Serotonergic Properties of Spiroxatrine Enantiomers

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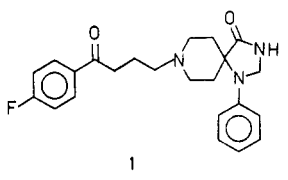
The neuroleptic drug spiperone (1) has proven very useful in the characterization of putative serotonin (5-hydroxytryptamine, 5-HT) receptors. Thus, 5-HT_1 receptors have been divided into subtypes based on their affinities for 1: 5-HT_{1A} sites have high affinity, while 5-HT_{1B} sites have low affinity. However, the usefulness of 1 for the pharmacological characterization of 5-HT_{1A} sites is limited because of its high affinity for 5-HT_2 (as well as D_2-dopaminergic) receptors. A close analogue of 1, (±)-spiroxatrine (2), has much higher affinity for 5-HT_{1A} receptors and much lower affinity for 5-HT_2 receptors. We report here the stereospecific synthesis of (*R*)-(+)- and (*S*)-(−)-spiroxatrine enantiomers and their evaluation at several 5-HT receptors and D_2-dopaminergic and α_1-adrenergic receptors.

In a search for selective antagonists for 5-HT_1 receptors, the compound initially studied was the neuroleptic drug spiperone (1). In addition to having high affinity for D_2 and 5-HT_2 receptors, spiperone also distinguishes between the subtypes of 5-HT_1 binding sites, having high affinity for 5-HT_{1A} sites and low affinity for 5-HT_{1B} sites.[1] A series of analogues related to spiperone obtained from Janssen Laboratories were examined for their potencies at 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 binding sites in an attempt to find an agent with greater selectivity for 5-HT_{1A} sites.[2] The most potent and selective of these compounds was spiroxatrine (2), which exhibits excellent discrimination between 5-HT_{1A} and 5-HT_{1B} sites, being 75 000 times more potent at the former. It also was 30 times more potent at 5-HT_{1A} sites than at 5-HT_2 sites. In order to further investigate the selectivity of 2, we have stereospecifically prepared the *R*-(+) and *S*-(−) enantiomers and examined their binding at 5-HT_{1A}, 5-HT_{1B}, 5-HT_2, and D_2-dopaminergic and α_1-adrenergic receptors.



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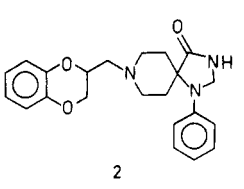
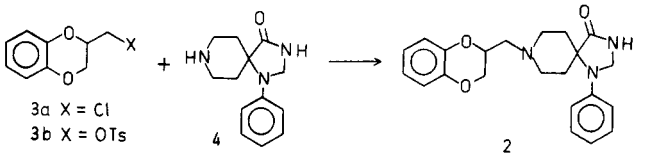


2

Chemistry

Racemic spiroxatrine, (±)-2, has been prepared by an S_N2 reaction between 2-(chloromethyl)-1,4-benzodioxan (**3a**) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one) (4) (Scheme I).[3] We have found that the tosylate (**3b**) of 2-(hydroxymethyl)-1,4-benzodioxane (6) reacted with 4 in DMSO at 25 °C over a period of 3 days gives a better yield of 2. The synthesis of (*R*)-(+)-2 and (*S*)-(−)-2 thus required the preparation of (*R*)-(+)-6 and (*S*)-(−)-6 as the key intermediates. This synthesis, which features the Sharpless epoxidation of allylic alcohol 5 as the critical step,[4] is outlined in Scheme II. The absolute configuration of the final product can be controlled by using (+)-diethyl tartrate or (−)-diethyl tartrate in the epoxidation reaction. Reductive debenzylation of 5 followed by base-catalyzed ring closure of the epoxy alcohols 8 gave the appropriate 2-oxiranyl-1,4-benzodioxans 9. The optical purities of the enantiomers (2*R*,1′*S*)-9 and (2*S*,1′*R*)-9 were found to be 95.3 and 94.2% ee based on earlier results.[4,5] The latter were converted to the amino alcohol 10 by reaction with *n*-propylamine. Periodate cleavage of 10 gave 1,4-benzodioxane-2-carboxaldehydes (11), which were reduced in situ to (*R*)-(+)- and (*S*)-(−)-6 with sodium borohydride. The

Scheme I



3a X = Cl
3b X = OTs

4

2

Table I. Comparison of the Affinities of the (+) and (−) Enantiomers of Spiroxatrine at Different Monoaminergic Receptor Binding Sites[a]

binding site	apparent K_i, nM (−)-spiroxatrine	(+)-spiroxatrine	*R*
5-HT_{1A}	1.9 ± 0.4 (4)	21 ± 6 (4)	11
5-HT_{1B}	$>10^{-5}$ M (2)	$>10^{-5}$ M (2)	
5-HT_2	113 ± 31 (3)	858 ± 46 (3)	8
D_2-dopaminergic	0.22 ± 0.02 (3)	1.45 ± 0.06 (3)	7
α_1-adrenergic	149 ± 17 (3)	1050 ± 190 (3)	7

[a] Each apparent K_i value is expressed as the mean ±SEM in nanomolar units, except at the 5-HT_{1B} site where no significant inhibition of binding was seen at concentrations up to 10^{-5} M. The number of separate experiments is given in parentheses. *R* is the ratio: (K_i of (+)-spiroxatrine)/(K_i of (−)-spiroxatrine).

tosylates of the optically pure 2-(hydroxymethyl)-1,4-benzodioxans (6)[5] were reacted with 1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one to give the corresponding spiroxatrine enantiomers (*R*)-(+)-2 and (*S*)-(−)-2.

Results and Discussion

As predicted from the activity of the racemate,[2] neither spiroxatrine enantiomer had significant affinity for the 5-HT_{1B} site. Both the *R*-(+) and *S*-(−) enantiomers of spiroxatrine (2) were more potent at the 5-HT_{1A} than the 5-HT_2 sites, having a similar degree of selectivity between these two types of receptors. Thus, the individual enantiomers did not show any improvement in selectivity over the racemic mixture.

It was interesting, however, that (*S*)-(−)-spiroxatrine was the most potent isomer, not only at the 5-HT receptor binding sites, but also at two other monoaminergic receptors, the D_2-dopaminergic and α_1-adrenergic receptors. Thse other two receptors were examined because it is common for many classes of antagonists to cross-react with these receptors and with certain of the 5-HT receptors. In

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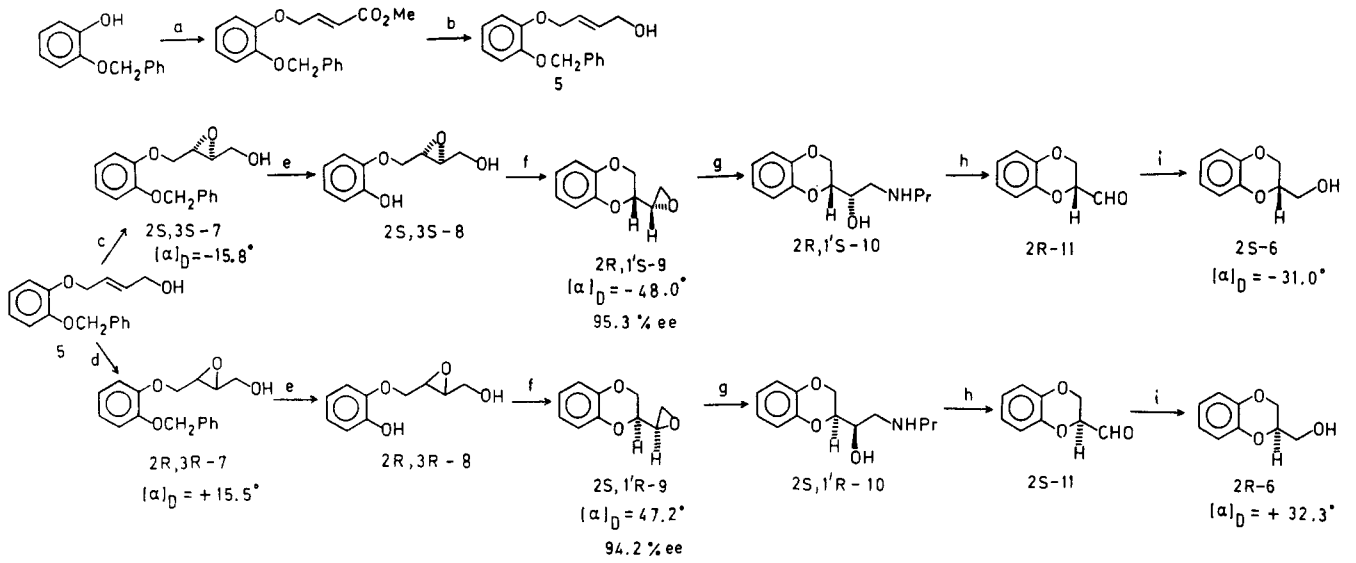
Scheme II[a]



[a] Reagents: (a) $BrCH_2CH_2{=}CHCO_2Et/K_2CO_3$/2-butanone; (b) Dibal/ether; (c) Ti(*i*-OPr)$_4$/(+)-diethyl tartrate/*t*-Bu hydroperoxide in toluene; (d) Ti(*i*-OPr)$_4$/(−)-diethyl tartrate/*t*-Bu hydroperoxide in toluene; (e) Pd/C, 10%; EtOH; (f) NaOH/H_2O; (g) *n*-Pr-NH_2; (h) $NaIO_4/H_2O$/MeOH; (i) $NaBH_4/H_2O$/MeOH.

addition to the *S*-(−) enantiomer being the most potent enantiomer at these sites, the ratio of the potency of the *R*-(+) enantiomer to the *S*-(−) enantiomer at each of the binding sites was very close (Table I). These results suggest the possibility that there may be similarities in the way that each of the different receptors recognizes spiroxatrine. Other studies, comparing disparate classes of drugs that are active in the central nervous system, have also noted common structural features required for recognition by the different receptor types. This has led Andrews and Llyod[6] to suggest that many of these may have evolved from a common ancestral protein. Such an idea is consistent with current findings, using molecular biologic techniques, of significant structural homologies between certain groups of receptors (for a review see Hall, 1987).[7] Thus, the pharmacologic similarities between 5-HT_{1A}, 5-HT_2, D_2-dopaminergic, and α_1-adrenergic receptors might suggest that these receptors could be the products of a related gene family, while the 5-HT_{1B} site, which shares little pharmacologic similarity with these other sites, might belong to a different or less closely related receptor family.

While there may be similarities between the way that certain of the different receptor types recognize spiroxatrine, there are also some significant differences. It has long been suggested that (±)-spiroxatrine is an antagonist at dopaminergic receptors,[8] and we have also shown that it is an antagonist at 5-HT_2 receptors measured in the rat aorta[9] and 5-HT_{1A}-like receptors in the canine basilar artery.[10] However, we have recently shown that (±)-spiroxatrine is an agonist at hippocampal 5-HT_{1A} receptors, which are negatively coupled to adenylate cyclase.[11] Preliminary results with the individual enantiomers are consistent with these previous findings, i.e., both (*R*)-(±)- and (*S*)-(−)-spiroxatrine appear to act as antagonists at 5-HT_2 receptors in the rat aorta and as agonists at 5-HT_{1A} receptors linked to adenylate cyclase (unpublished observations). This property of spiroxatrine, i.e., agonist activity at 5-HT_{1A} receptors and antagonist activity at other monoaminergic receptors, is similar to a number of compounds of current interest, such as buspirone, ipsapirone, gepirone, and WB4101, which have been proposed as novel antianxiety agents, whose mechanism of action may be mediated through the 5-HT_{1A} receptor.[12] Thus, analogues of the enantiomers of spiroxatrine should be of interest in future studies aimed at gaining further insight into the structural features that determined agonist versus antagonist activity at monoaminergic receptors, as well as studies of new classes of antianxiety agents.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by MicAnal Inc., Tucson, AZ, and were within ± 0.40% of the calculated values. NMR spectra were recorded on a JEOL FX90Q (90 MHz) spectrometer and absorptions are reported as downfield from Me_4Si. IR spectra were recorded on a Beckman 1100 spectrophotometer, and optical rotations were determined on a Perkin-Elmer 241 MC polarimeter.

(2*R*,1′*S*)-2-Oxiranyl-1,4-benzodioxan (9). The compound was prepared according to the reported procedure:[4] mp 62–64 °C (lit. mp 62–63 °C); $[\alpha]^{25}_D$ −48.0°, 95.3% ee based on literature[4] ($[\alpha]^{25}_D$ −46.3°, 94% ee).

(2*S*,1′*R*)-2-Oxiranyl-1,4-benzodioxan (9). The compound was prepared according to the reported procedure:[4] mp 62–64 °C (lit. mp 62–64 °C); $[\alpha]^{25}_D$ +47.2°, 94.2% ee based on literature[4] ($[\alpha]^{25}_D$ +48.1°, 94% ee).

(2*S*)-2-(Hydroxymethyl)-1,4-benzodioxan (6). The product was obtained by using (2*R*,1′*S*)-2-oxiranyl-1,4-benzodioxan described earlier as the starting material: yield 85%; mp 73–74 °C (lit.[4] mp 74–74.5 °C); $[\alpha]^{25}_D$ −31.0° (*c* 0.050, ethanol) [lit. $[\alpha]^{25}_D$ −33.0° (*c* 0.07, ethanol)].

(2*R*)-2-(Hydroxymethyl)-1,4-benzodioxan (6). The product was obtained by using (2*S*,1′*R*)-2-oxiranyl-1,4-benzodioxan described earlier: yield 96%; mp 72–74 °C (lit.[4] mp 71–73 °C); $[\alpha]^{25}_D$ +32.3° (*c* 0.050, ethanol) [lit. $[\alpha]^{25}_D$ 34.0° (*c* 0.1, ethanol)].

(2*S*)-2-(Hydroxymethyl)-1,4-benzodioxan Tosylate (3b). The tosylate was obtained by adding tosyl chloride (0.380 g, 2

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mmol) to a cold solution of (2S)-2-(hydroxymethyl)-1,4-benzodioxan (0.210 g, 1.6 mmol) in pyridine (4 mL). Usual workup gave the pure tosylate as a yellow oil: yield 0.190 g, 37.2%.

(2R)-2-(Hydroxymethyl)-1,4-benzodioxan Tosylate (3b). The tosylate was obtained by adding tosyl chloride (0.500 g) to a cold solution of (2R)-2-(hydroxymethyl)-1,4-benzodioxan (0.431 g, 2.6 mmol) in pyridine (4 mL): yield 0.587 g, 73.3%.

Spiroxatrine (−), 1-Phenyl-4-oxo-8-[(2S)-(1,4-benzodioxan-2-yl)methyl]-1,3,8-triazaspiro[4.5]decane (2). A solution of (2S)-2-(hydroxymethyl)-1,4-benzodioxan tosylate (0.172 g, 0.537 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.231 g, 1 mmol) in DMSO (5 mL) was stirred at room temperature for 3 days. The solution turned turbid after 1 day. The white suspension was poured in cold water and triturated until a free-flowing white solid was obtained. The precipitate was filtered and crystallized from ethanol: yield 0.082 g, 40.4%; mp 214–217 °C (lit. racemic mp 215.8–218 °C); $[\alpha]^{25}_D$ −32.3° (c 0.0141, ethanol). Anal. ($C_{22}H_{25}N_3O_3$) C, H, N.

Spiroxatrine (+), 1-Phenyl-4-oxo-8-[(2R)-1,4-benzodioxan-2-yl)methyl]-1,3,8-triazaspiro[4.5]decane (2). (2R)-(+)-2-(Hydroxymethyl)-1,4-benzodioxan tosylate (0.587 g, 1.83 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.577 g, 2.5 mmol) in DMSO (7 mL) were combined and processed as described above for the (−) enantiomer: yield 0.326 g, 46.9%; mp 215–217 °C; $[\alpha]^{25}_D$ +34.3° (c 0.0113, ethanol). Anal. ($C_{22}H_{25}N_3O_3$) C, H, N.

Tissue Preparation for the Binding Assays. Male Sprague–Dawley rats (150–225 g) were used as the source of tissue for the different radioligand binding assays. The brain regions used were cerebral cortex (dorsal to the rhinal sulcus) for the [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H-8-OH-DPAT) and [^{3}H]WB4101 binding assays; corpus striatum for the [^{3}H]-sulpiride and [^{125}I]iodocyanpindolol ([^{125}I]ICYP) binding assays; and frontal cerebral cortex for the [^{3}H]ketanserin binding assay. The animals were decapitated, and the brains were rapidly chilled and dissected to obtain the regions of interest. For the [^{3}H]-8-OH-DPAT and [^{3}H]WB4101 assays, the cerebral cortices were homogenized in 40 volumes of ice-cold Tris·HCl buffer (50 mM, pH 7.4 at 22 °C) with a Brinkmann Polytron (setting 5 for 15 s), and the homogenate was centrifuged at 48000*g* for 10 min. The resulting pellet was then resuspended in the same buffer, and the centrifugation and resuspension process was repeated three additional times to wash the membranes. Between the second and third washes the resuspended membranes were incubated for 10 min at 37 °C to facilitate the removal of endogenous ligands.[13] The final pellet was resuspended in the buffer to a final concentration of 10 mg of tissue (original wet weight)/mL for use in the binding assay. For the [^{3}H]sulpiride assay, the corpora striata were processed in the same way as the tissues for the [^{3}H]-8-OH-DPAT and [^{3}H]WB4101 assays except that the composition of the homogenization medium was: 108 mM NaCl, 4.7 mM KCl, and 1.3 mM $CaCl_2$ in the 50 mM Tris buffer, and the final pellet was resuspended to a concentration of 4 mg of tissue (original wet weight)/mL for use in the binding assay. When prepared for the [^{125}I]ICYP binding assay the corpora striata were homogenized in 10 volumes of 0.32 M sucrose with a Brinkmann Polytron (setting 5 for 15 s) and centrifuged at 900*g* for 10 min. The resulting supernatant was diluted with an excess of 50 mM Tris·HCl buffer (pH 7.4) and processed as described above for the other assays. The final tissue pellet was resuspended in Tris·HCl (pH 7.4) to give a concentration of 3.3 mg of original tissue wet weight per milliliter of buffer. The tissue preparation for the [^{3}H]ketanserin binding assay was based on that described by Leysen et al.[14] The frontal cortices were homogenized in 10 volumes of ice-cold 0.25 M sucrose, using a Teflon glass homogenizer, and then centrifuged for 10 min at 1100*g*. The resulting pellet was rehomogenized in 5 volumes of 0.25 M sucrose and again centrifuged at 1100*g* for 10 min. The combined supernatant from the two centrifugations was diluted in Tris·HCl buffer (50 mM, pH 7.6) to 1:80, w/v (based on the original wet weight of tissue), and centrifuged at 48000*g* for 10 min. The resulting pellet was washed once by resuspension and centrifugation. The final pellet was resuspended in Tris buffer to a concentration of 5 mg of tissue (original wet weight)/mL for use in the binding assay.

5-HT$_{1A}$ Binding Assay. The conditions of the [^{3}H]-8-OH-DPAT binding assay were essentially as described previously.[10] To each assay tube the following were added: 0.1 mL of drug dilution (or water if no competing drug was added), 0.9 mL of [^{3}H]-8-OH-DPAT in buffer (containing Tris, $CaCl_2$, and pargyline to achieve final assay concentrations of 50 mM, 3 mM, and 100 μM, respectively, pH 7.4), and 1 mL of resuspended membranes. The final concentration of [^{3}H]-8-OH-DPAT in the assays was 1 nM. The tubes were incubated for 15 min at 37 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters (pretreated by soaking for 2 h in a 0.1% v/v solution of polyethylenimine and then dried) followed by two 4-mL rinses with ice-cold 50 mM phosphate buffer. The filters were dried, 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 10 μM 5-HT.

5-HT$_{1B}$ Binding Assay. [^{125}I]ICYP binding was determined by using a modification of the method of Hoyer et al.[15] The binding reaction was carried out in polypropylene incubation tubes that contained 50 μL of [^{125}I]ICYP (to give a final concentration of 150 pM), 50 μL of competing drug or water, and 150 μL of tissue suspension. The composition of the assay mixture was the same as for the [^{3}H]-8-OH-DPAT assay with the addition of (−)-isoproterenol (60 μM) to prevent the binding of [^{125}I]ICYP to β-adrenergic receptors. Incubations were carried out at 37 °C for 15 min and then terminated by dilution of the incubation mixture with 5 mL of ice-cold sodium/potassium phosphate buffer (pH 7.4), followed by rapid vacuum filtration onto Whatman GF/B filters. The filters were then washed with two 5-mL rinses of buffer and counted in a gamma counter to determine the amount of trapped radioactivity. Nonspecific binding was determined using 10 μM 5-HT.

5-HT$_2$ Binding Assay. This assay was modified from that of Leysen et al.[14] as described by Taylor et al.[10] The conditions for the assay were the same as described above for the 5-HT$_{1A}$ assay except that polypropylene assay tubes were used, the buffer was 50 mM Tris·HCl, pH 7.6 (with no added $CaCl_2$ or pargyline), the final concentration of [^{3}H]ketanserin was 0.5 nM, and nonspecific binding was defined by 10 μM methysergide.

D$_2$-Dopaminergic Binding Assay. The binding assay for [^{3}H]sulpiride was modified from that described by Sokoloff et al.[16] Each polypropylene assay tube contained 100 μL of drug dilution, 1 mL of tissue resuspension, and 0.9 mL of buffer (108 mM NaCl, 4.7 mM KCl, and 1.3 mM $CaCl_2$ in 50 mM Tris·HCl buffer, pH 7.4) containing [^{3}H]sulpiride to achieve a final concentration of 2 nM. The tubes were incubated for 20 min at 37 °C. Termination of the incubation was by vacuum filtration as described above. Specific binding was defined by the use of 1 μM spiperone.

α$_1$-Adrenergic Binding Assay. The [^{3}H]WB4101 binding assay was carried out as described above for the [^{3}H]-8-OH-DPAT binding with the following modifications. The final concentration of [^{3}H]WB4101 was 0.5 nM, 1 μM 5-HT was included as a mask to prevent binding of [^{3}H]WB4101 to 5-HT$_{1A}$ sites,[17] and nonspecific binding was defined by 1 μM prazosin.

Analysis of Data. IC_{50} values from the competition assays were determined graphically and converted to apparent K_i values for comparison between the different radioligands by using the Cheng–Prusoff equation:[18]

$$\text{apparent } K_i = \frac{IC_{50}}{1 + L/K_d}$$

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where L = free ligand concentration and K_d = the dissociation constant for the radioligand determined from saturation studies.

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Registry No. (+)-**2**, 115649-79-1; (-)-**2**, 115649-80-4; (2*R*)-**3b**, 62501-70-6; (2*S*)-**3b**, 62501-73-9; **4**, 1021-25-6; **5**, 98571-96-1; (2*R*)-**6**, 62501-72-8; (2*S*)-**6**, 98572-00-0; (2*R*,3*R*)-**7**, 98633-62-6; (2*S*,3*S*)-**7**, 98571-97-2; (2*R*,3*R*)-**8**, 115563-58-1; (2*S*,3*S*)-**8**, 115649-81-5; (2*S*,1′*R*)-**9**, 81703-47-1; (2*R*,1′*S*)-**9**, 72522-21-5; (2*S*,1′*R*)-**10**, 115563-59-2; (2*R*,1′*S*)-**10**, 115563-61-6; (2*R*)-**11**, 115563-60-5; (2*S*)-**11**, 115563-62-7; (+)-diethyl tartrate, 87-91-2; (-)-diethyl tartrate, 13811-71-7; *n*-propylamine, 107-10-8; tosyl chloride, 98-59-9.

Arylpiperazine Derivatives as High-Affinity 5-HT_{1A} Serotonin Ligands

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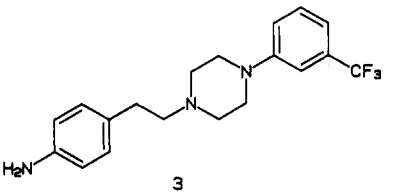
Although simple arylpiperazines are commonly considered to be moderately selective for 5-HT_{1B} serotonin binding sites, N4-substitution of such compounds can enhance their affinity for 5-HT_{1A} sites and/or decrease their affinity for 5-HT_{1B} sites. A small series of 4-substituted 1-arylpiperazines was prepared in an attempt to develop agents with high affinity for 5-HT_{1A} sites. Derivatives where the aryl portion is phenyl, 2-methoxyphenyl, or 1-naphthyl, and the 4-substituent is either a phthalimido or benzamido group at a distance of four methylene units away from the piperazine 4-position, display high affinity for these sites. One of these compounds, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (**18**), possesses a higher affinity than 5-HT and represents the highest affinity (K_i = 0.6 nM) agent yet reported for 5-HT_{1A} sites.

The discovery of multiple populations of central serotonin (5-hydroxytryptamine; 5-HT) binding sites has rekindled a new interest in this neurotransmitter (see Glennon[1] and Fozard[2] for recent reviews). To date, three populations of sites have been identified (i.e., 5-HT_1, 5-HT_2, 5-HT_3) and there is good evidence of heterogeneity for at least one of these sites (i.e., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D} sites).[1,2] Serotonin itself binds at the various 5-HT_1 sites with nearly equal affinity;[1] obviously, it is rather important to investigate the structure–activity relationships of agents that bind at these sites (i.e., structure–affinity relationships; SAFIR) so that these results may ultimately lead to the development of high-affinity site-selective agents. Amongst agents that bind at the various 5-HT_1 sites, arylpiperazines are the most notable.[1] Two such derivatives that have been extensively investigated are TFMPP (**1**; 1-[3-(trifluoromethyl)phenyl]-piperazine) and its chloro analogue mCPP (**2**); although they possess only a modest selectivity for 5-HT_{1B} sites, they are, nonetheless, considered to be 5-HT_{1B}-selective agents (5-HT_{1B} K_i = 30–50 nM).[1,3] At one time it was thought that arylpiperazine moiety might confer selectivity for 5-HT_{1B} sites.[4] However, the discovery of 1-[3(trifluoromethyl)phenyl]-4-[2-(4-aminophenyl)ethyl]piperazine (PAPP, **3**), which binds fairly selectively to 5-HT_{1A} sites,[5] questions this generality. The second-generation anxiolytic (SGA) buspirone (**4**), an arylpiperazine, also binds with high affinity and selectivity at 5-HT_{1A} sites.[6,7] We observed that arylpiperazines (and, indeed, other serotonergic agents) possess a lower affinity for 5-HT_{1B} versus 5-HT_{1A} sites when the terminal amine is a tertiary amine.[1] Recently, we have even demonstrated that conversion of 5-HT to a tertiary amine (i.e., the *N*,*N*-di-*n*-propyl derivative) dramatically reduces its affinity for 5-HT_{1B} sites but has little effect on its affinity at 5-HT_{1A} sites.[8]

We had earlier concluded that arylpiperazines constitute one of the most versatile structural templates for the investigation of serotonergic agents and that selectivity might



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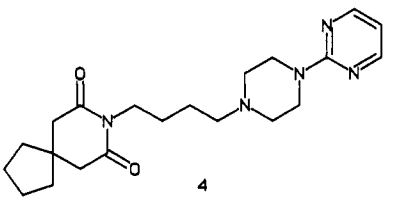

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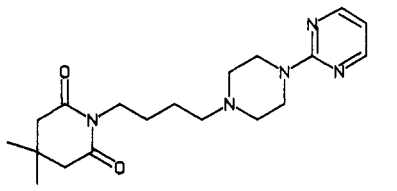

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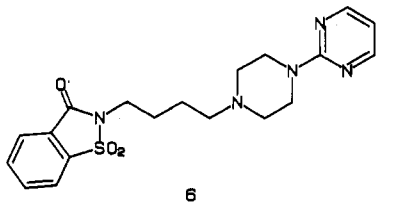

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be achieved by the incorporation of the appropriate substituent groups.[1,9] Knowing now that simple tertiary-

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