated under reduced pressure to give a crude residue that was chromatographed (petroleum ether/AcOEt, 1:1, as eluent). Pure **16** was obtained as a colorless oil in 56% yield. Spectral properties of this compound were fully consistent with those reported in the literature.[30]

4-(3-Methoxyphenyl)-1-butyl Methanesulfonate (17). Triethylamine (1.1 mL, 8.0 mmol) and methanesulfonyl chloride (0.5 mL, 6.5 mmol) were added to a solution of **16** (0.95 g, 5.3 mmol) in CH$_2$Cl$_2$ cooled at −10 °C. The mixture was stirred at room temperature for 6 h. Then the reaction mixture was

Table 4. Agonistic Activity (ED$_{50}$ ± SE*^{a}* in μg/kg iv) of Compounds *trans*-**8a** Tested on Postsynaptic 5-HT$_{1A}$ Receptors (Induction of Fore-paw Treading in Rats)

	ED$_{50}$ ± SE for fore-paw treading induction in rats	
compound	iv	subcutaneous
trans-**8a**	0.721 ± 0.005	1.957 ± 0.203
8-OH-DPAT	0.084 ± 0.012	0.137 ± 0.011

^{a} SE ≡ standard error.

first washed with a saturated aqueous solution of NaHCO$_3$ and then with 3 N HCl. The separated organic phase was dried over Na$_2$SO$_4$ and concentrated under reduced pressure, yielding a crude residue. Pure **17** was obtained after chromatography (petroleum ether/AcOEt, 4:1, as eluent) as a colorless oil in 44% yield. ^{1}H NMR (90 MHz): δ 1.60–1.90 [m, 4H, CH$_2$-(C*H$_2$*)$_2$CH$_2$], 2.50–2.75 (m, 2H, ArC*H$_2$*), 2.95 (s, 3H, SO$_2$CH$_3$), 3.78 (s, 3H, OCH$_3$), 4.21 (m, 2H, CH$_2$O), 6.60–7.35 (m, 4H, aromatic). GC/MS: *m/z* 260 (M$^+$ + 2, 2), 259 (M$^+$ + 1, 5), 258 (M$^+$, 39), 161 (49), 134 (100), 121 (72).

N-Benzyl-2-(2-pyridyloxy)ethylamine (19). A mixture of 2-chloropyridine (4.00 g, 35.2 mmol), *N*-benzylethanolamine (**18**) (12.70 g, 84.0 mmol), powdered KOH (3.95 g, 70.4 mmol), and 18-crown-6 (3.70 g, 14.0 mmol) in toluene (100 mL) was vigorously stirred under reflux. After 6 h, the reaction mixture was cooled and washed with H$_2$O (40 mL). The separated aqueous phase was extracted with Et$_2$O (40 mL), and the combined organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/CH$_3$OH, 19:1, as eluent) to give amine **19** as a yellow oil (7.08 g, 88% yield). ^{1}H NMR (90 MHz): δ 1.91 (s, 1H, NH, D$_2$O exchanged), 2.98 (t, 2H, *J* = 6.0 Hz, C*H$_2$*-CH$_2$), 3.87 (s, 2H, benzylic), 4.45 (t, 2H, *J* = 6.0 Hz, CH$_2$O), 6.65–8.25 (m, 9H, aromatic). GC/MS: *m/z* 133 (84), 132 (54), 120 (22), 91 (100).

Ethyl 6-Methoxy-1,2,3,4-tetrahydro-2-naphthalenecarboxylate (22). A solution of 6-methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (**21**) (1.75 g, 8.5 mmol) in anhydrous ethanol (50 mL) was refluxed overnight in the presence of concentrated H$_2$SO$_4$ (0.5 mL). Then the solvent was removed under reduced pressure, and the residue was partitioned between CHCl$_3$ and 20% aqueous Na$_2$CO$_3$. The separated organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was chromatographed (petroleum ether/AcOEt, 4:1, as eluent) to give ester **22** as a colorless oil (1.76 g, 88% yield). ^{1}H NMR (90 MHz): δ 1.23 (t, 3H, *J* = 6.0 Hz, CH$_2$C*H$_3$*), 1.50–3.05 (m, 7H, tetralinic), 3.75 (s, 3H, OCH$_3$), 4.15 (q, 2H, *J* = 6.0 Hz, C*H$_2$*CH$_3$), 6.50–7.15 (m, 3H, aromatic).

6-Methoxy-1,2,3,4-tetrahydro-2-naphthalenemethanol (23). Alcohol **23** was prepared from ester **22** by reduction with LiAlH$_4$ through the same procedure reported for the synthesis of compound **16**. Pure **23** was obtained by column chromatography (petroleum ether/AcOEt, 3:2, as eluent) as a colorless oil in 76% yield. Spectral properties of this compound were fully consistent with those reported in the literature.[31]

6-Methoxy-1,2,3,4-tetrahydro-2-naphthalenemethyl Methanesulfonate (24). Title compound was prepared from alcohol **23** following the same procedure described above for compound **17**. Pure **24** was obtained after workup as a colorless oil in 80% yield. ^{1}H NMR (90 MHz): δ 1.85–2.90 (m, 7H, tetralinic), 3.00 (s, 3H, SO$_2$CH$_3$), 3.77 (s, 3H, OCH$_3$), 4.18 (d, 2H, *J* = 6.0 Hz, CH$_2$O), 6.55–7.15 (m, 3H, aromatic). GC/MS: *m/z* 272 (M$^+$ + 2, 4), 271 (M$^+$ + 1, 10), 270 (M$^+$, 59), 174 (59), 159 (100).

General Procedure for the Preparation of Compounds 3a, 3b, 4a, 5a, 5b, 6a, and 20. A stirred mixture of alkylating agent (2.0 mmol), amine (4.0 mmol), and a slight excess of K$_2$CO$_3$ in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H$_2$O was added to the residue. The aqueous phase was extracted twice with AcOEt. The collected organic layers were dried over Na$_2$SO$_4$ and evaporated under reduced pressure. The crude residue was chromatographed as indicated below to give the target compounds.

4-[3-(5-Methoxy-1-naphthalenyl)propyl]-1-(2-pyridinyl)-piperazine (3a). Eluted with CHCl$_3$/AcOEt, 1:1; 44% yield. ^{1}H NMR: δ 1.92−2.03 (m, 2H, CH$_2$CH_2CH$_2$), 2.46−2.58 [m, 6H, benzylic, CH$_2$N(CH_2)$_2$], 3.09 [t, 2H, J = 7.7, CH_2N(CH$_2$)$_2$], 3.55 [br t, 4H, (CH_2)$_2$NAr], 3.98 (s, 3H, CH$_3$), 6.57−7.64 (m, 9H, aromatic), 8.13−8.18 (m, 1H, aromatic N=CH). GC/MS: m/z 362 (M$^+$ + 3), 361 (M$^+$, 12), 267 (39), 254 (44), 252 (34), 242 (22), 240 (35), 107 (100).

3-(5-Methoxy-1-naphthalenyl)-*N*-[2-(2-pyridyloxy)eth-yl]propanamine (3b). Eluted with CHCl$_3$/CH$_3$OH, 19:1; 60% yield. ^{1}H NMR: δ 1.88−2.01 (m, 3H, CH$_2$CH_2CH$_2$, NH, 1H D$_2$O exchanged), 2.78 (t, 2H, J = 7.2 Hz, benzylic), 2.98−3.02 (m, 2H, CH_2CH$_2$O), 3.09 (br t, 2H, CHHN), 3.98 (s, 3H, CH$_3$), 4.38−4.42 (m, 2H, CH$_2$CH_2O), 6.71−7.62 (m, 9H, aromatic), 8.10−8.17 (m, 1H, aromatic N=CH). GC/MS: m/z 336 (M$^+$, 1), 241 (100), 226 (42), 198 (38), 171 (30).

4-[4-(3-Methoxyphenyl)butyl]-1-(2-pyridinyl)pipera-zine (4a). Eluted with CHCl$_3$/AcOEt, 1:1; 83% yield. ^{1}H NMR: δ 1.50−1.73 [m, 4H, CH$_2$(CH_2)$_2$CH$_2$], 2.39 (t, 2H, J = 7.4 Hz, benzylic), 2.51−2.55 [br t, 4H, CH$_2$N(CH_2)$_2$], 2.60 [t, 2H, J = 7.3 Hz, CH_2N(CH$_2$)$_2$], 3.53 [br t, 4H, (CH_2)$_2$NAr], 3.78 (s, 3H, CH$_3$), 6.57−7.48 (m, 7H, aromatic), 8.15−8.18 (m, 1H, aromatic N=CH). GC/MS: m/z 326 (M$^+$ + 1), 325 (M$^+$, 16), 231 (42), 218 (32), 206 (28), 204 (53), 121 (44), 107 (100).

4-[(6-Methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)me-thyl]-1-(2-pyridinyl)piperazine (5a). Eluted with CHCl$_3$/AcOEt, 1:1; 60% yield. ^{1}H NMR: δ 1.39−1.41 (m, 1H, CHH-CHCHHCH$_2$), 1.95−2.02 (m, 2H, CHHCHCHHCH$_2$), 2.32−2.41 [m, 3H, CHHCHCHHCH$_2$, CH_2N(CH$_2$)$_2$], 2.52−2.59 [m, 4H, CH$_2$N(CH_2)$_2$], 2.77−2.91 (m, 3H, CHHCHCHHCH_2), 3.55 [br t, 4H, (CH_2)$_2$NAr], 3.75 (s, 3H, CH$_3$), 6.57−7.48 (m, 6H, aromatic), 8.16−8.19 (m, 1H, aromatic N=CH). GC/MS: m/z 338 (M$^+$ + 1, 5), 337 (M$^+$, 19), 243 (73), 230 (74), 176 (66), 147 (40), 121 (49), 107 (100).

N-[(6-Methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)me-thyl]-2-(2-pyridyloxy)ethylamine (5b). Eluted with CHCl$_3$/CH$_3$OH, 19:1; 20% yield. ^{1}H NMR: δ 1.34−1.45 (m, 1H, CHHCHCHHCH$_2$), 1.92−1.99 (m, 3H, CHHCHCHHCH$_2$, NH, 1H D$_2$O exchanged), 2.33−2.42 (m, 1H, CHHCHCHHCH$_2$), 2.68 (d, 2H, J = 6.7 Hz, CH_2NH), 2.76−2.87 (m, 3H, CHH-CHCHHCH_2), 3.01−3.05 (m, 2H, CH_2CH$_2$O), 3.74 (s, 3H, CH$_3$), 4.40−4.44 (m, 2H, CH$_2$CH_2O), 6.59−7.59 (m, 6H, aromatic), 8.11−8.14 (m, 1H, aromatic N=CH). GC/MS: m/z 313 (M$^+$ + 1, 2), 312 (M$^+$, 7), 217 (47), 174 (100), 159 (67), 151 (54), 122 (48).

4-[2-(7-Methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)-ethyl]-1-(2-pyridinyl)piperazine (6a). Eluted with CHCl$_3$/AcOEt, 1:1; 72% yield. ^{1}H NMR: δ 1.34−1.95 (m, 5H, CH$_2$-CHCH$_2$), 2.39−2.86 [m, 10H, CH$_2$N(CH$_2$)$_2$, benzylic], 3.55 (br t, 4H, (CH_2)$_2$NAr), 3.75 (s, 3H, CH$_3$), 6.58−7.48 (m, 6H, aromatic), 8.16−8.18 (m, 1H, aromatic N=CH). GC/MS: m/z 352 (M$^+$ + 1, 3), 351 (M$^+$, 10), 257 (33), 244 (51), 121 (29), 107 (100).

4-(3-Methoxyphenyl)-*N*-benzyl-*N*-[2-(2-pyridyloxy)eth-yl]butanamine (20). Eluted with CHCl$_3$/AcOEt, 1:1; 30% yield. ^{1}H NMR (90 MHz): δ 1.35−1.85 [m, 4H, CH$_2$(CH_2)$_2$CH$_2$], 2.00 [br s, 2H, benzylic], 2.35−2.73 (m, 2H, CH$_2$N), 2.83 (t, 2H, J = 6.0 Hz, OCH$_2$CH_2), 3.70 (s, 2H, CH_2Ph), 3.78 (s, 3H, CH$_3$), 4.30 (t, 2H, J = 6.0 Hz, OCH_2CH$_2$), 6.55−8.25 (m, 13H, aromatic). GC/MS: m/z 282 (40), 280 (86), 91 (100).

4-(3-Methoxyphenyl)-*N*-[2-(2-pyridyloxy)ethyl]butan-amine (4b). The compound 20 (0.51 g, 1.3 mmol) was dissolved in ethanol and hydrogenated at normal pressure and room temperature in the presence of 10% Pd/C (0.1 g) until the uptake ceased. The catalyst was removed by filtration through Celite, and the solvent was evaporated in vacuo to give a crude residue that was chromatographed (CHCl$_3$/CH$_3$OH, 19:1, as eluent) to provide pure compound 4a as a pale-yellow oil (0.12 g, 31% yield). ^{1}H NMR: δ 1.51−1.76 [m, 4H, CH$_2$(CH_2)$_2$CH$_2$], 1.80 (s, 1H, NH, D$_2$O exchanged), 2.59 (t, 2H, J = 7.4 Hz, benzylic), 2.68 (t, 2H, J = 7.1 Hz, CH_2HN), 2.96−3.00 (m, 2H, CH_2CH$_2$O), 3.77 (s, 3H, CH$_3$), 4.38 (t, 2H, J = 5.2, CH$_2$CH_2O), 6.63−7.57 (m, 7H, aromatic), 8.09−8.12 (m, 1H, aromatic N=CH). GC/MS: m/z 121 (43), 78 (34), 58 (100).

General Procedure for the Preparation of Compounds 26a−e. A mixture of 1,4-cyclohexanedione mono-ethylene ketal (25) (20 mmol) and the appropriate amine (24 mmol) in anhydrous toluene (100 mL) was refluxed overnight in the presence of a catalytic amount of *p*-toluenesolfonic acid, and the formed water was azeotropically distilled off and collected by a Dean−Stark trap. After the mixture was cooled, the solvent was evaporated, the crude enamine was solubilized in anhydrous THF, and gaseous HCl was added until precipitation of enamine hydrochloride was complete. Then to the suspension was added in one portion a solution of NaBH$_3$CN (19 mmol) in 10 mL of absolute CH$_3$OH under stirring. The resulting solution was stirred for 30 min at 25 °C. Then the solvent was concentrated in vacuo and the residue was taken up with 0.1 N KOH (60 mL). The aqueous suspension was extracted with AcOEt (2 × 30 mL). The combined organic phases were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/AcOEt, 1:1, as eluent) to give compounds 26a−e as semisolids in 70−80% yield.

8-[4-(2-Pyridinyl)piperazin-1-yl]-1,4-dioxaspiro-[4,5]-decane (26a). ^{1}H NMR: δ 1.45−2.00 (m, 8H, cyclohexyl CH$_2$), 2.13 (br s, 1H, CH), 2.73 [br t, 4H, CHN(CH_2)$_2$], 3.60 [m, 4H, (CH_2)$_2$NAr], 3.95 [s, 4H, O(CH$_2$)$_2$], 6.53−7.63 (m, 3H, aromatic), 8.20−8.33 (m, 1H, aromatic N=CH). GC/MS: m/z 305 (M$^+$ + 2, 1), 304 (M$^+$ + 1, 9), 303 (M$^+$, 41), 209 (41), 196 (47), 184 (62), 107 (100).

8-[*N*-Benzyl-2-(2-pyridyloxy)ethylamino]-1,4-dioxaspiro-[4,5]-decane (26b). ^{1}H NMR (90 MHz): δ 1.40−1.95 (m, 8H, cyclohexyl CH$_2$), 2.53−2.80 (m, 1H, CH), 2.95 (t, 2H, J = 6.0 Hz, NCH_2CH$_2$), 3.80 (s, 2H, benzylic), 3.95 [s, 4H, O(CH$_2$)$_2$], 4.30 (t, 2H, J = 6.0 Hz, NCH$_2$CH_2), 6.60−7.67 (m, 9H, aromatic), 8.00−8.25 (m, 1H, aromatic N=CH). GC/MS: m/z 273 (79), 272 (29), 260 (91), 134 (24), 91 (100).

8-(4-Phenylpiperazin-1-yl)-1,4-dioxaspiro-[4,5]-de-cane (26c). ^{1}H NMR (90 MHz): δ 1.50−2.00 (m, 8H, cyclo-hexyl CH$_2$), 2.43 (br s, 1H, CH), 2.66−2.85 [m, 4H, CHN-(CH_2)$_2$], 3.30−3.33 [m, 4H, (CH_2)$_2$NAr], 3.95 [s, 4H, O(CH$_2$)$_2$], 6.80−7.47 (m, 5H, aromatic). GC/MS: m/z 304 (M$^+$ + 2, 3), 303 (M$^+$ + 1, 27), 302 (M$^+$, 81), 209 (41), 201 (49), 132 (33), 101 (100).

8-[4-(2-Methoxyphenyl)piperazin-1-yl]-1,4-dioxaspiro-[4,5]-decane (26d). ^{1}H NMR (90 MHz): δ 1.55−2.15 (m, 8H, cyclohexyl CH$_2$), 2.70 (br s, 1H, CH), 2.90−3.10 [m, 4H, CHN-(CH_2)$_2$], 3.18−3.35 [m, 4H, (CH_2)$_2$NAr], 3.90 (s, 3H, CH$_3$), 4.00 [s, 4H, O(CH$_2$)$_2$], 7.03 (br s, 4H, aromatic). GC/MS: m/z 334 (M$^+$ + 2, 4), 333 (M$^+$ + 1, 28), 332 (M$^+$, 100), 231 (96), 162 (33), 149 (35).

8-[4-(1-Naphthalenyl)piperazin-1-yl]-1,4-dioxaspiro-[4,5]-decane (26e). ^{1}H NMR (90 MHz): δ 1.45−2.15 (m, 8H, cyclohexyl CH$_2$), 2.50 (br s, 1H, CH), 2.75−2.95 [m, 4H, CHN-(CH_2)$_2$], 3.03−3.28 [m, 4H, (CH_2)$_2$NAr], 3.95 [s, 4H, O(CH$_2$)$_2$], 7.00−8.23 (m, 7H, aromatic). GC/MS: m/z 354 (M$^+$ + 2, 3), 353 (M$^+$ + 1, 26), 352 (M$^+$, 99), 251 (52), 154 (31), 101 (100).

General Procedure for the Preparation of Ketones 27a−e. In a typical run, one of compounds 26a−e (10 mmol) was refluxed for 3 h with 3 N HCl (50 mL) in acetone. Then the mixture was concentrated under reduced pressure and the aqueous residue was alkalized with 20% aqueous Na$_2$CO$_3$. The aqueous phase was extracted with AcOEt (2 × 30 mL). The organic phase was separated, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/AcOEt, 1:1, as eluent) to give the corresponding ketone 27 as a white semisolid in 90% yield.

4-[4-(2-Pyridinyl)piperazin-1-yl]cyclohexan-1-one (27a). ^{1}H NMR (90 MHz): δ 1.65−2.60 (m, 9H, cyclohexyl), 2.73 [br t, 4H, CHN(CH_2)$_2$], 3.60 [br t, 4H, (CH_2)$_2$NAr], 6.55−7.70 (m, 3H, aromatic), 8.15−8.35 (m, 1H, aromatic N=CH). GC/MS: m/z 260 (M$^+$ + 1, 2), 259 (M$^+$, 10), 107 (100), 140 (24).

4-[*N*-Benzyl-2-(2-pyridyloxy)ethylamino]cyclohexan-1-one (27b). ^{1}H NMR (90 MHz): δ 1.55−2.60 (m, 9H, cyclohexyl), 2.93 (t, 2H, J = 6.0 Hz, NCH_2CH$_2$), 3.80 (s, 2H, benzylic), 4.35 (t, 2H, J = 6.0 Hz, NCH$_2$CH_2), 6.60−7.70 (m,

8H, aromatic), 8.07–8.27 (m, 1H, aromatic N=CH). GC/MS: *m/z* 229 (42), 216 (35), 91 (100).

4-(4-Phenylpiperazin-1-yl)cyclohexan-1-one (27c). ^{1}H NMR (90 MHz): δ 1.65–2.55 (m, 9H, cyclohexyl), 2.80 [br t, 4H, CHN(C*H$_2$*)$_2$], 3.27 [br t, 4H, (C*H$_2$*)$_2$NAr], 6.80–7.50 (m, 5H, aromatic). GC/MS: *m/z* 260 (M$^+$ + 2, 1), 259 (M$^+$ + 1, 14), 258 (M$^+$, 68), 201 (100), 173 (64), 132 (47).

4-[4-(2-Methoxyphenyl)piperazin-1-yl]cyclohexan-1-one (27d). ^{1}H NMR (90 MHz): δ 1.70–2.67 (m, 9H, cyclohexyl), 2.80 [br t, 4H, CHN(C*H$_2$*)$_2$], 3.15 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.87 (s, 3H, CH$_3$), 6.97 (br s, 4H, aromatic). GC/MS: *m/z* 290 (M$^+$ + 2, 3), 289 (M$^+$ + 1, 22), 288 (M$^+$, 95), 231 (100), 203 (66), 162 (48), 136 (68).

4-[4-(1-Naphthalenyl)piperazin-1-yl]cyclohexan-1-one (27e). ^{1}H NMR (90 MHz): δ 1.55–2.75 (m, 9H, cyclohexyl), 2.90 [br t, 4H, CHN(C*H$_2$*)$_2$], 3.23 [br t, 4H, (C*H$_2$*)$_2$NAr], 7.05–8.40 (m, 7H, aromatic). GC/MS: *m/z* 310 (M$^+$ + 2, 3), 309 (M$^+$ + 1, 25), 308 (M$^+$, 100), 251 (96), 223 (53), 154 (45).

General Procedure for the Synthesis of Alkenes 7a–e, 28a, and 29a. In a typical run, to a stirred solution of Grignard reagent prepared from Mg turnings (24 mmol) and the appropriate bromoanisole (15.9 mmol) in anhydrous THF (30 mL) was added dropwise one of ketones **27a–e** (11.6 mmol) in the same solvent (15 mL), and the mixture was refluxed for 7–8 h. After the mixture was cooled at room temperature, a cooled saturated aqueous solution of NH$_4$Cl (40 mL) was added to the reaction mixture. Extraction with Et$_2$O and evaporation of the organic layer gave a crude residue that was warmed at 70 °C for 6 h with 20% H$_2$SO$_4$ (30 mL). After the mixture was cooled, the aqueous solution was alkalized with 10% aqueous NaOH and the suspension was extracted with AcOEt (2 × 10 mL). The collected organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/AcOEt, 1:1, as eluent) to give the corresponding title compound as a white solid in 10–15% yield.

4-[1-(3-Methoxyphenyl)cyclohexen-4-yl]-1-(2-pyridinyl)-piperazine (7a). ^{1}H NMR: δ 1.57–1.69, 2.13–2.29, and 2.39–2.81 [m, 11H, cyclohexene, CHN(C*H$_2$*)$_2$], 3.58 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.79 (s, 3H, CH$_3$), 6.06 (t, 1H, *J* = 2.7 Hz, vinylic), 6.57–7.49 (m, 7H, aromatic), 8.17–8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 350 (M$^+$ + 1, 5), 349 (M$^+$, 18), 242 (32), 107 (100).

N-Benzyl-N-[1-(3-methoxyphenyl)cycloexen-4-yl]-2-(2-pyridyloxy)ethylamine (7b). ^{1}H NMR (90 MHz): δ 1.55–2.65 (m, 7H, cyclohexene), 2.97 (t, 2H, *J* = 6.0 Hz, C*H$_2$*CH$_2$O), 3.75 (s, 2H, benzylic), 3.80 (s, 3H, CH$_3$), 4.33 (t, 2H, *J* = 6.0 Hz, CH$_2$C*H$_2$*O), 6.00–6.25 (m, 1H, vinylic), 6.75–7.65 (m, 12H, aromatic), 8.17–8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 415 (M$^+$ + 1, 2), 414 (M$^+$, 5), 319 (34), 306 (27), 278 (23), 159 (100), 158 (32).

4-[1-(3-Methoxyphenyl)cyclohexen-1-yl]-1-phenylpiperazine (7c). ^{1}H NMR: δ 1.55–1.69, 2.14–2.27, and 2.29–2.85 [m, 11H, cyclohexene, CHN(C*H$_2$*)$_2$], 3.23 [br t, 4H, (C*H$_2$*)$_2$-NAr], 6.08 (t, 1H, *J* = 2.6 Hz, vinylic), 6.75–7.28 (m, 9H, aromatic). GC/MS: *m/z* 350 (M$^+$ + 2, 2), 349 (M$^+$ + 1, 13), 348 (M$^+$, 48), 188 (100), 132 (95).

4-[1-(3-Methoxyphenyl)cyclohexen-4-yl]-1-(2-methoxy-phenyl)piperazine (7d). ^{1}H NMR: δ 1.57–1.71, 2.13–2.46, and 2.50–2.87 [m, 11H, cyclohexene, CHN(C*H$_2$*)$_2$], 3.15 [br s, 4H, (C*H$_2$*)$_2$NAr], 3.80 and 3.86 (2 s, 6H, 2 CH$_3$), 6.07 (t, 1H, *J* = 2.5 Hz, vinylic), 6.74–7.24 (m, 8H, aromatic). GC/MS: *m/z* 380 (M$^+$ + 2, 2), 379 (M$^+$ + 1, 16), 378 (M$^+$, 58), 218 (97), 162 (100), 149 (47).

4-[1-(3-Methoxyphenyl)cyclohexen-4-yl]-1-(1-naphtha-lenyl)piperazine (7e). ^{1}H NMR: δ 1.65–1.75, 2.22–2.37, and 2.49–2.94 [m, 11H, cyclohexene, CHN(C*H$_2$*)$_2$], 3.20 [br s, 4H, (C*H$_2$*)$_2$NAr], 3.81 (s, 3H, CH$_3$), 6.11 (t, 1H, *J* = 2.4 Hz, vinylic), 6.76–8.23 (m, 11H, aromatic). GC/MS: *m/z* 400 (M$^+$ + 2, 3), 399 (M$^+$ + 1, 13), 398 (M$^+$, 42), 238 (100), 237 (30), 182 (69), 169 (35).

4-[1-(2-Methoxyphenyl)cyclohexen-4-yl]-1-(2-pyridinyl)-piperazine (28a). ^{1}H NMR (90 MHz): δ 1.45–2.90 [m, 11H, cyclohexene, CHN(C*H$_2$*)$_2$], 3.55 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.80 (s, 3H, CH$_3$), 5.77 (br s, 1H, vinylic), 6.50–7.68 (m, 7H, aromatic), 8.17–8.30 (m, 1H, aromatic N=CH). GC/MS: *m/z* 351 (M$^+$ + 2, 1), 350 (M$^+$ + 1, 4), 349 (M$^+$, 14), 242 (38), 107 (100).

4-[1-(4-Methoxyphenyl)cyclohexen-4-yl]-1-(2-pyridinyl)-piperazine (29a). ^{1}H NMR (90 MHz): δ 1.45–2.90 [m, 11H, cyclohexene, CHN(C*H$_2$*)$_2$], 3.60 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.83 (s, 3H, CH$_3$), 6.03 (br s, 1H, vinylic), 6.50–7.17 (m, 7H, aromatic), 8.18–8.33 (m, 1H, aromatic N=CH). GC/MS: *m/z* 350 (M$^+$ + 1, 2), 349 (M$^+$, 9), 242 (24), 189 (28), 120 (25), 107 (100).

General Procedure for the Synthesis of Compounds 8a–e, 9a, and 10a. One of the alkenes **7a–e**, **28a**, or **29a** (1.3 mmol) was dissolved in ethanol and hydrogenated at normal pressure and room temperature in the presence of 10% Pd/C (0.1 g) until the uptake ceased. The catalyst was removed by filtration through Celite, and the solvent was evaporated in vacuo to give a crude residue. The latter was chromatographed using CH$_2$Cl$_2$/AcOEt, 1:1, as eluent containing 0.1% ammonia to give the cis isomer as the faster-moving component (fraction A) and the trans isomer as the slower-moving component (fraction B).[32] Each amine was obtained in 25–30% yield.

cis-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-(2-pyridinyl)-piperazine (cis-8a). ^{1}H NMR: δ 1.52–1.63 [m, 4H, CH-(CH*H*CH*H*)$_2$], 1.97–2.05 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.31 [br s, 1H, C*H*N(CH$_2$)$_2$], 2.61–2.71 [m, 5H, benzylic CH, CHN(C*H$_2$*)$_2$], 3.55 [br s, 4H, (C*H$_2$*)$_2$NAr], 3.78 (s, 3H, CH$_3$), 6.58–7.49 (m, 7H, aromatic), 8.16–8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 352 (M$^+$ + 1, 4), 351 (M$^+$, 16), 257 (33), 232 (62), 188 (52), 107 (100).

trans-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-(2-pyridi-nyl)piperazine (trans-8a). ^{1}H NMR: δ 1.41–1.56 [m, 4H, CH(CH*H*CH*H*)$_2$], 1.92–2.08 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.42–2.48 [m, 2H, benzylic CH, C*H*N(CH$_2$)$_2$], 2.73 [br t, 4H, CHN-(C*H$_2$*)$_2$], 3.58 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.78 (s, 3H, CH$_3$), 6.58–7.45 (m, 7H, aromatic), 8.16–8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 352 (M$^+$ + 1, 5), 351 (M$^+$, 20), 257 (35), 244 (41), 232 (59), 230 (28), 188 (31), 121 (35), 107 (100).

cis-N-[4-(3-Methoxyphenyl)cyclohexyl]-2-(2-pyridyloxy)-ethylamine (cis-8b). ^{1}H NMR: δ 1.55–1.88 [m, 9H, CH-(C*H*HC*H*H)$_2$, NH, 1H D$_2$O exchanged], 2.48–2.58 (m, 1H, C*H*NH), 2.94 (br t, 1H, benzylic CH), 2.99 (t, 2H, *J* = 5.4 Hz, C*H$_2$*CH$_2$O), 3.78 (s, 3H, CH$_3$), 4.42 (t, 2H, *J* = 5.3 Hz, CH$_2$C*H$_2$*O), 6.68–7.58 (m, 8H, aromatic), 8.11–8.14 (m, 1H, aromatic N=CH). GC/MS: *m/z* 231 (61), 188 (100), 134 (52), 124 (38).

trans-N-[4-(3-Methoxyphenyl)cyclohexyl]-2-(2-pyri-doxy)ethylamine (trans-8b). ^{1}H NMR: δ 1.27 [dq, 2H, *J* = 11.2, 2.9 Hz, axial ArCH(CH*H*CH*H*)$_2$], 1.49 [dq, 2H, *J* = 12.9, 2.6 Hz, axial ArCH(CH*H*CHH)$_2$], 1.79 (br s, 1H, NH, D$_2$O exchanged], 1.92 [app br d, 2H, equatorial ArCH(CHHC*H*H)$_2$], 1.92 [app br d, 2H, equatorial ArCH(CHHC*H*H)$_2$], 2.07 [app br d, 2H, equatorial ArCH(C*H*HCHH)$_2$], 2.48 [dt, *J* = 12.0, 3.4 Hz, 1H, axial C*H*N(CH$_2$)$_2$], 2.59 [dt, *J* = 11.0, 3.7 Hz, 1H, benzylic CH], 3.06 (t, 2H, *J* = 5.2 Hz, C*H$_2$*CH$_2$O), 3.78 (s, 3H, CH$_3$), 4.41 (t, 2H, *J* = 5.2 Hz, CH$_2$C*H$_2$*O), 6.69–7.58 (m, 8H, aromatic), 8.11–8.14 (m, 1H, aromatic N=CH). GC/MS: *m/z* 231 (54), 188 (100), 134 (54), 121 (39).

cis-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-phenylpiper-azine (cis-8c). ^{1}H NMR: δ 1.53–1.61 [m, 4H, CH(CH*H*-CH*H*)$_2$], 1.96–2.15 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.32–2.34 [m, 1H, C*H*N(CH$_2$)$_2$], 2.64–2.71 [m, 5H, benzylic CH, CHN(C*H$_2$*)$_2$], 3.21 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.79 (s, 3H, CH$_3$), 6.69–7.28 (m, 9H, aromatic). GC/MS: *m/z* 352 (M$^+$ + 2, 2), 351 (M$^+$ + 1, 16), 350 (M$^+$, 58), 201 (100), 132 (22).

trans-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-phenylpi-perazine (trans-8c). ^{1}H NMR: δ 1.41–1.58 [m, 4H, CH-(CH*H*CH*H*)$_2$], 1.97–2.10 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.42–2.50 [m, 2H, benzylic CH, C*H*N(CH$_2$)$_2$], 2.79 [br t, 4H, CHN(C*H$_2$*)$_2$], 3.23 [br t, 4H, (C*H$_2$*)$_2$NAr], 3.78 (s, 3H, CH$_3$), 6.70–7.28 (m, 9H, aromatic). GC/MS: *m/z* 352 (M$^+$ + 2, 2), 351 (M$^+$ + 1, 14), 350 (M$^+$, 53), 201 (100), 132 (19).

cis-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-(2-methoxy-phenyl)piperazine (*cis*-8d). ^{1}H NMR: δ 1.52−1.60 [m, 4H, CH(CH*H*CH*H*)$_2$], 1.97−2.05 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.34 [br s, 1H, C*H*N(CH$_2$)$_2$], 2.64−2.70 [m, 5H, benzylic CH, CHN-(C*H*$_2$)$_2$], 3.09 [br s, 4H, (C*H*$_2$)$_2$NAr], 3.79 and 3.85 (2 s, 6H, 2 CH$_3$), 6.69−7.24 (m, 8H, aromatic). GC/MS: *m/z* 382 (M$^+$ + 2, 4), 381 (M$^+$ + 1, 26), 380 (M$^+$, 80), 232 (23), 231 (100), 162 (26), 149 (28).

trans-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-(2-meth-oxyphenyl)piperazine (*trans*-8d). ^{1}H NMR: δ 1.38−1.58 [m, 4H, CH(CH*H*CH*H*)$_2$], 1.97−2.11 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.42−2.49 [m, 2H, benzylic CH, C*H*N(CH$_2$)$_2$], 2.82 [br t, 4H, CHN(C*H*$_2$)$_2$], 3.12 [br s, 4H, (C*H*$_2$)$_2$NAr], 3.78 and 3.85 (2 s, 6H, 2 CH$_3$), 6.70−7.24 (m, 8H, aromatic). GC/MS: *m/z* 382 (M$^+$ + 2, 3), 381 (M$^+$ + 1, 18), 380 (M$^+$, 68), 232 (18), 231 (100), 162 (21), 149 (23).

cis-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-(1-naphthale-nyl)piperazine (*cis*-8e). ^{1}H NMR: δ 1.63−1.75 [m, 4H, CH-(CH*H*CH*H*)$_2$], 1.99−2.12 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.45 [br s, 1H, C*H*N(CH$_2$)$_2$], 2.65−2.82 [m, 5H, benzylic CH, CHN(C*H*$_2$)$_2$], 3.17 [br s, 4H, (C*H*$_2$)$_2$NAr], 3.80 (s, 3H, CH$_3$), 6.70−8.20 (m, 11H, aromatic). GC/MS: *m/z* 402 (M$^+$ + 2, 3), 401 (M$^+$ + 1, 19), 400 (M$^+$, 63), 252 (20), 251 (100).

trans-4-[4-(3-Methoxyphenyl)cyclohexyl]-1-(1-naph-thalenyl)piperazine (*trans*-8e). ^{1}H NMR: δ 1.48−1.5 [m, 4H, CH(CH*H*CH*H*)$_2$], 2.00−2.10 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.45−2.55 [m, 2H, benzylic CH, C*H*N(CH$_2$)$_2$], 2.92 [br s, 4H, CHN(C*H*$_2$)$_2$], 3.18 [br s, 4H, (C*H*$_2$)$_2$NAr], 3.80 (s, 3H, CH$_3$), 6.71−8.22 (m, 11H, aromatic). GC/MS: *m/z* 402 (M$^+$ + 2, 3), 401 (M$^+$ + 1, 18), 400 (M$^+$, 58), 252 (19), 251 (100).

cis-4-[4-(2-Methoxyphenyl)cyclohexyl]-1-(2-pyridinyl)-piperazine (*cis*-9a). ^{1}H NMR: δ 1.52−1.62 [m, 4H, CH-(CH*H*CH*H*)$_2$], 1.83−2.10 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.28 [br s, 1H, C*H*N(CH$_2$)$_2$], 2.61 [br t, 4H, CHN(C*H*$_2$)$_2$], 3.04−3.14 (m, 1H, benzylic CH), 3.54 [br s, 4H, (C*H*$_2$)$_2$NAr], 3.80 (s, 3H, CH$_3$), 6.57−7.49 (m, 7H, aromatic), 8.17−8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 353 (M$^+$ + 2, 1), 352 (M$^+$ + 1, 4), 351 (M$^+$, 17), 257 (34), 244 (56), 232 (80), 188 (39), 134 (33), 121 (59), 107 (100).

trans-4-[4-(2-Methoxyphenyl)cyclohexyl]-1-(2-pyridi-nyl)piperazine (*trans*-9a). ^{1}H NMR: δ 1.44−1.56 [m, 4H, CH(CH*H*CH*H*)$_2$], 1.90−2.07 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.47 [br t, 1H, C*H*N(CH$_2$)$_2$], 2.76 [br t, 4H, CHN(C*H*$_2$)$_2$], 2.87−2.92 (m, 1H, benzylic CH), 3.58 [br t, 4H, (C*H*$_2$)$_2$NAr], 3.80 (s, 3H, CH$_3$), 6.58−7.49 (m, 7H, aromatic), 8.17−8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 353 (M$^+$ + 2, 1), 352 (M$^+$ + 1, 5), 351 (M$^+$, 19), 257 (30), 244 (48), 232 (71), 230 (32), 188 (27), 134 (25), 121 (55), 107 (100).

cis-4-[4-(4-Methoxyphenyl)cyclohexyl]-1-(2-pyridinyl)-piperazine (*cis*-10a). ^{1}H NMR 1.51−1.63 [m, 4H, CH-(CH*H*CH*H*)$_2$], 1.90−2.00 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.29 [br s, 1H, C*H*N(CH$_2$)$_2$], 2.58−2.66 [m, 5H, benzylic CH, CHN(C*H*$_2$)$_2$], 3.53 [br t, 4H, (C*H*$_2$)$_2$NAr], 3.77 (s, 3H, CH$_3$), 6.57−7.48 (m, 7H, aromatic), 8.17−8.19 (m, 1H, aromatic N=CH). GC/MS: *m/z* 353 (M$^+$ + 2, 1), 352 (M$^+$ + 1, 4), 351 (M$^+$, 15), 257 (31), 244 (39), 232 (60), 188 (30), 134 (45), 121 (45), 107 (100).

trans-4-[4-(4-Methoxyphenyl)cyclohexyl]-1-(2-pyridi-nyl)piperazine (*trans*-10a). ^{1}H NMR: δ 1.39−1.49 [m, 4H, CH(CH*H*CH*H*)$_2$], 1.94−2.15 [m, 4H, CH(C*H*HC*H*H)$_2$], 2.38−2.42 [m, 2H, benzylic CH, C*H*N(CH$_2$)$_2$], 2.72 [br t, 4H, CHN-(C*H*$_2$)$_2$], 3.55 [br t, 4H, (C*H*$_2$)$_2$NAr], 3.76 (s, 3H, CH$_3$), 6.58−7.48 (m, 7H, aromatic), 8.16−8.18 (m, 1H, aromatic N=CH). GC/MS: *m/z* 353 (M$^+$ + 2, 1), 352 (M$^+$ + 1, 4), 351 (M$^+$, 15), 257 (29), 244 (34), 232 (51), 230 (21), 134 (30), 121 (42), 107 (100).

Partition Coefficient. The experimental partition coefficient of *trans*-8a was measured with the shake-flask method as described in the literature.[33] 1-Octanol saturated with water buffer and water buffer (50 mM sodium phosphate buffer, pH 7.4) saturated with 1-octanol were used. The sample (8−17 mg, free base) was shaken overnight with a mixture of 1-octanol (4 mL) and water buffer (4 mL). After centrifugation (3000 rpm × 10 min) of the mixture, the two layers were separated and the concentration of the partitioned substance in the aqueous layer was quantified by HPLC (column Phenomenex Prodigy ODS-3 RP-18 5 μm, 4.6 mm × 250 mm; mobile phase CH$_3$OH/H$_2$O/EtN$_3$, 9:1:0.05; λ = 249 nm; flow rate, 1 mL/min). The concentration of the compound in the 1-octanol layer was assumed to be unchanged after shaking. The mean log *P* value was determined after three separate determinations.

Biological Methods. 1. General. 8-OH-DPAT·HBr was from RBI (Research Biochemicals International, Natick, MA). GDP and GTPγS were from SIGMA-Aldrich (Milan, Italy). 5-HT was from Merck (Milan, Italy). [^{3}H]Prazosin, [^{3}H]8-OH-DPAT, and [^{35}S]guanosine-5′-(γ-thio)triphosphate ([^{35}S]GTPγS) were obtained from NEN Life Science Products (Milan, Italy).

For receptor binding studies, the compounds were dissolved in absolute alcohol. For iv administration in rats and mice, *trans*-8a was dissolved in *N,N*-dimethylformamide (20% v/v) and TWEEN 80 (10% v/v) in demineralized water, 1a was dissolved in *N,N*-dimethylformamide (4% v/v) and TWEEN 80 (8% v/v) in demineralized water, and 8-OH-DPAT was dissolved in demineralized water.

Male Wistar Hannover rats (200−250 g) from Harlan, Italy, were used for the binding experiments on rat brain homogenates. Male Sprague Dawley rats (Crl:CD(SD)BR, 175−200 g body weight (bw)), and male mice (Crl:CD-1(ICR)BR, 28−38 g bw) from Charles River, Italy, were used for the in vivo experiments. Animals were housed with free access to food and water and were maintained on a forced 12 h light−dark cycle at 22−24 °C for at least 1 week before the experiments were carried out. The animals were handled according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, O.J. No. L358, December 18, 1986).

2. Radioligand Binding Assay at Rat Hippocampal Membranes 5-HT$_{1A}$ Receptors. Binding experiments were performed according to Borsini et al.[34] with minor modifications. Rats were killed by decapitation, the brain was quickly removed, and the hippocampus was dissected. The hippocampus (1.0 g) was homogenized with a Brinkman polytron (setting 5 for 3 × 15 s) in 25 mL of 50 mM Tris buffer, pH 7.6. The homogenate was centrifuged at 48 000*g* for 15 min at 4 °C. The surnatant was discarded, and the pellet was resuspended in 25 mL of buffer, then preincubated for 10 min at 37 °C. The homogenate was centrifuged at 48 000*g* for 15 min at 4 °C. The supernatant was discarded, and the final pellet was stored at −80 °C until used. Each tube received in a final volume of 1 mL of 50 mM Tris (pH 7.6) hippocampus membranes suspension and 1 nM [^{3}H]-8-OH-DPAT. For competitive inhibition experiments various concentrations of drugs studied were incubated. Nonspecific binding was defined using 1 μM 8-OH-DPAT. Samples were incubated at 37 °C for 20 min and then filtered on Whatman GF/B glass microfiber filters. The K_d value determined for 8-OH-DPAT was 8.8 nM.

3. Radioligand Binding Assay at Rat Striatal Membranes D$_2$ Receptors. Binding experiments were performed 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]spiroperidol. 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. The K_d value determined for spiroperidol was 0.05 nM.

4. Radioligand Binding Assay at Rat Cortical Membranes α_1-adrenoceptors. Binding experiments were performed according to Glossman and Hornung[36] with minor modifications. Rats were killed by decapitation, the brain was

quickly removed, and the cerebral cortex was dissected. The cerebral cortex (1.0 g) was homogenized with a Brinkman polytron (setting 5 for 3 × 15 s) in 25 mL of buffer (50 mM Tris, 0.1 mM PMSF, pH 7.4). The homogenated was centrifuged at 1000g for 15 min at 4 °C. The surnatant was recovered and centrifuged at 50 000g for 30 min at 4 °C. The final pellet was stored at −80 °C until used. Each tube received in a final volume 1 mL of 50 mM Tris (pH 7.4) rat cerebral cortical membranes suspension and 1 nM [^{3}H]prazosin. For competitive inhibition experiments various concentrations of drugs studied were incubated. Nonspecific binding was defined using 10 μM phentolamine. Samples were incubated at 25 °C for 50 min and then filtered on Whatman GF/B glass microfiber filters. The filters were presoaked for 50 min in Tris-HCl-polyethylenimine 0.5%. The K_d value determined for prazosin was 0.5 nM.

5. Radioligand Binding Assay at Human Cloned 5HT$_{1A}$ Serotonergic Receptors. Genomic clone G-21 coding for the human 5HT$_{1A}$ serotonergic receptor is stably transfected in a human cell line (HeLa).[37] 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) amd 7% CO$_2$ at 37 °C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lised in ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4). Homogenates were centrifuged at 40000g for 20 min, and pellets were resuspended in a small volume of ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4) and immediately frozen and stored at −70 °C until use. On the experimental day, cell membranes were resuspended in binding buffer 50 mM Tris (pH 7.4), 2.5 mM MgCl$_2$, and 10 μM pargiline.[38] 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.

6. Radioligand Binding Assay at Human Cloned α_1-Adrenoceptors. Binding to cloned human α_1-adrenoceptor subtypes was performed in membranes from Chinese hamster ovary (CHO) cells transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoceptor subtype. Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described.[39] CHO cell membranes were incubated in 50 mM Tris, pH 7.4, with 0.2 nM [^{3}H]prazosin in a final volume of 1.0 mL for 30 min at 25 °C in the absence or presence of competing drugs (1 pM to 10 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The incubation was stopped by addition of ice-cold Tris buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

7. 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[40] with minor modifications. On the experimental day, 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 further 30 min at 30 °C. Nonspecific binding was determined in the presence of 10 μM GTPγS. The incubation was stopped by addition of ice-cold HEPES buffer and rapid filtration on Schleicher & Schuell GF52 filters, using a Brandel cell harvester. The filters were washed three times with a total of 5 mL of the same buffer.

Radioactivity was counted by liquid scintillation spectrometry with an efficiency greater than 90%.

8. In Vivo Activity at Postsynaptic 5-HT$_{1A}$ Receptors. The in vivo activity on postsynaptic 5-HT$_{1A}$ receptors was evaluated as an induction of fore-paw treading in rats.[28] On the day of the experiment, rats were placed singly in clear plastic boxes, 10−15 min before intravenous or subcutaneous injection of test compounds. Groups of four to eight rats per dose of test compounds were used. Only the major component of the 5-HT$_{1A}$ syndrome was evaluated by an observer "blind" to drug pretreatments: the fore-paw treading (FT). Observation sessions of 30 s began 3 min after treatment and were repeated every 3 min over a period of 15 min (five observation sessions). The appearance of fore-paw treading was noted, and its intensity was scored using the following ranked intensity scale: 0 = absent; 1 = equivocal; 2 = present; 3 = intense. The maximal cumulative score attainable was 15 per rat.

9. Statistical Analysis. The inhibition curves on the different binding sites of the compounds reported in Table 1 were analyzed by nonlinear curve fitting utilizing the Graph-Pad Prism program.

The inhibition curves on the different binding sites of compounds reported in Table 3 were analyzed by nonlinear curve fitting of the logistic equation according to the method reported by De Lean et al.,[41] utilizing the ALLFIT program (from N.I.H.). The IC$_{50}$ values and pseudo-Hill slope coefficients were estimated by the program.

The value for the inhibition constant, K_i, was calculated by using the Cheng−Prusoff equation.[42]

Stimulation of [^{35}S]GTPγS binding induced by the compounds tested was expressed as the percentage increase in binding above the basal value because the maximal stimulation observed with 5-HT was 100%. The concentration−response curves of the agonistic activity were analyzed by ALLFIT as reported above. The maximal percent stimulation of [^{35}S]GTPγS binding (E_{max}) achieved for each compound and the concentration required to obtain 50% of E_{max} (pD$_2$ = −log EC$_{50}$ value) were evaluated.

In in vivo models, the sigmoidal dose−response curves were analyzed by the nonlinear curve fitting of the logistic equation as reported above. E_{max} was always considered to be 100%. The extrapolated doses corresponding to 50% E_{max} were also evaluated.

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Supporting Information Available: Two figures showing the induction of fore-paw treading in rats. This material is available free of charge via the Internet at http://pubs.acs.org.

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