New 1-Aryl-3-(4-arylpiperazin-1-yl)propane Derivatives, with Dual Action at 5-HT$_{1A}$ Serotonin Receptors and Serotonin Transporter, as a New Class of Antidepressants

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In a search toward new and efficient antidepressants, 1-aryl-3-(4-arylpiperazin-1-yl)propane derivatives were designed, synthesized, and evaluated for 5-HT reuptake inhibition and 5-HT$_{1A}$ receptor antagonism. This dual pharmacological profile should lead, in principle, to a rapid and pronounced enhancement in serotoninergic neurotransmission and consequently to a more efficacious treatment of depression. The design was based on coupling structural moieties related to inhibition of serotonin reuptake, such as γ-phenoxypropylamines, to arylpiperazines, typical 5-HT$_{1A}$ ligands. In binding studies, several compounds showed affinity at the 5-HT transporter and 5-HT$_{1A}$ receptors. Antidepressant-like activity was initially assayed in the forced swimming test with those compounds with K_i < 200 nM in both binding studies. Functional characterization was performed by measuring the intrinsic effect on rectal temperature in mice and also the antagonism to 8-OH-DPAT-induced hypothermia. The most efficacious compounds (**12f**, **23g*E***, **28a**, and **28b**) were further explored for their ability to antagonize 8-OH-DPAT-induced inhibition of forskolin-stimulated cAMP formation in a cell line expressing the 5-HT$_{1A}$ receptor. Furthermore, the antidepressant-like properties of **12f**, **28a**, and **28b**, which exhibited 5-HT$_{1A}$ receptor antagonistic property in the latter study, were also evaluated in the learned helplessness test in rats. Among these three compounds, **28b** (1-benzo[*b*]thiophene-3-yl)-3-[4-(2-methoxyphenyl)-1-ylpropan-1-ol) showed the higher affinity at both the 5-HT transporter and 5-HT$_{1A}$ receptors (K_i = 20 nM in both cases) and was also active in the other pharmacological tests. Such a pharmacological profile could lead to a new class of antidepressants with a dual mechanism of action and a faster onset of action.

Introduction

Major depression is one of the most frequent psychiatric disorders, with an incidence of about 4% and a lifetime prevalence of 10−15%. Selective serotonin (5-HT) reuptake inhibitors (SSRIs) have less severe side effects than first-generation drugs, tricyclic antidepressants (TCA), and nonselective monoamine oxidase inhibitors (MAOI) and are the most widely prescribed antidepressants in several countries. Yet, in spite that 5-HT reuptake blockade takes place within a few hours,[1,2] an administration of at least 2 weeks is required before inducing a clinically significant improvement. This suggests that adaptive changes account for the antidepressant activity of SSRIs. It has been repeatedly suggested that somatodendritic 5-HT$_{1A}$ autoreceptors are desensitized by long-term SSRI administration, leading to an enhancement of 5-HT neurotransmission.[3] It has been shown that the concomitant administration of a 5-HT$_{1A}$ antagonist such as WAY 100635 or pindolol and a SSRI increases extracellular 5-HT levels in terminal regions of the serotoninergic system because of a prevention of the attenuation by the SRRI of the firing activity of 5-HT neurons.[4−7] Accordingly, when major depression patients are treated with a SSRI and pindolol, a reduction in the latency period for the therapeutic effect is observed.[8−12]

The aim of this work was the design, synthesis, and biological evaluation of new compounds able to inhibit 5-HT reuptake and also to block somatodendritic 5-HT$_{1A}$ autoreceptors. This dual action could result in a rapid increase in the concentration of extracellular 5-HT in the terminal areas and, presumably, in an acceleration of the onset of the antidepressant effect. Such an approach has received much attention in different laboratories, but few pharmacological data are available.[13]

The design of the present compounds was inspired in the structure of drugs showing the sought activities. Fluoxetine (a γ-phenoxypropylamine derivative) is a potent antidepressant drug[14] which exerts its therapeutic action by selectively inhibiting 5-HT reuptake. There are many other SSRIs that share this structural feature and also inhibit the 5-HT transporter with high selectivity (Chart 1). On the other hand, although several structurally different compounds possess high affinity and selectivity for 5-HT$_{1A}$ receptors (for reviews see

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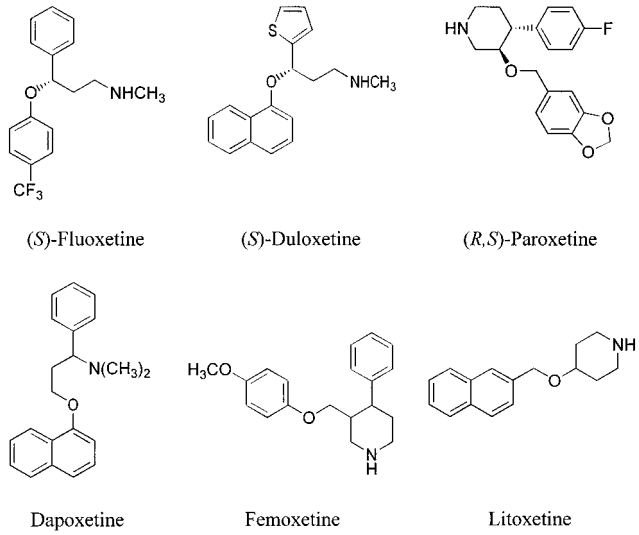
Chart 1. γ-Phenoxypropylamine Derivatives with Affinity for the 5-HT Transporter



Nelson[15] and Glennon[16]), the class of arylpiperazines has yielded many potent 5-HT ligands such as buspirone, gepirone, tandospirone, ipsapirone, NAN-190, BMY 7378 (partial agonists), flesinoxan (agonist), MP 3022, WAY 100135, and WAY 100635 (antagonists) (Chart 2). Most of the arylpiperazines contain a long-chain substituent at the basic nitrogen of the piperazine ring, which is important for the high 5-HT_{1A} receptor affinity and selectivity of these compounds.[17,18]

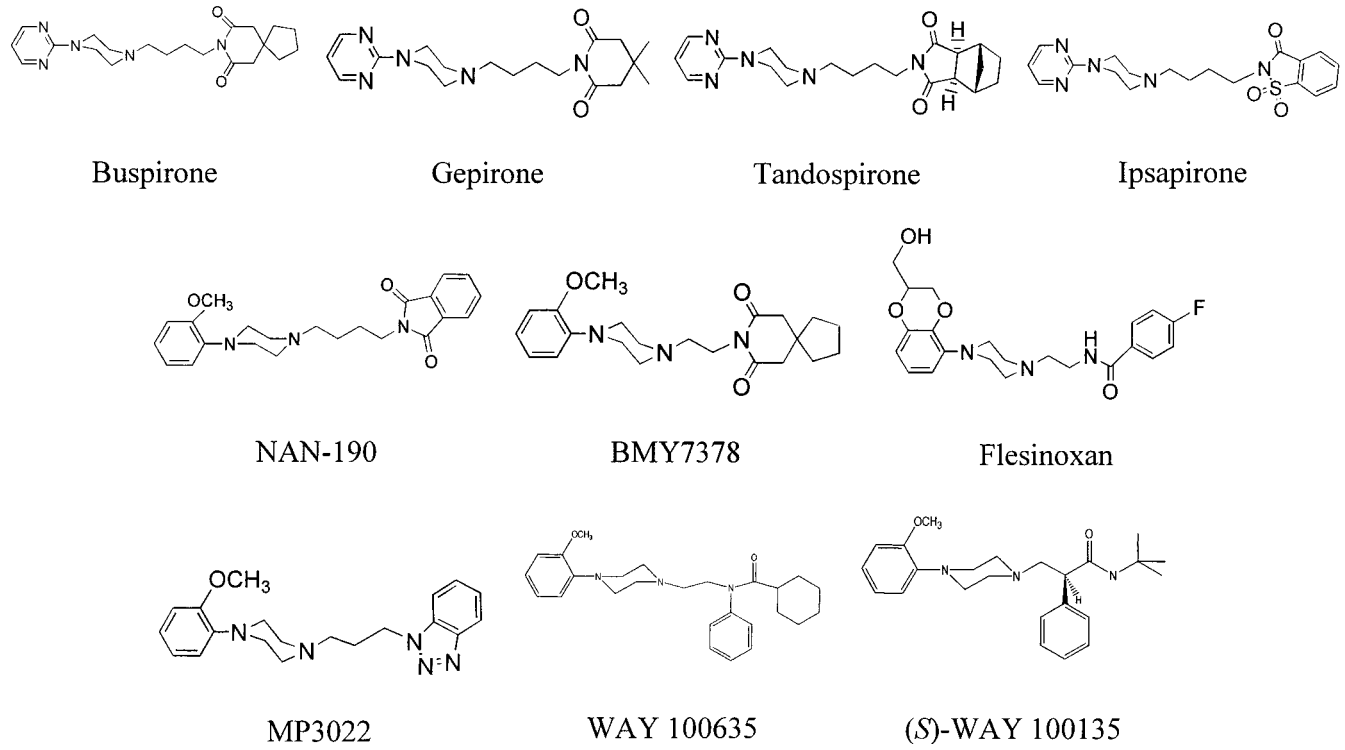
Therefore, we prepared a series of compounds of general structure A (Chart 3) in which the nitrogen of the γ-phenoxypropylamine moiety was included in the piperazine ring. Several structural changes were made on both sites A and B (Chart 3) in order to obtain compounds with the sought dual affinity. We considered new series of arylpiperazines derivatives **I**, **II**, **III**, and **IV**, in which we explored the influence of different aromatic rings, such as benzene, thiophene, naphthalene, and benzo[*b*]thiophene, at Ar_2. With the aim of obtaining a better insight on the influence of structural changes on biological activity, we evaluated in the binding studies not only the initial target compounds (aryl ethers) but also their precursors (ketones and alcohols) and some oxime derivatives, also less voluminous than the ethers. The general structure of the synthesized compounds can be therefore expressed as general structure B (Chart 3).

The approach to the synthetic objectives was carried out dynamically, in successive cycles of design, synthesis, biological evaluation, analysis of results, and continuous correction of objectives (biodirected synthesis). We herein report the methods used in the synthesis of the compounds and the determination of their binding affinity at the 5-HT transporter and 5-HT_{1A} receptor. Structure−activity relationships (SARs) afforded insights for obtaining new molecules with dual activity. Several compounds were further evaluated for antidepressant-like properties and agonist/antagonist activity at 5-HT_{1A} receptors.

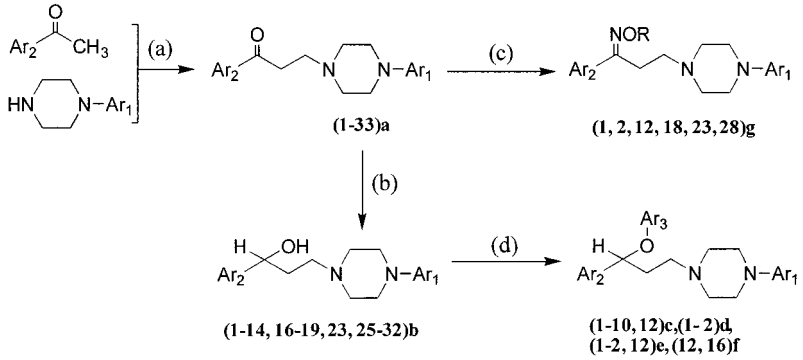
Chemistry

The preparation of 3-(4-arylpiperazin-1-yl)-1-arylpropane derivatives was carried out according to Scheme 1. Ketone derivatives were prepared by Mannich reaction of the corresponding aryl methyl ketones with different arylpiperazines, paraformaldehyde, and concentrated hydrochloric acid in refluxing ethanol.[19] All of the phenylpiperazines were commercially available except for 1-naphthylpiperazine,[20] 4-chloro-2-methoxyphenylpiperazine,[21] and 4-fluoro-2-methoxyphenylpiperazine,[22] which were synthesized according to the procedure depicted in Scheme 2. The arylamine derivative reacted with bis(2-chloroethyl)amine in refluxing

Chart 2. Arylpiperazine Derivatives with Affinity for 5-HT_{1A} Receptors

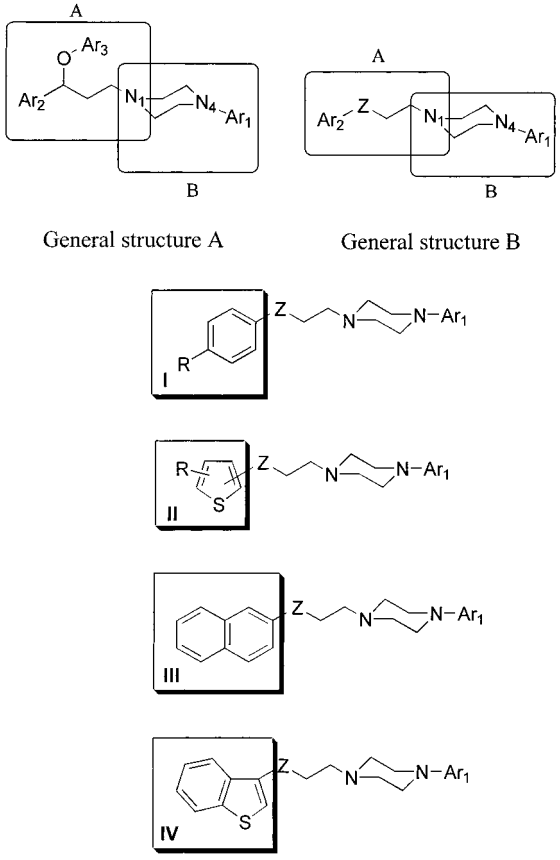


Scheme 1[a]



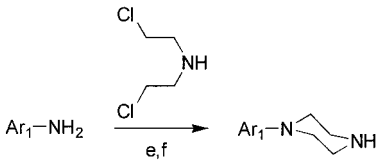
[a] Reagents: (a) $(CH_2O)_n$, HCl, EtOH; (b) $NaBH_4$, MeOH; (c) H_2NOR, EtOH; (d) NaH, Ar_3F, DMF (**1c−10c, 12c, 12f, 16f**) or MsCl, Ar_3OH, NaOH (**1d, 2d, 1e, 2e, 12e**).

Chart 3. General Structures and Series of Compounds



Series **I**, **II**, **III** and **IV** of compounds.

Scheme 2[a]



[a] Reagents: (e) K_2CO_3/C_6H_5Cl; (f) *p*-TosOH/C_6H_5Cl.

chlorobenzene in acidic (*p*-toluenesulfonic acid)[23] or basic medium (potassium carbonate).[24] For 1-naphthylpiperazine better results were obtained in basic medium, while disubstituted phenylpiperazines were synthesized in acidic medium. The reduction of ketones with sodium borohydride in methanol at 0 °C gave the corresponding hydroxyl derivatives. Aryl ethers were prepared by two different pathways: 4-trifluoromethylphenoxy and 1-naphthyl derivatives were prepared by the treatment of hydroxyl derivatives with sodium hydride and 4-fluorotrifluoromethylbenzene or 1-fluoronaphthalene. Other phenolic ethers were obtained from the mesylation of the corresponding hydroxyl derivative followed by their reaction with the corresponding phenol in ethanolic basic medium (sodium hydroxide).[19] Oxime derivatives were prepared from the carbonyl derivatives with hydroxylamine or *O*-alkylhydroxylamine in refluxing ethanol. When possible, the separation of each one of the two isomers was accomplished by column chromatography and they were identified by ^{1}H NMR[25] and ^{13}C NMR.[26,27]

All compounds were characterized by physical constants, elemental analysis, IR, ^{1}H NMR, and MS spectra.

Hydroxyl and aryl ether derivatives reported in this work were tested as racemates. The composition of the mixture was studied by analytical HPLC, using a Chirex 3020 column. Further separation and biological evaluation of each enantiomer will be carried out in the future for the most active compounds.

Biological Results and Discussion

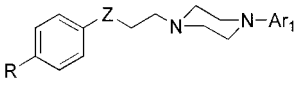
All of the synthesized compounds (86 piperazine derivatives) were evaluated for their affinity at 5-HT_{1A} receptors and 5-HT transporter. Structure and binding data of the compounds are shown in Tables 1−4. The inhibition constant (K_i) was obtained from the IC_{50} value by the Cheng−Prusoff equation.[28]

Inspection of binding data with compounds from series **I** and **II** (benzene and thiophene derivatives) allowed the assessment of the following SARs:

With respect to the functional group Z present in the propyl chain (Chart 3), many different groups were considered, including ketone, alcohol, oxime, and aryl ethers. Ketones such as **2a**, **12a**, **13a**, **15a**, **18a**, **19a**, **20a**, **21a**, and **22a** and alcohols such as **2b**, **6b**, **9b**, **10b**, **11b**, **12b, 13b**, **16b**, **18b**, **19b**, and **23b** showed dual affinity (K_i < 5000 nM). Compounds **12a** and **18b** were selected for further studies.

With regard to the oxime derivatives, only *E* isomers were formed in most cases; however, when both isomers were formed and separated (**18g** and **23g**), *E* isomers showed higher affinities than *Z* isomers. Among all oxime derivatives, only compound **23g*E*** (Table 2) showed

Table 1. Compounds of Series **I**: Structure and Affinity for 5-HT_{1A} Receptors and 5-HT Transporter



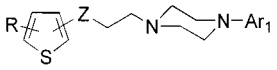
				K_i (nM)	
compd	R	Z	Ar_1	5-HT_{1A} receptor	5-HT transporter
1a	H	CO	2-methoxyphenyl	50	>5000
1b	H	CHOH	2-methoxyphenyl	47.5	>5000
1c	H	CHO-4-$CF_3C_6H_4$	2-methoxyphenyl	450	650
1d	H	CHO-4-$CH_3OC_6H_4$	2-methoxyphenyl	90	1450
1e	H	CHO-3,4-OCH_2O-C_6H_3	2-methoxyphenyl	20	500
1g	H	CNOH	2-methoxyphenyl	17.5	5000
2a	H	CO	4-chlorophenyl	800	300
2b	H	CHOH	4-chlorophenyl	800	350
2c	H	CHO-4-$CF_3C_6H_4$	4-chlorophenyl	>5000	350
2d	H	CHO-4-$CH_3OC_6H_4$	4-chlorophenyl	1450	500
2e	H	CHO-3,4-OCH_2O-C_6H_3	4-chlorophenyl	550	375
2g	H	CNOH	4-chlorophenyl	>5000	5000
3a	H	CO	4-methoxyphenyl	5000	>5000
3b	H	CHOH	4-methoxyphenyl	>5000	1150
3c	H	CHO-4-$CF_3C_6H_4$	4-methoxyphenyl	>5000	>5000
4a	H	CO	2-pyrimidyl	120	>5000
4b	H	CHOH	2-pyrimidyl	375	>5000
4c	H	CHO-4-$CF_3C_6H_4$	2-pyrimidyl	1600	1600
5a	H	CO	2-chlorophenyl	180	>5000
5b	H	CHOH	2-chlorophenyl	115	>5000
5c	H	CHO-4-$CF_3C_6H_4$	2-chlorophenyl	>5000	>5000
6a	H	CO	4-fluorophenyl	800	>5000
6b	H	CHOH	4-fluorophenyl	145	4700
6c	H	CHO-4-$CF_3C_6H_4$	4-fluorophenyl	>5000	500
7a	H	CO	2-pyridyl	50	>5000
7b	H	CHOH	2-pyridyl	155	>5000
7c	H	CHO-4-$CF_3C_6H_4$	2-pyridyl	1600	1500
8a	H	CO	4-nitrophenyl	>5000	>5000
8b	H	CHOH	4-nitrophenyl	>5000	1150
8c	H	CHO-4-$CF_3C_6H_4$	4-nitrophenyl	>5000	2500
9a	phenyl	CO	2-methoxyphenyl	3650	700
9b	phenyl	CHOH	2-methoxyphenyl	850	175
9c	phenyl	CHO-4-$CF_3C_6H_4$	2-methoxyphenyl	>5000	>5000
10a	methoxy	CO	2-methoxyphenyl	1750	>5000
10b	methoxy	CHOH	2-methoxyphenyl	325	500
10c	methoxy	CHO-4-$CF_3C_6H_4$	2-methoxyphenyl	1000	650
11a	nitro	CO	2-methoxyphenyl	50	>5000
11b	nitro	CHOH	2-methoxyphenyl	10	2500

good affinity in both assays and was selected. Four different aryl ether derivatives were synthesized. In the phenyl ring of the aryl ether group (Ar_3), the change of an electron-withdrawing substituent (4-trifluoromethyl) by an electron-donating substituent (4-methoxy or 3,4-methylenedioxy) improved the affinity at 5-HT_{1A} receptors (**1c** vs **1e**, Table 1). On the other hand, the insertion of a substituent with high π delocalization, such as a naphthalene ring, improved both binding affinities (**12f** vs **12c**). Due to this good result, compound **12f** was selected among the ether derivatives for further studies. It can be seen that compounds with any of the functional groups showed affinity for both 5-HT_{1A} receptors and 5-HT transporter. Consequently, the presence of an aryl ether moiety in Z is not essential to achieve the dual activity, which can be obtained with any other smaller functional group. In general, higher affinity values were obtained with the hydroxyl derivatives.

With regard to the arylpiperazine moiety, different aromatic rings were introduced in Ar_1, including heterocycles (pyridine and pyrimidine), mono- and disubstituted benzenes (4-chlorophenyl and 2-methoxy-4-chlorophenyl), and benzocondensed rings (such as naphthalene). It was also observed that the introduction of *ortho*-substituents in Ar_1 is more favorable to the 5-HT_{1A} affinity than the *para*-substituents (Table 1, **1a** vs **3a**). On the other hand, the presence of chloro at the *para*-position enhanced the affinity at the 5-HT transporter (see, for example, **1a**−**e** vs **2a**−**e**, Table 1, or **16a**,**b** vs **17a**,**b**, Table 2). Taking into account these findings, it was considered interesting the coupling of both classes of substituents, such as 4-chloro-2-methoxy or 4-fluoro-2-methoxy, in Ar_1. These changes resulted in an enhancement of the affinity at the 5-HT transporter rather than at the 5-HT_{1A} receptor but did not afford good activity at both sites (**18a** vs **21a** and **22a**, Table 2). The best dual activity was obtained with 2-methoxyphenylpiperazine derivatives. Compounds with 1-naphthyl and 4-chlorophenyl moieties also showed a good affinity, so all of these substituents were selected for further synthetic approaches.

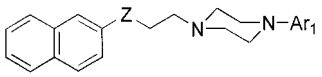
The nature of Ar_2 was also important for affinity. Insertion of substituents that increased electronic charge or π delocalization in Ar_2 resulted in improved affinity at the 5-HT transporter (**9a,b** and **10a,b** vs **1a,b**, Table 1; **18b** vs **12b,** Table 2). In general, higher affinity was found with compounds from series **II** (thiophene derivatives), particularly 3-thiophene derivatives, than with compounds from series **I** (benzene derivatives). Taking into account these results, new naphthalene (series **III**) and benzo[*b*]thiophene (series **IV**) compounds Ar_2 were synthesized (Tables 3 and 4). Naphthalene derivatives

Table 2. Compounds of Series **II**: Structure and Affinity for 5-HT_{1A} Receptors and 5-HT Transporter



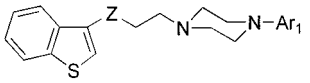
compd	R	Z	position	Ar_1	K_i (nM) 5-HT_{1A} receptor	K_i (nM) 5-HT transporter
12a	H	CO	3	2-methoxyphenyl	16	112
12b	H	CHOH	3	2-methoxyphenyl	50	385
12c	H	CHO-4-$CF_3C_6H_4$	3	2-methoxyphenyl	255	460
12g	H	CNOH	3	2-methoxyphenyl	6.5	950
12e	H	CHO-3,4-OCH_2O-C_6H_3	3	2-methoxyphenyl	55	6500
12f	H	CHO-1-$C_{10}H_7$	3	2-methoxyphenyl	180	150
13a	H	CO	3	4-chlorophenyl	700	220
13b	H	CHOH	3	4-chlorophenyl	2750	200
14a	H	CO	3	2-chlorophenyl	200	>5000
14b	H	CHOH	3	2-chlorophenyl	200	>5000
15a	H	CO	3	1-naphthyl	35	800
16a	H	CO	2	2-methoxyphenyl	10	>5000
16b	H	CHOH	2	2-methoxyphenyl	19	1250
16f	H	CHO-1-$C_{10}H_7$	2	2-methoxyphenyl	220	>5000
17a	H	CO	2	4-chlorophenyl	>5000	60
17b	H	CHOH	2	4-chlorophenyl	>5000	290
18a	2,5-dimethyl	CO	3	2-methoxyphenyl	5	1350
18b	2,5-dimethyl	CHOH	3	2-methoxyphenyl	12	115
18g*E*	2,5-dimethyl	CNOH (*E*)	3	2-methoxyphenyl	85	5000
18g*Z*	2,5-dimethyl	CNOH (*Z*)	3	2-methoxyphenyl	130	>5000
19a	2,5-dimethyl	CO	3	2-hydroxyphenyl	7.5	800
19b	2,5-dimethyl	CHOH	3	2-hydroxyphenyl	90	2500
20a	2,5-dimethyl	CO	3	1-naphthyl	100	370
21a	2,5-dimethyl	CO	3	4-fluoro-2-methoxyphenyl	13	750
22a	2,5-dimethyl	CO	3	4-chloro-2-methoxyphenyl	500	275
23a	5-methyl	CO	2	2-methoxyphenyl	17.5	>5000
23b	5-methyl	CHOH	2	2-methoxyphenyl	34	5000
23g*Z*	5-methyl	CNOH (*Z*)	2	2-methoxyphenyl	75	5000
23g*E*	5-methyl	CNOH (*E*)	2	2-methoxyphenyl	130	65
24a	5-nitro	CO	2	2-methoxyphenyl	340	>5000

Table 3. Benzocondensed Derivatives of Series **III**: Structure and Affinity for 5-HT_{1A} Receptors and 5-HT Transporter



compd	Z	Ar_1	K_i (nM) 5-HT_{1A} receptor	K_i (nM) 5-HT transporter
25a	CO	2-methoxyphenyl	250	1000
25b	CHOH	2-methoxyphenyl	420	380
26a	CO	4-chlorophenyl	>5000	240
26b	CHOH	4-chlorophenyl	>5000	10
27a	CO	2-hydroxyphenyl	1000	2500
27b	CHOH	2-hydroxyphenyl	190	190

Table 4. Benzocondensed Derivatives of Series **IV**: Structure and Affinity for 5-HT_{1A} Receptors and 5-HT Transporter



compd	Z	Ar_1	K_i (nM) 5-HT_{1A} receptor	K_i (nM) 5-HT transporter
28a	CO	2-methoxyphenyl	44	105
28b	CHOH	2-methoxyphenyl	20	20
28g	CNOH	2-methoxyphenyl	60	95
29a	CO	4-chlorophenyl	>5000	5000
29b	CHOH	4-chlorophenyl	5000	50
30a	CO	2-hydroxyphenyl	110	490
30b	CHOH	2-hydroxyphenyl	18	39
31a	CO	4-chloro-2-methoxyphenyl	500	275
31b	CHOH	4-chloro-2-methoxyphenyl	361	35
32a	CO	4-fluoro-2-methoxyphenyl	500	500
32b	CHOH	4-fluoro-2-methoxyphenyl	500	12
33a	CO	1-naphthyl	100	500

showed moderate to high affinity at the 5-HT transporter with both carbonyl and hydroxyl derivatives, so the change of benzene to naphthalene in Ar_2 appeared to increase affinity at the 5-HT transporter (**25a**,**b**, Table 3, vs **1a**,**b**, Table 1). However, only one of the naphthalene derivatives (**27b**, Table 3) could be selected for further design and synthesis.

The introduction of the 3-benzo[*b*]thiophene ring in Ar_2 improved the affinity data. Good dual affinity was shown by compounds **28a**,**b**,**g** and **30b**. Best results were obtained when Ar_1 was a 2-methoxyphenyl moiety and there was a hydroxyl group at Z. The higher affinity at both sites was found with compound **28b** (K_i = 20 nM for both 5-HT_{1A} receptors and 5-HT transporter, Table 4).

A number of compounds that exhibited moderate to high affinity at the 5-HT_{1A} receptor and 5-HT transporter ($K_i \leq 200$ nM) were selected to test their antidepressant-like activity in the forced swimming test (FST) in mice. Four compounds from series **II** (**12b**,**f**, **18b** and **23g*E***), one from series **III** (**27b**), and four compounds from series **IV** (**28a**,**b**,**g** and **30b**) were included. Results are shown in Table 5. Compound **27b** significantly reduced the immobility time at the higher dose tested (10 mg/kg, ip) while **12a**,**f** and **28a** reduced the immobility time over a dose range of 1−10 mg/kg. Compound **28b** produced antiimmobility effects in a dose range from 0.001−0.1 mg/kg; higher doses were however ineffective. The SSRIs fluoxetine (10−30 mg/

Table 5. Effects of Selected Antidepressants and Several Compounds on Immobility Time in the FST in Mice[a]

treatment	dose (mg/kg)	immobility time (s)	antidepressant-like activity (%)
amitriptyline	0	128.2 ± 16.8	
	0.1	128.1 ± 14.7	0
	1	130.5 ± 15.6	0
	10	19.3 ± 0.1**	100
fluoxetine	0	166.9 ± 9.2	
	1	160.1 ± 12.3	5
	10	131.5 ± 12.6	23
	30	32.8 ± 13.5	90
paroxetine	0	143.7 ± 12.1	
	1	86.8 ± 15.7*	44
	10	50.5 ± 8.7**	73
12a	0	170.1 ± 8.7	
	0.1	169.5 ± 11.1	0
	1	118.8 ± 12.1*	34
	10	44.9 ± 9.5**	83
12f	0	157.1 ± 11.9	
	0.1	194.4 ± 12.5	0
	1	91.6 ± 18.6**	48
	10	91.1 ± 15.6**	49
18b	0	124.3 ± 17.2	
	1	111.0 ± 25.6	13
	10	103.0 ± 18.2	20
23g*E*	0	142.7 ± 15.3	
	0.1	151.6 ± 21.6	0
	1	79.2 ± 12.6**	51
	10	126.2 ± 9.0	13
27b	0	1.57 ± 11.9	
	0.1	1.60 ± 8.9	0
	1	178.3 ± 16.5	0
	10	24.4 ± 11.2	93
28a	0	169.4 ± 6.9	
	0.1	141.4 ± 25.3	18
	1	77.1 ± 14.4**	60
	10	86.6 ± 21.3**	54
28b	0	145.0 ± 21.2	
	0.001	58.8 ± 15.4**	67
	0.01	54.4 ± 18.0**	70
	0.1	99.5 ± 12.8**	35
	1	101.8 ± 18.1	33
	10	143.5 ± 26.2	1
28g	0	188.1 ± 7.4	
	0.1	181.1 ± 9.2	0
	1	150.1 ± 19.2	22
	10	102.0 ± 20.1**	50
30b	0	178.0 ± 11.8	
	0.01	186.1 ± 8.8	0
	0.1	112.5 ± 16.6*	40
	1	183.3 ± 13.5	0
	10	160.9 ± 18.6	11

[a] Values are means ± SEM of 10 mice. Compounds were administered ip 30 min before the test. *P < 0.05, **P < 0.01 vs vehicle (Student's t-test). Amitriptyline (10 mg/kg) was taken as reference compound, and the antidepressant-like activity of all other compounds was comparatively calculated.

kg) and paroxetine (1−10 mg/kg) also showed antidepressant-like activity in this test, in keeping with previous data.[29] In the few studies aimed at evaluating the mechanism of action of SSRIs in the FST, it has been suggested that an indirect activation of postsynaptic 5-HT_{1B}[30,31] and presynaptic 5-HT_{1A}[31] receptors could be involved. It is known that 5-HT_{1A} receptor antagonists lack antiimmobility effects in the FST[31,32] while, by contrast, there is good evidence that 5-HT_{1A} agonists are active in this test at lower doses than SSRIs via activation of postsynaptic 5-HT_{1A} receptors.[30−33] The present results indicate that some compounds with dual affinity at the 5-HT transporter and 5-HT_{1A} receptors were also active in this behavioral test, one of them (**28b**) at lower doses than typical SSRIs. It would be no doubt of interest to further explore the interplay between 5-HT reuptake inhibition and agonist/antagonist activity at 5-HT_{1A} receptors in this animal model of depression.

All compounds tested in the FST were also assayed for effects on body temperature in mice. Intrinsic hypothermia effects, an index of agonist activity at presynaptic 5-HT_{1A} receptors,[34] and antagonism to the hypothermic effect of 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist, were measured (Table 6). This study showed that none of the nine selected compounds had intrinsic hypothermic effects. By contrast, antagonism studies revealed that compounds **23g*E*** (5 mg/kg), **12f** and **28a** (0.5 mg/kg) and, in a wider dose range, **28b** (0.01−0.5 mg/kg) partially antagonized 8-OH-DPAT-induced hypothermia suggesting antagonistic properties at presynaptic 5-HT_{1A} receptors (Table 6). However, **12f** and **28a**,**b** were ineffective at the higher dose tested (5 mg/kg), so it cannot be discarded that, after this high dose, other nonspecific actions of these compounds could be involved.

The selected compounds were also examined for agonist/antagonist properties at the 5-HT_{1A} receptor by measuring their ability to inhibit forskolin-stimulated cAMP formation or to antagonize 8-OH-DPAT-mediated inhibition of forskolin-stimulated cAMP formation, respectively[35] (Table 7). Compound **23g*E*** produced significant inhibition (75%) of forskolin-stimulated cAMP formation, similar to the inhibition (65%) induced by the full 5-HT_{1A} receptor agonist 8-OH-DPAT (0.1 μM). Compound **28b** (1 μM) partially inhibited forskolin-stimulated cAMP formation (36%) indicating a weak agonist activity at 5-HT_{1A} receptors; no inhibition was however observed with compounds **12f** and **28a**. In antagonism studies, WAY 100635 (1 μM) markedly prevented (78% inhibition) the 8-OH-DPAT-induced effect, a result in keeping with previous studies.[35] Compounds **12f** and **28a**,**b**, 1 μM each, were weaker antagonists at 5-HT_{1A} receptors and prevented by 25%, 55%, and 40%, respectively, the 8-OH-DPAT-induced inhibition of forskolin-stimulated cAMP formation.

Compounds showing antagonist properties at 5-HT_{1A} receptors in the latter studies were further explored for antidepressant-like properties in the learned helplessness test in rats (Table 8), another behavioral model with a higher validity than the FST because of the analogy between the behavioral characteristics of helpless animals and signs of depression in humans.[36] The results obtained appeared to confirm the antidepressant-like properties of **12f** and **28a**,**b** previously shown in the FST. The effect of two typical SSRIs, fluoxetine and paroxetine, were also comparatively studied in the learned helplessness test, and it was found that they were only active at higher doses than the new compounds.

A study of the selected compounds was finally carried out on their binding profile at different receptors (Table 9). Compounds **12f** and **28a**,**b** were devoid of affinity at other 5-HT receptor subtypes (Table 9), and they did not either show any affinity at muscarinic receptors (K_i > 5000 nM). A moderate affinity was however found at α_1-adrenoceptors, although they were weaker ligands than the reference compound, prazosin, by at least 1

Table 6. Time-Dependent Effects of WAY 100635 and Several Compounds on 8-OH-DPAT-Induced Hypothermia in Mice[a]

pretreatment	dose (mg/kg)	change in body temperature (°C)[b] 15 min	30 min	60 min
saline		−2.61 ± 0.35	−2.36 ± 0.40	−1.47 ± 0.20
WAY 100635	1	−0.71 ± 0.21**	−0.95 ± 0.15**	−0.35 ± 0.09**
12a	0.5	−2.52 ± 0.29	−2.04 ± 0.20	−1.48 ± 0.18
	5	−2.76 ± 0.15	−2.18 ± 0.19	−1.88 ± 0.45
12f	0.1	−2.12 ± 0.35	−1.54 ± 0.32	−1.16 ± 0.16
	0.5	−1.72 ± 0.34*	−1.35 ± 0.46*	−0.52 ± 0.16*
	5	−2.73 ± 0.22	−2.23 ± 0.28	−1.22 ± 0.15
18b	0.5	−2.53 ± 0.20	−2.13 ± 0.27	−1.21 ± 0.14
	5	−2.01 ± 0.19	−1.86 ± 0.13	−1.26 ± 0.18
23g*E*	0.5	−2.12 ± 0.15	−2.05 ± 0.19	−1.11 ± 0.13
	5	−1.41 ± 0.15*	−1.31 ± 0.11*	−0.94 ± 0.15
27b	0.5	−2.52 ± 0.15	−2.03 ± 0.18	−1.12 ± 0.19
	5	−1.97 ± 0.15	−2.41 ± 0.13	−0.93 ± 0.13
28a	0.1	−2.14 ± 0.28	−1.66 ± 0.13	−1.13 ± 0.17
	0.5	−1.58 ± 0.28*	−0.86 ± 0.27**	−0.47 ± 0.12*
	5	−2.53 ± 0.32	−1.47 ± 0.21*	−1.27 ± 0.14
28b	0.01	−1.26 ± 0.10**	−1.30 ± 0.20*	−0.73 ± 0.10
	0.1	−1.60 ± 0.20*	−1.30 ± 0.20*	−0.4 ± 0.20*
	0.5	−1.23 ± 0.31*	−1.38 ± 0.30**	−0.63 ± 0.20*
	5	−2.20 ± 0.10	−2.50 ± 0.10	−1.16 ± 0.10
28g	0.5	−2.16 ± 0.30	−2.18 ± 0.25	−1.7 ± 0.26
	5	−2.77 ± 0.22	−2.99 ± 0.41	−1.31 ± 0.13
30b	0.5	−2.54 ± 0.23	−2.01 ± 0.23	−1.38 ± 0.15
	5	−3.17 ± 0.25	−2.20 ± 0.15	−1.32 ± 0.19

[a] 8-OH-DPAT given always at a single dose of 0.5 mg/kg sc. All other drugs given 30 min before 8-OH-DPAT. [b] Values (means ± SEM) correspond to changes in rectal temperature measured immediately before first drug injection and at different times after 8-OH-DPAT. *P < 0.05, **P < 0.01 vs saline group (one-way ANOVA followed by Student−Newman−Keuls test).

Table 7. Effect of 8-OH-DPAT, WAY 100635, **12f**, **23g*E***, **28a**, and **28b** on Forskolin-Stimulated cAMP Formation, in HeLa Cells Transfected with the 5-HT_{1A} Receptor[a]

treatment	concn (μM)	cAMP (pmol/well)	cAMP increase (%)
control		1.5 ± 0.2	
forskolin	10	29.7 ± 0.7	100
8-OH-DPAT	0.1	10.5 ± 0.4**	35
WAY 100635	1	22.6 ± 3.8	76
12f	1	28.5 ± 1.6	96
23g*E*	1	7.4 ± 0.9**	25
28a	1	27.0 ± 0.3	91
28b	1	19.1 ± 1.5*	64
WAY 100635 + 8-OH-DPAT	1 + 0.1	25.3 ± 1.3$^{\nabla\nabla}$	85
12f + 8-OH-DPAT	1 + 0.1	15.4 ± 1.1$^{\nabla}$	52
23g*E* + 8-OH-DPAT	1 + 0.1	9.8 ± 1.0	33
28a + 8-OH-DPAT	1 + 0.1	20.8 ± 1.2$^{\nabla}$	70
28b + 8-OH-DPAT	1 + 0.1	18.1 ± 1.4$^{\nabla}$	61

[a] Data (pmol cAMP/well) are the mean ± SEM of 5−6 independent experiments. *P < 0.05, **P < 0.01 vs forskolin and $^{\nabla}P$ < 0.05, $^{\nabla\nabla}P$ < 0.01 vs 8-OH-DPAT (one-way ANOVA followed by Student−Newman−Keuls *t*-test).

order of magnitude (Table 9). Microdialysis studies with compounds that exhibit a dual activity at α_1 and 5-HT_{1A} receptors have shown that α_1-adrenoceptor-mediated enhancement in 5-HT release is counteracted by simultaneous activation of somatodendritic 5-HT_{1A} receptors.[37,38] Yet, it is known that the role of α_1-adrenoceptors in the control of extracellular 5-HT levels is shadowed when drugs, such as BMY 7378 and S 16924, which are also active at presynaptic 5-HT_{1A} receptors, are given systemically.[38,39] Probably, this would be also the case with compounds **12f** and **28a**,**b**. It is well-known, on the other hand, that the better safety profile of SSRIs over TCAs is due to the lack of anticholinergic and cardiovascular side effects, derived from the blockade of muscarinic and α_1-adrenergic receptors, respectively.[40] It can be consequently suggested that the present new compounds may show, like SSRIs, a better safety profile than TCAs.

Table 8. Effect of Compounds **12f**, **28a**, and **28b** and Selected Antidepressants on Helpless Behavior[a]

treatment	dose (mg/kg/day)	number of escape failures SB_1	SB_2	SB_3
amitriptyline	saline	18.5 ± 2.7	20.3 ± 3.1	20.6 ± 4.0
	10	9.0 ± 3.0*	3.3 ± 1.7**	1.2 ± 1.0**
fluoxetine	saline	18.5 ± 2.7	20.3 ± 3.1	20.6 ± 4.0
	30	13.0 ± 1.0	4.0 ± 0.8*	3.2 ± 1.0*
paroxetine	saline	18.5 ± 2.7	20.3 ± 3.1	20.6 ± 4.0
	10	3.3 ± 1.4*	3.0 ± 0.9*	2.2 ± 0.6*
12f	saline	13.4 ± 3.2	11.4 ± 3.5	10.2 ± 4.2
	1	5.8 ± 1.4*	1.3 ± 1.1**	0.5 ± 0.5**
28a	saline	19.2 ± 3.1	14.0 ± 3.9	17.7 ± 3.8
	1	9.8 ± 0.5**	4.0 ± 1.2**	2.8 ± 1.6**
28b	saline	17.4 ± 4.1	15.4 ± 5.1	17.0 ± 5.2
	1	8.0 ± 1.5	2.3 ± 3.2**	1.8 ± 1.2**
	10	6.6 ± 1.2*	3.2 ± 1.5*	4.0 ± 0.9*

[a] Data are means ± SEM of escape failures in each of the three consecutive shuttle-box sessions (SB_{1-3}). Compounds were ip injected on 4 consecutive days, 6 h after inescapable shocks on day 1 and then twice a day (30 min before shuttle-box sessions and 6 h later). *P < 0.05, **P < 0.01 vs saline (Mann−Whitney *U*-test).

Conclusions

In this study, we synthesized different series of arylpiperazine derivatives in order to obtain new compounds with affinity at both the 5-HT_{1A} receptor and the 5-HT transporter, for potential use in the treatment of depression. The study of the functional group Z, the aromatic ring attached to the piperazine (Ar_1), and the aromatic ring Ar_2 (see Chart 3) allowed us to suggest the following SAR conclusions. First, the introduction of an aryl ether moiety at Z was not necessary to achieve the sought dual activity; even more, the best results were obtained with hydroxyl derivatives. Second, among all of the arylpiperazines tested the highest affinities were obtained with 2-methoxyphenylpiperazine derivatives. Third, in regard to the aromatic ring Ar_2, affinities were clearly improved when the benzene ring was

Table 9. Affinity of **28a**, **28b**, and **12f** at 5-HT Receptor Subtypes, Dopamine D_2 Receptors, Adrenoceptors (α_1, α_2, and β), and Muscarinic Receptors[a]

	K_i (nM)			
receptor	standard ligand	**12f**	**28a**	**28b**
5-HT_{1D}	sumatriptan, 12 ± 1.9	1250 ± 120	5000 ± 580	2650 ± 110
5-HT_2	ketanserine, 0.7 ± 0.09	>5000	5000 ± 250	250 ± 25
5-HT_3	granisetron, 0.3 ± 0.01	>5000	>5000	>5000
dopamine D_2	haloperidol, 1.5 ± 1.2	2500 ± 125	500 ± 60	150 ± 10
α_1-adrenoceptor	prazosin, 0.5 ± 0.01	135 ± 15	6 ± 0.5	35 ± 9.3
α_2-adrenoceptor	clonidine, 8 ± 0.1	5000 ± 230	280 ± 13	2000 ± 250
β-adrenoceptor	DHA, 3.5 ± 0.3	>5000	>5000	750 ± 56
muscarinic	atropine, 2.5 ± 0.1	>5000	>5000	>5000

[a] The values represent the mean ± SEM from at least three independent experiments. DHA, dihydroalprenolol.

replaced by thiophene (series **I** and **II**, respectively) and also in the series of benzocondensed derivatives when naphthalene was replaced by benzothiophene (series **III** and **IV**, respectively). Compounds **12f**, **28a**, and, particularly, **28b** with affinity at both 5-HT_{1A} receptors and 5-HT transporter and efficiency in animal models of depression are potential antidepressants. Body temperature measurements in mice and cAMP assays in a cell line transfected with the 5-HT_{1A} receptor revealed also an antagonist activity at this 5-HT receptor subtype. Among these three compounds, the sought pharmacological profile was particularly evident in compound **28b,** a 5-HT reuptake inhibitor with antagonist activity at presynaptic 5-HT_{1A} receptors in mice. This hybrid profile should facilitate serotonergic neurotransmission, and further pharmacological characterization of this compound is in progress to evaluate its real antidepressant potential.

Experimental Section

Chemistry. Melting points were determined using a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3−4 mmHg, 24 h, at ca. 80−100 °C) with a Leco CHN-900 instrument and are within ± 0.4% of the calculated values except where otherwise stated. Infrared spectra were recorded on a Perkin-Elmer 681 apparatus, using potassium bromide tablets for solids products and sodium chloride plates for oil products; the frequencies are expressed in cm^{-1}. ^1H NMR spectra were recorded on a Brucker AC-200E (200 MHz) instrument, with tetramethylsilane (TMS) as the internal reference, at a concentration of ca. 0.1 g/mL and with dimethyl sulfoxide-d_6 (DMSO-d_6) or chloroform ($CDCl_3$) as solvents; chemical shifts are expressed in parts per million (ppm) relative to internal TMS in δ units, and the coupling constant (J) values are in hertz (Hz). The mass spectra were recorded on a Hewlett-Packard 5988-A instrument at 70 eV.

Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates with the indicated solvents, revealed with iodine, and the plates were scanned under ultraviolet light at 254 and 366 nm. Column chromatography was carried out with Merk silica gel 60 (70−230 mesh ASTM).

Arylpiperazines are commercially available except for 1-naphthylpiperazine, 4-chloro-2-methoxyphenylpiperazine and 4-fluoro-2-methoxyphenylpiperazine, which were prepared as described in refs 20−22, respectively.

General Procedure for Preparation of 3-(4-Arylpiperazin-1-yl)-1-aryl-1-propanone Derivatives 1a−33a. A mixture of aryl methyl ketone derivative (30 mmol), piperazine hydrochloride (30 mmol) and concentrated hydrochloric acid in absolute ethanol (40 mL) was heated at reflux and paraformaldehyde (90 mmol) was added in four equal portions over a period of 40 min. The reaction mixture was further refluxed for 3−48 h.

Method a: The reaction mixture was poured onto crushed ice. The separated solid was filtered and recrystallized from 2-propanol.

Method b: A dissolution of sodium hydroxide (10%) was added until basic pH was reached. It was then extracted with ethyl acetate (3 × 20 mL), washed with brine (3 × 10 mL) and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure. The impure oil was purified by column chromatography (SP: silica gel), eluting with *n*-hexane/ethyl acetate 50:50 (v:v). In some cases the hydrochloride salt was formed by adding some drops of concentrated hydrochloric acid to a solution of the ketone derivative in acetone.

3-[4-(2-Methoxyphenyl)piperazin-1-yl]-1-aryl-1-propanone Hydrochloride (1a). Method a; 40% yield; mp 180 °C. IR (KBr): 2932, 1677, 1237, 751 cm^{-1}. ^1H NMR ($CDCl_3$) δ (free base): 2.77−2.74 (m, 4H, $N^1(CH_2)_2$); 2.91 (t, 2H, CH_2-N^1, J = 7.2); 3.03−3.15 (m, 4H, $N^4(CH_2)_2$); 3.23 (t, 2H, CH_2-CO, J = 7.2); 3.84 (s, 3H, OCH_3); 6.84 (d, 1H, o-OCH_3-*Ph*, H_6, J = 7.3); 6.89−7.01 (m, 3H, o-OCH_3-*Ph*, H_3+H_4+H_5); 7.41−7.59 (m, 3H, *Ph*, H_3+H_4+H_5); 7.97 (d, 2H, *Ph*, H_2+H_6, J = 7.1). Anal. ($C_{20}H_{24}N_2O_2$·HCl) C,H,N.

3-[4-(2-Chlorophenyl)piperazin-1-yl]-1-phenyl-1-propanone (5a). Method b; 70% yield; mp 181 °C. IR (KBr): 2950, 1677, 750, 689 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.71−2.73 (m, 4H, $N^1(CH_2)_2$); 2.92 (t, 2H, N^1CH_2, J = 7.2); 3.02−3.19 (m, 4H, N^4-$(CH_2)_2$); 3.23 (t, 2H, CH_2-CO, J = 7.2); 7.05−6.95 (m, 2H, o-Cl-*Ph*, H_4+H_6); 7.20 (t, 1H, o-Cl-*Ph*, H_5, J = 7.7); 7.34 (d, 1H, o-Cl-*Ph*, H_3, J = 7.7); 7.56−7.42 (m, 3H, *Ph*, H_3+H_4+H_5); 7.97 (d, 2H, *Ph*, H_2+H_6, J = 7.5). Anal. ($C_{19}H_{21}N_2ClO$) C,H,N.

General Procedure for Preparation of 3-(4-Arylpiperazin-1-yl)-1-arylpropanol Derivatives 1b−14b, 16b−19b, 23b, 25b−32b. An excess of sodium borohydride was added to a well-stirred solution or suspension of the corresponding 3-(4-arylpiperazin-1-yl)-1-aryl-1-propanone (3 mmol) in methanol, over a period of 15 min at 0 °C. The stirring was continued for another 4−8 h. The reaction mixture was poured onto water. It was then extracted with ethyl acetate (3 × 20 mL), washed with water (3 × 10 mL), and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure. The product obtained was further purified by recrystallization or by column chromatography (SP: silica gel), eluting with *n*-hexane/ethyl acetate 1:1 (v/v).

3-[4-(2-Methoxyphenyl)piperazin-1-yl]-1-phenyl-1-propanol (1b). Recrystallized from ethyl ether/*n*-hexane; 60% yield; mp 85 °C. IR (KBr): 3283; 2943, 1242, 748 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.89−1.99 (m, 2H, C**H₂**CH); 2.54−2.76 (m, 6H, $CH_2N^1(CH_2)_2$); 3.13−3.15 (m, 4H, $N^4(CH_2)_2$); 3.79 (s, 3H, OCH_3); 4.89 (t, 1H, CH, J = 5.7); 6.77−6.98 (m, 4H, o-OCH_3-*Ph*); 7.15−7.34 (m, 5H, *Ph*). Anal. ($C_{20}H_{26}N_2O_2$) C,H,N.

General Procedure for Preparation of 3-(4-Arylpiperazin-1-yl)-1-aryl-1-(4-trifluoromethylphenoxy)propane Derivatives 1c−10c, 12c. The corresponding 3-(4-arylpiperazin-1-yl)-1-aryl-1-propanol derivative (2.5 mmol) was dissolved in *N*,*N*-dimethylacetamide (15 mL) and heated to 75 °C. Sodium hydride (2.5 mmol) was then added and the reaction mixture was maintained at 75 °C for 2 h to allow the formation of the salt. After this period of time, 1-fluoro-4-trifluoromethylbenzene was added (2.5 mmol) and the resulting mixture was heated at 110 °C for 4 h. The reaction mixture was poured onto crushed ice. It was then extracted with diethyl ether (4 × 10 mL), washed with brine (3 × 10 mL) and dried with anhydrous sodium sulfate. The solvent was removed under

reduced pressure. The impure oil was purified by column chromatography (SP: silica gel), eluting with *n*-hexane/ethyl acetate 1:1 (v:v). In some cases the hydrochloride salt of the product was formed by adding some drops of concentrated hydrochloric acid to a solution of the aryl ether derivative in acetone.

3-[4-(2-Methoxyphenyl)piperazin-1-yl]-1-phenyl-1-(4-trifluoromethylphenoxy)propane Dihydrochloride (1c). 25% yield; mp 80 °C. IR (KBr): 2932, 1322, 756 cm^{-1}.^{1}H NMR ($CDCl_3$) δ (free base): 1.98−2.07 (m, 1H, C**H$_a$**CH); 2.14−2.23 (m, 1H, C**H$_b$**CH); 2.45−2.58 (m, 6H, $CH_2N^1(CH_2)_2$); 2.92−3.02 (m, 4H, $N^4(CH_2)_2$); 3.76 (s, 3H, OCH_3); 5.23 (dd, 1H, CH, J = 7.3, J' = 5.2); 6.75−6.85 (m, 6H, o-OCH_3-*Ph*, p-CF_3-*Ph*, H_2+H_6); 7.16−7.36 (m, 7H, *Ph*, p-CF_3-*Ph*, H_3+H_5). Anal. ($C_{27}H_{29}F_3N_2O_2$·2HCl) C,H,N.

General Procedure for Preparation of 3-(4-Arylpiperazin-1-yl)-1-aryl-1-(1-naphthyloxy)propane Derivatives 12f, 16f. The corresponding 3-(4-arylpiperazin-1-yl)-1-thiopheneyl-1-propanol derivative (2 mmol) was dissolved in *N*,*N*-dimethylacetamide (15 mL) and heated to 75 °C. Sodium hydride (2 mmol) was then added and the reaction mixture was stirred at 75 °C for 2 h to allow the formation of the salt. After this period of time, 1-fluoronaphthalene was added (2 mmol) and the resulting mixture was heated at 110 °C for 8−24 h. The reaction mixture was then diluted with water. It was then extracted with diethyl ether (4 × 10 mL), washed with brine (3 × 10 mL) and dried (anhydrous sodium sulfate). The solvent was removed under reduce pressure. The impure oil was purified by column chromatography (SP: silica gel) eluting with *n*-hexane/ethyl acetate 1:1 (v/v). The hydrochloride salt was formed by adding some drops of concentrated hydrochloric acid to a solution of the aryl ether derivative in acetone.

3-[4-(2-Methoxyphenyl)piperazin-1-yl]-1-(1-naphthyloxy)-1-(thiophene-3-yl)propane Hydrochloride (12f). 50% yield; mp 86 °C. IR (KBr): 1239 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.48−2.66 (m, 2H, C**H$_2$**CH); 3.04−3.67 (m, 10H, CH_2); 3.78 (s, 3H, OCH_3); 5.85 (dd, 1H, CH); 6.92−7.08 (m, 4H, o-OCH_3-*Ph*); 7.27−7.55 (m, 7H, *naphthyl*); 7.58 (s; 1H, H_2); 7.85 (dd, 1H, H_4 J_{45} = 6.1, J_{25} = 3.1); 8.35 (dd, 1H, $H_{5''}$ J_{78} = 7.2, J_{68} = 3.3). Anal. ($C_{28}H_{30}N_2O_2S$·HCl) C,H,N.

General Procedure for Preparation of 3-(4-Arylpiperazin-1-yl)-1-aryl-1-(4-methoxyphenoxy or 3,4-methylenedioxyphenoxy)propane Derivatives 1d, 2d, 1e, 2e, 12e. Mesyl chloride (5 mmol) was added to a precooled suspension (0 °C, 10 min) of the corresponding 3-(4-arylpiperazin-1-yl)-1-aryl-1-propanol (2 mmol), dry acetone, (10 mL) and potassium carbonate (5 mmol) over a period of 10 min. The reaction mixture was stirred for another 48 h at 0−4 °C. It was then diluted with water (20 mL), extracted with chloroform (20 mL × 3), washed with water (10 mL × 2) and dried (anhydrous sodium sulfate). The solvent was reduced under reduce pressure and the resultant oil was used for the next reaction without further purification.

The corresponding phenol (2 mmol) was added to a solution of sodium hydroxide, (2.5 mmol) in ethanol (absolute, 5 mL) with stirring at room temperature. After 10 min, a solution of mesylate (1.5 mmol) in ethanol (absolute, 5 mL) was added dropwise with stirring. The reaction mixture was stirred for another 48 h, at room temperature and poured onto crushed ice. It was then extracted with chloroform (4 × 20 mL), washed with brine (2 × 10 mL) and dried (anhydrous sodium sulfate). The solvent was then removed under reduced pressure. The obtained oil was purified by column chromatography (SP: silica gel) eluting with chloroform/ethyl acetate 50:50 (v:v). In the cases in which the hydrochloride salt was formed, this was prepared by adding an hydrogen chloride ethereal solution to a ethereal solution of piperazine derivate.

1-Phenyl-1-(3,4-methylenedioxyphenoxy)-3-[4-(2-methoxyphenyl)piperazin-1-yl]propane Hydrochloride (1e). 21% yield; mp 90 °C. IR (KBr): 2942, 1240, 748 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ (free base): 2.02−2.09 (m, 1H, C**H$_a$**CH); 2.11−2.20 (m, 1H, C**H$_b$**CH); 2.51−2.64 (m, 6H, $CH_2N^1(CH_2)_2$); 3.08−3.11 (m, 4H, $N^4(CH_2)_2$); 3.84 (s, 3H, OCH_3); 5.08 (dd, 1H, CH, J = 8.0, J' = 4.9); 5.83 (s, 2H, OCH_2O); 6.25 (dd, 1H, OCH_2O-*Ph*, H_6, J = 8.4, J' = 1.6); 6.45 (d, 1H, OCH_2O-*Ph* H_2, J = 1.6); 6.57 (d, 1H, OCH_2O-*Ph*, H_5, J = 8.4); 6.83 (d, 1H, o-OCH_3-*Ph*, H_6, J = 7.5); 6.85−6.98 (m, 3H, OCH_3-*Ph*, H_3+H_4+H_5); 7.24−7.33 (m, 5H, *Ph*). Anal. ($C_{27}H_{30}N_2O_4$·HCl·0.5H_2O) C,H,N.

Table 10. Radioligand Binding Studies and References[a]

receptor/site	radioligand	tissue	ref
5-HT_{1A}	[^{3}H]8-OH-DPAT	rat cortex	41, 42
5-HT transporter	[^{3}H]paroxetine	rat cortex	43
5-HT_{1D}	[^{3}H]5-HT	calf caudate	44
5-HT_2	[^{3}H]ketanserin	rat frontal cortex	45
5-HT_3	[^{3}H]granisetron	rat frontal cortex	46
dopamine D_2	[^{3}H]spiroperidol	rat striatum	47
α_1-adrenoceptor	[^{3}H]prazosin	rat cortex	48
α_2-adrenoceptor	[^{3}H]clonidine	rat cortex	49
β-adrenoceptor	[^{3}H]DHA	rat cortex	50
muscarinic	[^{3}H]QNB	rat cortex	51

[a] [^{3}H]DHA, [^{3}H]dihydroalprenolol; [^{3}H]QNB, [^{3}H]quinuclidinyl benzilate.

General Procedure for Preparation of Oxime Derivatives. A mixture of 3-(4-arylpiperazin-1-yl)-1-aryl-1-propanone derivative (2.6 mmol) and hydroxylamine hydrochloride (1 g) in absolute methanol (25 mL) was heated at reflux for 1.5 h. After that the reaction mixture was lead to pH 8−9 with a solution of sodium hydroxide (10%). The reaction mixture was further refluxed for 30 min and cooled. The excess of ethanol was removed under reduced pressure. The residue was extracted with ethyl acetate (10 mL × 3), washed with brine (10 mL × 3) and dried (anhydrous sodium sulfate). The solvent was removed under reduce pressure. The two oxime isomers was separated by column chromatography (SP: silica gel) eluting with dichloromethane/methanol 9:1 (v:v).

Oxime from 1-Phenyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-1-propanone (1g). 35% yield; mp 140 °C. IR (KBr): 3423**,** 2827, 1591, 1242, 750 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.50−2.53 (m, 6H, $CH_2N^1(CH_2)_2$); 2.83−3.07 (m, 6H, CH_2−CN, $N^4(CH_2)_2$); 3.76 (s, 3H, OCH_3); 6.86−6.90 (m, 4H, o-OCH_3-*Ph*); 7.37−7.40 (m, 3H, *Ph*, H_3+H_4+H_5); 7.66 (dd, 2H, *Ph*, H_2+H_6, J = 7.8, J' = 2.6); 11.28 (s, 1H, OH). Anal. ($C_{20}H_{25}N_3O_2$) C,H,N.

Radioligand Binding Experiments. Binding studies to different receptors were performed as summarized in Table 10, in which the radioligand used, the source of tissue, and the corresponding reference are indicated.[41−51] IC_{50} values were calculated from at least three experiments by logit−log analysis and the inhibition constant (K_i) was obtained from the IC_{50} by the Cheng−Prusoff equation.[28]

8-OH-DPAT-Induced Hypothermia in Mice. The procedures used for these studies were based on previously described methods.[34] Briefly, male Swiss mice (23−28 g) were housed in groups of five and body temperature was measured with a lubricated digital thermometer probe (pb0331, Panlab, Barcelona) inserted to a depth of 2 cm into the rectum of the mice. Temperature was recorded at 15, 30, and 60 min, after injection of 8-OH-DPAT or the compound to be tested. To study the antagonism to 8-OH-DPAT-induced hypothermia, compounds or vehicle (control) were administered ip 30 min before the injection of 8-OH-DPAT (0.5 mg/kg sc). The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature recorded in this period. The results were expressed as change in body temperature (Δt) with respect to basal temperature, measured at the beginning of the experiment. The obtained data were analyzed by Anova followed by Student−Newman−Keuls test.

cAMP Formation in HeLa Cells Transfected with the Human 5-HT_{1A} Receptor.[52] Cell culture: A HeLa cell line permanently expressing the human 5-HT_{1A} receptor gene (kindly donated by Cajal Institute, Madrid) was cultured in DMEM supplemented with 2 mM glutamine, 1 mM pyruvate and 10% heat-inactivated fetal calf serum. Subcultures were made by using 0.025% trypsin in PBS. Cultures were maintained at 37 °C in an air/CO_2 (95:5) water-saturated atmo-

sphere. cAMP experiments were carried out with cultures grown for 2−3 days in 8-well culture plates with 2 mL medium/well.

Forskolin-induced cAMP formation: Cultures (about 7.5×10^4 cells/well) were washed with PBS and incubated for 10 min with 1 mL of PBS containing 0.5 mM isobutylmethylxanthine, 10 μM forskolin in the presence or absence of test compounds. The medium was then aspirated and the reaction stopped by addition of 600 μL ice-cold ethanol. Two hours later ethanol was taken into an Eppendorf tube to be lyophilized and the resulting pellet was resuspended in 100 μL of assay buffer (Kit Amersham SPA, RPA 538) and cAMP was quantified by RIA. To study the antagonism to 8-OH-DPAT-induced inhibition of forskolin-induced cAMP formation, test compounds were preincubated 20 min before the addition of forskolin and 8-OH-DPAT.

FST or Porsolt Test.[53] Mice were placed individually for 6 min into a glass cylinders (height 24 cm, diameter 13 cm) containing 14 cm of water, maintained at 22−23 °C. This procedure was repeated for 2 consecutive days. On the second day, the animals were treated 30 min before water immersion and the duration of immobility was recorded the second day during the last 4 min of the 6-min testing period. A mouse was considered to be immobile when it floated in an upright position and made only small movements to keep its head above water. The tricyclic antidepressant amitriptyline was used as reference compound. Results were analyzed by using the Student's *t*-test.

Learned Helplessness Test. The test was performed as previously described[54] with minor modifications. Experimental condition were as follows:

Helpless induction: On day 1, rats were placed individually in an operand conditioning chamber with a grid floor connected to a scrambled shock generator (Coulbourn Instruments). Each rat was then exposed to inescapable electric foot shocks (1.1 mA, 10 s) every 30 s during 30 min.

Conditioned avoidance training: Animals were placed individually into a shuttle-box (Letica Instruments, Spain) consisting of two compartments of the same size separated by a door. Shocks delivered through the grid floor were terminated as soon as the animal entered into the other compartment. The assay consisted of 30 stimulus shock trials of 8 s with a 30-s resting period between each trial. During the first 5 s of each trial, a light and a sound were on (conditioned stimulus). If the animal did not cross within this period to the other compartment, a shock (1 mA, 3-s maximal duration) was delivered. Avoidance sessions were performed during 3 consecutive days and the number of escape failures and of intertrial crossings were recorded.

Animals received either saline or treatment throughout the 4-day period. On day 1, the drug was given 6 h after exposure to the inescapable shocks and on days 2−4 the drug was administered twice a day, 30 min before shuttle-box exposure and 6 h after. On each day of avoidance testing, differences between control and treated rats were evaluated by the Mann−Whitney *U*-test.

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Supporting Information Available: ^{1}H NMR, IR, combustion analyses, and physical data of the prepared compounds (mp, yield, purification method, and formula). This material is available free of charge via the Internet at http://pubs.acs.org.

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