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Synthesis of New 1,2,3-Benzotriazin-4-one-arylpiperazine Derivatives as 5-HT_{1A} Serotonin Receptor Ligands

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Abstract—A series of novel 1,2,3-benzotriazin-4-one derivatives was prepared and evaluated as ligands for 5-HT receptors. Radioligand binding assays proved that the majority of the novel compounds behaved as good to excellent ligands at the 5-HT_{1A} receptor, some of which were selective with respect 5-HT_{2A} and 5-HT_{2C} receptors. Six analogues (**1a, 2a, 2b, 2c, 2e** and **2i**) were selected and further evaluated for their binding affinities on D_1, D_2 dopaminergic and α_1-, α_2-adrenergic receptors. A *o*-OCH_3 derivative (**2e**) bound at 5-HT_{1A} sites with subnanomolar affinity ($IC_{50} = 0.059$ nM) and shows high selectivity over all considered receptors and may offer a new lead for the development of therapeutically efficacious agents.

Introduction

Serotonin (5-HT) is an important molecule in medicinal chemistry, and several 5-HT receptor subtypes have been found by molecular biological methods as well as by specific agonists or antagonists.[1,2] Particular attention, over the last decade, has been focused on 5-HT_{1A} because this receptor plays an important role in the central nervous system (CNS) modulating a number of behaviours such as impulsivity, sexual behaviour and food intake.[3] Moreover, several agonists for this receptor have been shown to possess anxiolytic and antidepressant properties in man.[4] It is potentially useful to develop ligands that are selective for the 5-HT_{1A} subtype to facilitate the study and characterization of this receptor. Continuing our study in this field,[5–8] recently we reported the synthesis and the pharmacological characterization of a new class of benzoyltriazole arylpiperazine derivatives,[9] which have 5-HT_{1A} receptor affinity while they showed low affinity versus two other considered serotonin receptors (5-HT_{2A}, 5-HT_{2C}). Taking into account these results, in an attempt to increase affinity and selectivity for the 5-HT_{1A} receptor, in order to clarify the role played by the heterocyclic nucleus in the interaction with a corresponding hydrophobic region of the 5-HT_{1A} receptor, and the importance of the nature of the polymethylene chain connecting the heterocyclic nucleus and piperazine rings, we replaced the benzoyltriazole nucleus by a 3-hydroxy-1,2,3-benzotriazin-4-one ring also introducing an oxygen atom in the polymethylene chain.

Substituents used on the arylpiperazine moiety (X = *o*-Cl, *m*-Cl, *p*-Cl, *o*-OCH_3, *p*-OCH_3, *o*-F, *p*-F and *m*-CF_3) are those previously reported[9] except for *m*-CF_3 which had not been considered in the previous work. The piperazine ring is connected to the 3-hydroxybenzotriazinone moiety through an ethylene (series **1**) or a propylene (series **2**) bridge.

In this article, we described the synthesis of new 1,2,3-benzotriazin-4-one arylpiperazines (**1a**–**i** and **2a**–**i**), their affinity and selectivity for 5-HT_{1A} receptor, obtained by radioligand binding studies. Several analogues showing interesting 5-HT_{1A} binding properties were further evaluated for their affinity versus dopaminergic D_1, D_2 and adrenergic α_1, α_2 receptors.

Chemistry

The synthesis of the 1,2,3-benzotriazinone derivatives **1a**–**i** and **2a**–**i** is summarized in Scheme 1. The 1-(2-chloroethyl) and 1-(3-chloropropyl)-4-phenylpiperazine intermediates **4** and **5**, substituted on the aromatic ring,

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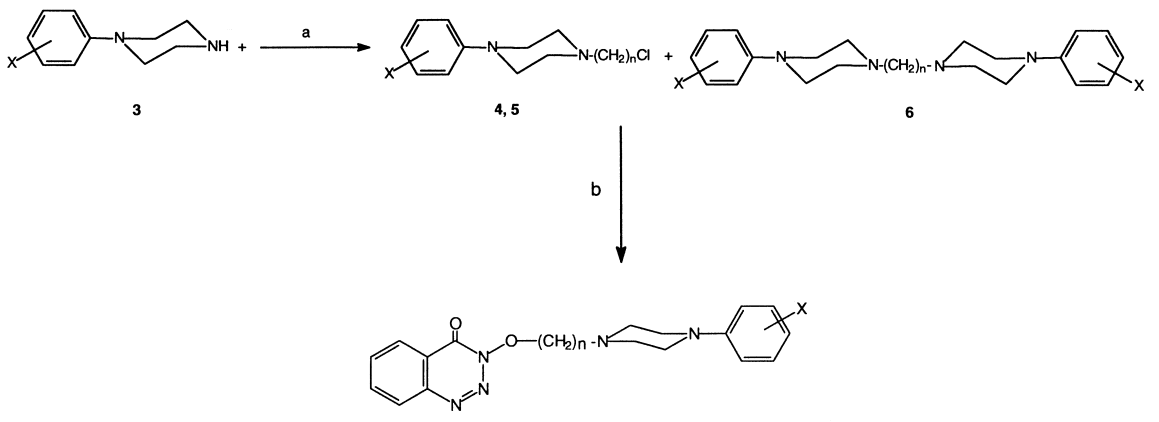
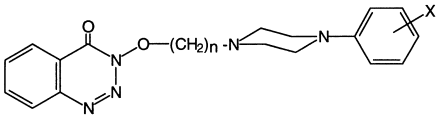


Scheme 1. Reagents and conditions: (a) $Br(CH_2)_nCl$, toluene, △; (b) 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one, NaOH, C_2H_5OH, △.

were prepared by alkylation of 1-bromo-2-chloroethane or 1-bromo-3-chloropropane, respectively, with various 4-phenylpiperazines **3,** commercially available. The reaction yielded the bis compound **6** as a by-product**.** To avoid this, the reagents were dissolved in toluene as an inert solvent, using a small excess of 1-bromo-2-chloroethane or 1-bromo-3-chloropropane. To reduce the production of the bis derivative **6**, the substituted piperazine had to be added when the reaction mixture was already under reflux. Condensation of the obtained compound **4** or **5**, with 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one in ethyl alcohol and in the presence of sodium hydroxide gave the expected compounds **1a**–**i** and **2a**–**i** (Table 1) in yields ranging between 40 and 65%. Analytical purification of each product, reported in Table 1, was obtained by chromatography on silica gel column and further by crystallization from the appropriate solvent. All new compounds gave satisfactory elemental analyses (C, H, Cl, F, N) and were characterized by ^1H NMR spectroscopy.

Table 1. Physicochemical properties of 1,2,3-benzotriazin-4-one derivatives **1a**–**i** and **2a**–**i**



Compound	X	n	Formula[a]	Mp (°C)	Cryst. solvent[b]	Yield[c] %
1a	H	2	$C_{19}H_{21}N_5O_2$	112–114	a	40
1b	*o*-Cl	2	$C_{19}H_{20}N_5O_2Cl$	84–85	a + e	41
1c	*m*-Cl	2	$C_{19}H_{20}N_5O_2Cl$	96–97	b	53
1d	*p*-Cl	2	$C_{19}H_{20}N_5O_2Cl$	140–141	a	45
1e	*o*-OCH_3	2	$C_{20}H_{23}N_5O_3$	68–69	a	50
1f	*p*-OCH_3	2	$C_{20}H_{23}N_5O_3$	141–142	b	55
1g	*o*-F	2	$C_{19}H_{20}N_5O_2F$	137–138	a + b	50
1h	*p*-F	2	$C_{19}H_{20}N_5O_2F$	145–147	a + b	48
1i	*m*-CF_3	2	$C_{20}H_{21}N_5O_2F_3$	101–102	a + b	40
2a	H	3	$C_{20}H_{23}N_5O_2$	124–125	a + d	42
2b	*o*-Cl	3	$C_{20}H_{22}N_5O_2Cl$	99–100	a	63
2c	*m*-Cl	3	$C_{20}H_{22}N_5O_2Cl$	116–117	a + b	45
2d	*p*-Cl	3	$C_{20}H_{22}N_5O_2Cl$	119–120	c	54
2e	*o*-OCH_3	3	$C_{21}H_{25}N_5O_3$	81–82	a	48
2f	*p*-OCH_3	3	$C_{21}H_{25}N_5O_3$	153–155	a	65
2g	*o*-F	3	$C_{20}H_{22}N_5O_2F$	140–141	a + b	48
2h	*p*-F	3	$C_{20}H_{22}N_5O_2F$	135–136	a + b	62
2i	*m*-CF_3	3	$C_{21}H_{23}N_5O_2F_3$	118–119	b	33

[a]Satisfactory microanalyses obtained: C, H, Cl, F, N values are within ±0.4% of the theoretical values.
[b]Cryst. solvent: a = diethyl ether; b = ethyl alcohol; c = methyl alcohol; d = chloroform; e = *n*/hexane.
[c]Yield related to last step.

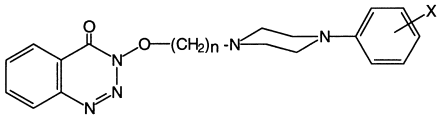
Pharmacology

The compounds reported in Table 1 (**1**–**2**) were tested for their in vitro affinity on serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors by radioligand binding assays. The more active compounds on serotonin receptors have been selected and evaluated for their affinity on dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. All the compounds were tested as water soluble, hydrochloride salts. The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptors, [^{3}H]-8-OH-DPAT, rat brain cortex membranes; (b) serotonin 5-HT_{2A} receptors, [^{3}H] ketanserin, rat brain cortex membranes; (c) serotonin 5-HT_{2C} receptors, [^{3}H] mesulergine, rat brain cortex membranes; (d) dopamine D_1 receptors, [^{3}H]SCH-23390, rat strial membranes; (e) dopamine D_2 receptors, [^{3}H]spiroperidol, rat strial membranes; (f) α_1-adrenergic receptors, [^{3}H]prazosin, rat brain cortex membranes; (g) α_2-adrenergic receptors, [^{3}H]yohimbine, rat brain cortex membranes. Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) were determined through four independent experiments with samples in triplicate using seven to nine different concentrations of the title compound. Specific binding, defined as described in the Experimental, represented more than 75% of total binding in all three assays. The obtained IC_{50} nM values are listed in Table 2 and 3.

Results and Discussion

The novel compounds (**1a**–**i** and **2a**–**i**) derived from benzoyltriazole derivatives[9] were prepared and preliminarily evaluated in vitro for their receptor binding affinity for 5-HT_{1A} receptors and their selectivity was

Table 2. Binding affinities and selectivities of 1,2,3-benzotriazin-4-one derivatives **1a–i** and **2a–i**



Compound	X	n	IC_{50} nM (± SEM)			Selectivity versus 5-HT_{1A} receptor IC_{50} ratio	
			5- HT_{1A} [^{3}H]8-OH-DPAT	5-HT_{2A} [^{3}H] ketanserin	5-HT_{2C} [^{3}H] mesulergine	5-HT_{2A}	5-HT_{2C}
1a	H	2	19 ± 2.0	15,000 ± 1350	8.4 ± 0.9	789	0.4
1b	*o*-Cl	2	95 ± 8.7	9100 ± 862	490 ± 52	96	5
1c	*m*-Cl	2	38 ± 3.6	6800 ± 700	97 ± 9.2	179	3
1d	*p*-Cl	2	3800 ± 382	9000 ± 897	1300 ± 110	2.4	0.3
1e	*o*-OCH_3	2	41 ± 3.8	$> 10^5$	1100 ± 980	$> 2\ 10^3$	27
1f	*p*-OCH_3	2	$> 10^5$	$> 10^5$	860 ± 82	1	$< 9\ 10^{-3}$
1g	*o*-F	2	470 ± 49	990 ± 95	$> 10^5$	2	$> 2\ 10^2$
1h	*p*-F	2	7700 ± 680	160 ± 20	$> 10^5$	0.02	> 13
1i	*m*-CF_3	2	2700 ± 300	130 ± 15	7400 ± 720	0.05	3
2a	H	3	7.0 ± 0.5	7700 ± 740	26 ± 2.3	1100	4
2b	*o*-Cl	3	1.0 ± 0.1	1000 ± 950	130 ± 11	1000	130
2c	*m*-Cl	3	2.4 ± 0.3	530 ± 55	40 ± 3.7	221	17
2d	*p*-Cl	3	20 ± 1.8	630 ± 60	47 ± 4.4	31	2.4
2e	*o*-OCH_3	3	0.059 ± 0.006	5500 ± 520	26 ± 2.5	93220	441
2f	*p*-OCH_3	3	770 ± 74	9300 ± 900	150 ± 18	12	0.2
2g	*o*-F	3	21 ± 2.0	16 ± 1.8	$> 10^5$	0.8	$> 5\ 10^3$
2h	*p*-F	3	160 ± 18	6.8 ± 0.7	4.2 ± 0.4	0.04	0.03
2i	*m*-CF_3	3	0.54 ± 0.04	0.0069 ± 0.0008	16 ± 1.4	0.01	30
8-OH-DPAT			2.1 ± 0.3	—	—	—	—
Ketanserin			—	1.7 ± 0.3	—	—	—
Cinanserin			—	1.6 ± 1.0	—	—	—
Mesulergine			—	—	1.2 ± 0.2	—	—

Table 3. Binding affinities for D_1, D_2, α_1 and α_2 receptors in compounds **1a**, **2a**, **2b**, **2c**, **2e** and **2i**

Compound	X	n	IC_{50} nM (SEM)			
			D_1 [^{3}H] SCH-23390	D_2 [^{3}H] spiroperidol	α_1 [^{3}H] prazosin	α_2 [^{3}H] yohimbine
1a	H	2	$> 10^5$	$> 10^5$	4400 350	400 38
2a	H	3	$> 10^5$	2600 ± 270	820 ± 85	1600 ± 180
2b	*o*-Cl	3	3400 ± 270	5200 ± 490	2.5 ± 0.4	15 ± 1.7
2c	*m*-Cl	3	1500 ± 150	1100 ± 110	8.4 ± 0.8	9900 ± 920
2e	*o*-OCH_3	3	4900 ± 410	$> 10^5$	340 ± 35	1500 ± 160
2i	*m*-CF_3	3	2.3 ± 0.3	9700 ± 890	17 ± 1.8	5300 ± 4.50
Spiroperidol			3.6 ± 0.5	4.6 ± 0.7		
Prazosin					1.3 ± 0.7	
Yohimbine						22 ± 2

compared to those of two other receptors, 5-HT_{2A} and 5-HT_{2C}. The obtained IC_{50} (nM) values are reported in Table 2. The values obtained with 8-OH-DPAT, ketanserine, and mesulergine were also reported as reference for 5-HT_{1A}-selective, 5-HT_{2A}-selective and 5-HT_{2C}-selective ligands.

The products selected on the preliminary binding screening were tested on an extended binding screening: dopaminergic D_1, D_2 and adrenergic α_1, α_2 receptors (Table 3). Biological data presented in these tables agree with our working hypothesis noted in the Introduction. In fact, most of the considered compounds demonstrated moderate to high affinity for the 5-HT_{1A} receptor.

The study of the results presented in Table 2 shows that the length of the alkyl chain plays an important role in the affinity and selectivity for the 5-HT_{1A} receptor. Among the ethylene series the compounds **1a**, **1c** and **1e** appear to be good ligands for the 5-HT_{1A} receptor since they show IC_{50S} values in the range of 15–50 nM. Moreover, these compounds have appreciable selectivity for the 5-HT_{1A} versus the 5-HT_{2A} receptor. Notably, **1e** is 1000-fold more potent on 5-HT_{1A} than towards 5-HT_{2A}. Unfortunately, 5-HT_{2C}/5-HT_{1A} IC_{50} ratios of **1a**, **1c** and **1e**, are all below 30.

The propylene derivatives (series **2**) showed receptor affinities higher than the corresponding ethylene homo-

logues. In fact it should be noted that several compounds (**2a**, **2b**, **2c**, **2e** and **2i**) on 5-HT_{1A} receptor exhibit IC_{50} values lower than 10 nM. Remarkably, **2e** and **2i**, exhibit subnanomolar affinity on 5-HT_{1A}, IC_{50} 0.059 and 0.54 nM, respectively. These results allowed us to conclude that the propylene chain was the better to furnish analogues with a good balance of affinity and selectivity.

Subsequently, we attempted the modification of the nature and the position of the substituents on the aromatic ring. Analysis of the structure endowed with highest 5-HT_{1A} affinity suggests that *ortho* (**1e**, **2b**, **2e** and **2g**) or *meta* (**1c**, **2c** and **2i**) by Cl, OCH_3 or F are favorable positions for substitution on the phenyl ring. Moving the substituent to the *para* position (**1f**, **2d** and **2f**) leads to a reduction of potency, especially in the case of the *p*-OCH_3 derivative (**1f** and **2f**). The same deleterious effect on affinity was observed in the benzoyltriazole series.[9] Note compounds **1a** and **2a** with no substituent also bind to the 5-HT_{1A} receptor with high affinity.

Compound **2e** shows the best affinity-selectivity profile for the 5-HT_{1A} receptor (5-HT_{2A}/5-HT_{1A} and 5-HT_{2C}/5-HT_{1A} IC_{50} ratios are about of 100,000 and 500, respectively). Compounds **2a** and **2b** are also significantly selective for 5-HT_{1A} over 5-HT_{2A} receptor (5-HT_{2A}/5-HT_{1A} IC_{50} ratios of both compounds are in the order of 1000).

As far as 5-HT_{2A} the results of these studies indicate that the affinity for this receptor are usually lower than the affinity for the 5-HT_{1A} receptor. The introduction of electron withdrawing substituents, such as the F or CF_3 group, increase the binding affinity for the 5-HT_{2A} receptor. In fact we obtained very interesting results with compound **2i**, which exhibits pM affinity (IC_{50} value is 6.9 10^{-3} nM). In addition, highly potent ligands are **2g** and **2h** showing IC_{50} values of 16 and 6.8 nM, respectively.

In particular, compounds **2i** and **2g** show the best selectivity for 5-HT_{2A} versus 5-HT_{2C} receptor (5-HT_{2A}/5-HT_{2C} IC_{50} ratios are 2300 and 6250, respectively) but they are moderately or scarcely selective versus the receptor 5-HT_{1A} (5-HT_{1A}/5-HT_{2C} IC_{50} ratios 78 and 1.3 nM, respectively). Compound **2h** is likewise nonselective for the 5-HT_{2A} receptor since it is only 20-fold more potent at the 5-HT_{2A} than at the 5-HT_{1A} receptor and has comparable affinity for the 5-HT_{2C} receptor.

As for the 5-HT_{2C} receptor, in addition to the above mentioned compound **2h** (IC_{50} = 4.2 nM) the compounds **2a**, **2c**, **2d**, **2e** and **2i**, exhibit IC_{50} values in the range of 10–50 nM. All these compounds are moderately selective on the 5-HT_{2C} versus 5-HT_{2A} receptor and are not selective versus 5-HT_{1A}.

As mentioned in the Introduction, to evaluate potential therapeutic uses it is crucial to check whether high potency is flanked by undesirable affinity for other receptors (e.g. dopaminergic and adrenergic receptors).[5–8] Thus, the most active compounds of the series **1** and **2** on 5-HT_{1A} (**1a**, **2a**, **2b**, **2c**, **2e** and **2i**), were further evaluated for their affinity at dopaminergic and adrenergic receptors. Results are summarized in Table 3.

As far as the dopaminergic system was concerned, the D_1 and D_2 receptor affinity consistently showed IC_{50} values of above 10^{-6} M except compound **2i** which exhibited a IC_{50} value of 2.3 nM at the D1 receptor. The affinities for α_1 receptors were in some cases quite considerable (**2b**, **2c** and **2i**). In particular **2b** and **2c** derivatives showed affinity for the α_1 similar to that shown concerning the 5-HT_{1A} receptor. In regards to the affinity for α_2-adrenergic receptors, the IC_{50} values were high for all selected compounds with the exception of **2b** (IC_{50} = 15 nM).

Our results confirm that the replacement of the benzoyltriazole ring of previously studied structures,[9] by a 3-hydroxy-1,2,3-benzotriazinone ring, proved compounds of series **1** and **2**, have led to the same binding profile. In fact these new compounds, appear to own the physicochemical requirements necessary to insure a higher affinity on the 5-HT_{1A} receptor. In particular compound **2e**, the most potent 5-HT_{1A} ligand, which showed the best selectivity profile versus all considered receptors, may offer a new lead for the development of new ligands.

In conclusion, we have synthesized and evaluated a new class of 5-HT_{1A} ligands having a 3-hydroxy-1,2,3-benzotriazinone skeleton. Although the compounds exhibit a good affinity for the 5-HT_{1A} receptor, further synthesis and biological in vitro studies are in progress to evaluate its intrinsic efficacy. The results of this work will be published in the near future.

Experimental

Chemistry

General. Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Kieselgel 60 was used for column chromatography and kieselgel 60 F_{254} plates from Merck were used for TLC. Where analyses are indicated only by the symbols of the elements, results obtained are within ±0.4% of the theoretical values. The purity of compounds was carefully assessed using analytical TLC and the structure verified spectroscopically by proton NMR spectra ($CDCl_3$) recorded on a Bruker AMX-500 instrument. Chemical shifts in ppm are referenced to the residue solvent signal at 7.27 ppm. Reagent grade materials were purchased from Aldrich. Appropriate aromatically substitued-4-phenylpiperazines and 3-hydroxy-1,2,3-benzotriazin-4(3)-one are commercially available from Aldrich.

1-(2-Chloroethyl)piperazine-4-substituted derivatives (4). A solution of the appropriate aromatically substituted-4-phenyl-piperazine (0.1 mol) in toluene (30 mL) was added dropwise over a 1 h period to a boiling solution of 1-bromo-2-chloroethane (0.15 mol) in 80 mL of toluene. The reaction mixture was stirred and refluxed for 5 h.

Successively, the mixture was cooled and partitioned between water, alkalinized with 2 N NaOH and chloroform. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude residue was purified by silica-gel column chromatography (eluent: diethyl ether) to obtain derivatives **4** in a yield ranging between 40 and 55%.

1-(3-Chloropropyl)piperazine-4-substituted derivatives (5). These compounds were prepared by the same procedure described above, from different 4-substituted-piperazine (0.1 mol) and 1-bromo-3-chloropropane (0.15 mol). All derivatives **5** were obtained in a yield ranging between 45 and 60%.

General procedure for the preparation of 3-{2-[4-(X-phenyl)-1-piperazinyl]etoxyl}benzo-triazinone derivatives (1a–i). To a stirred mixture of absolute ethanol (100 mL) and sodium hydroxide (0.1 mol) were added 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (0.1 mol) and appropriate 1-(2-chloroethyl)piperazine-4-substituted (0.1 mol). The mixture was refluxed for 24 h, diluted with H_2O, and extracted with CH_2Cl_2. The combined organic layers were dried on anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (eluent: CH_2Cl_2:EtOH 9:1 (v/v)). Further purification was performed by crystallization from the appropriate solvent to give the final products **1a–i** in the yields provided in Table 1. NMR spectra for all intemediates and final compounds were consistent with the proposed structures.

General procedure for the preparation of 3-{3-[4-(X-phenyl)-1-piperazinyl]propoxy}-benzotriazinone derivatives (2a–i). These compounds were prepared by the same procedure described above from 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (0.1 mol) and the appropriate 1-(3-chloropropyl)piperazine-4-substituted (0.1 mol). Compounds **2a–i** were obtained in the yields provided in Table 1. NMR spectra for all intemediates and final compounds were consistent with the proposed structures.

Pharmacology

5-HT_{1A} binding assay. Radioligand binding assay was performed following a published procedure.[10] Cerebral cortex from male Sprague–Dawley rats (180–220 g) was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7 at 22 °C) with a Brinkmann Polytron (setting 5 for 15 s), and the homogenate was centrifuged at 50,000 *g* for 10 min. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37 °C, and centrifuged at 50,000 *g* for 10 min. The final pellet was resuspended in 80 volumes of the Tris–HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. To each assay tube were added the following: 0.1 mL of the drug dilution (0.1 mL of distilled water if no competing drug was added), 0.1 mL of [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]-8-OH-DPAT) in buffer (containing Tris, $CaCl_2$, pargyline and ascorbate) to achieve a final assay concentration of 0.1 nM, and 0.8 mL of resuspended membranes. The tubes were incubated for 30 min at 37 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters. The filters were washed twice with 5 mL of ice-cold Tris–HCl buffer, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [^{3}H]-8-OH-DPAT binding was defined as the difference between binding in the absence and presence of 5-HT (10 μM).

5-HT_{2A} and 5-HT_{2C} binding assays. Radioligand binding assays were performed as previously reported by Herndon et al.[11] Briefly, frontal cortical regions of male Sprague–Dawley rats (200–250 g, Charles River) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris–HCl, 0.5 mM EDTA and 10 mM $MgCl_2$ at pH 7.4) and centrifuged at 3000 *g* for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37 °C for 15 min and then centrifuged twice more at 3000 *g* for 10 min (with resuspension between centrifugation). The final pellet was resuspended in buffer that also contained 0.1% ascorbate and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0 mL volume containing 5 mg wet weight of tissue and 0.4 nM [^{3}H] ketanserin (76 Ci/mmol; New England Nuclear) for 5-HT_{2A} receptor assays, and 10 mg wet weight of tissue and 1 nM [^{3}H] mesulergine (75.8 Ci/mmol; Amersham) for 5-HT_{2C} receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT_{2A} assay. In the 5-HT_{2C} assays, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block binding to 5-HT_{2A} receptors. Tubes were incubated for 15 min at 37 °C, filtered on Schliecher and Schuell (Keene, NH) glass fiber filters presoaked in poly(ethylene imine), and washed with 10 mL of ice-cold buffer. Filters were counted at an efficiency of 50%.

D_1 dopaminergic binding assay. The binding assay for D_1 dopaminergic receptors was that described by Billard et al.[12] Corpora striata were homogenized in 30 vol (w/v) ice-cold 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50 000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL [^{3}H]SCH-23390 to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris–HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]SCH-23390 binding was defined as the difference between binding in the absence or in the presence of 0.1 μM piflutixol.

D_2 dopaminergic binding assay. The procedure used in the radioligand binding assay was reported in detail by Creese et al.[13] Corpora striata were homogenized in

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

α_1-Adrenergic binding assay. The procedure used in the radioligand binding assay has been reported in detail by Greengrass and Bremner.[14] Brain cortex was homogenized in 30 vol (w/v) ice-cold 50 mM Tris–HCl buffer, (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50,000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl, (pH 7.4 at 25 °C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]prazosin to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris–HCl, buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]prazosin binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

α_2-Adrenergic binding assay. The procedure used in the radioligand binding assay was reported in detail by Perry and U'Prichard.[15] Brain cortex was homogenized in 30 vol (w/v) ice-cold 5 mM Tris–HCl, 5 mM EDTA buffer (pH 7.3 at 25 °C) using a polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged three times for 10 min at 50,000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl, 0.5 mM EDTA (pH 7.5 at 25 °C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]yohimbine to achieve a final concentration of 1 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris–HCl, 0.5 mM EDTA buffer (pH 7.5 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]yohimbine binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

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