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Thieno[3,2-*b*]- and Thieno[2,3-*b*]pyrrole Bioisosteric Analogues of the Hallucinogen and Serotonin Agonist *N*,*N*-Dimethyltryptamine

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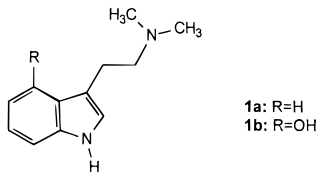
The synthesis and biological activity of 6-[2-(*N*,*N*-dimethylamino)ethyl]-4*H*-thieno[3,2-*b*]pyrrole (**3a**) and 4-[2-(*N*,*N*-dimethylamino)ethyl]-6*H*-thieno[2,3-*b*]pyrrole (**3b**), thienopyrroles as potential bioisosteres of *N*,*N*-dimethyltryptamine (**1a**), are reported. Hallucinogen-like activity was evaluated in the two-lever drug discrimination paradigm using LSD- and DOI-trained rats. Neither **3a** nor **3b** substituted for LSD or DOI up to doses of 50 μmol/kg. By comparison, **1a** fully substituted in LSD-trained rats. However, **3a** and **3b** fully substituted for the 5-HT_{1A} agonist LY293284 ((−)-(4*R*)-6-acetyl-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz[*c*,*d*]indole). Both **3a** and **3b** induced a brief "serotonin syndrome" and salivation, an indication of 5-HT_{1A} receptor activation. At the cloned human 5-HT_{2A} receptor **3b** had about twice the affinity of **3a**. At the cloned human 5-HT_{2B} and 5-HT_{2C} receptors, however, **3a** had about twice the affinity of **3b**. Therefore, thiophene lacks equivalence as a replacement for the phenyl ring in the indole nucleus of tryptamines that bind to 5-HT_2 receptor subtypes and possess LSD-like behavioral effects. Whereas both of the thienopyrroles had lower affinity than the corresponding **1a** at 5-HT_2 receptors, **3a** and **3b** had significantly greater affinity than **1a** at the 5-HT_{1A} receptor. Thus, thienopyrrole does appear to serve as a potent bioisostere for the indole nucleus in compounds that bind to the serotonin 5-HT_{1A} receptor. These differences in biological activity suggest that serotonin receptor isoforms are very sensitive to subtle changes in the electronic character of the aromatic systems of indole compounds.

Bioisosteres are isosteric molecules that have similar or antagonistic properties in biological systems.[1] In our continuing studies of the structure−activity relationships of hallucinogenic agents, we have recently become interested in the molecular determinants of receptor binding and activation for the tryptamine class of hallucinogenic agents.[2] The simplest of these compounds is *N*,*N*-dimethyltryptamine (**1a**, DMT). While there is extensive literature dealing with effects of ring substituents on biological activity in tryptamines, our particular focus in this case was on bioisosteres of the indole nucleus of the tryptamines.



Previously, benzo[*b*]thiophenes have been examined as bioisosteres of indoles in several types of biologically active molecules. For example, the benzo[*b*]thiophene analogue of **1a** was prepared and evaluated in vitro many years ago. While it had in vitro pharmacology similar to that of **1a**, its behavioral effects in rabbits differed.[3] The benzo[*b*]thiophene analogue of 4-hydroxy-DMT (**1b**, psilocin) was also prepared by Campaigne and co-workers,[3] but its pharmacology differed somewhat from that of **1b**. In a similar vein, the benzo[*b*]thiophene analogue of 5,6-dihydroxytryptamine, a serotonin neurotoxin, failed to affect serotonin levels but did cause depletion of norepinephrine.[4]

On the other hand, thiophene replacement of the annulated benzene ring in derivatives of piroxicam, an antiinflammatory agent used in arthritis patients, had no effect on activity.[5] Similarly, the thiophene analogue of amphetamine retains complete amphetamine-like activity.[6] In addition, replacement of the phenyl ring of dopaminergic phenyltetrahydroisoquinolines led to compounds with biological activity virtually identical to that of their phenyl counterparts.[7]

Thus, replacing the nitrogen atom in the indole with sulfur (or oxygen)[2] gives compounds that do not predictably retain biological properties analogous to their indole counterparts. On the other hand, replacement of the phenyl ring of indole with a thiophene, which leads to thienopyrroles, appeared more likely to result in bioisosteres with comparable biological properties. However, because the resonance stabilization energy for thienopyrrole is less than that for indole, charge-transfer or edge-to-face aromatic ring-stacking interactions involved in molecular recognition at serotonin receptors might be affected.

Molecular orbital calculations indicate that the stabilities of the thienopyrrole isomers differ significantly.[8,9] Annulation at 2,3 bonds (**2a**, **2b**) produces

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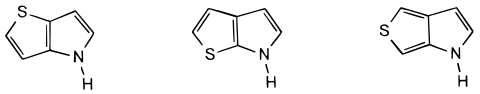
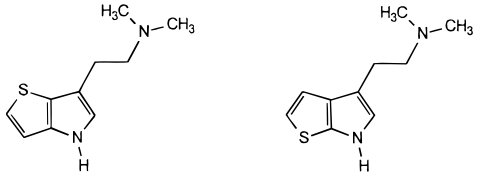
more stable thienopyrroles than annulation at the 3,4 bond of thiophene and one 2,3 bond of pyrrole (e.g., **2c**). Annulation at both 3,4 bonds results in considerable instability. Compounds **2a** and **2b** themselves are air-sensitive at room temperature.[9] Recently, two of the three possible thienopyrrole analogues of tryptophan were synthesized.[10] The synthesis of the third isomer, having **2c** as the thienopyrrole nucleus, was not completed due to the instability of this positional isomer. The *N*-BOC-protected ethyl acetic acid derivatives containing the nuclei **2a**, **2b**, and **2c** were also previously prepared, but deprotection of the respective derivative of **2c** led to decomposition.[11]



2a **2b** **2c**

Taking into account these theoretical and experimental stability considerations, we chose to synthesize 6-[2-(*N*,*N*-dimethylamino)ethyl]-4*H*-thieno[3,2-*b*]pyrrole (**3a**) and 4-[2-(*N*,*N*-dimethylamino)ethyl]-6*H*-thieno[2,3-*b*]-pyrrole (**3b**) as thienopyrrole positional isomers of DMT (**1a**). This report describes the synthesis of these two bioisosteres and their comparison with **1a** for affinity at the rat 5-HT_{1A} serotonin receptor and at the cloned human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. The compounds were also compared with **1a** using the two-lever drug discrimination assay in rats trained to discriminate the hallucinogen LSD as well as LY293284 ((−)-(4*R*)-6-acetyl-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz[*c*,*d*]indole), a potent serotonin 5-HT_{1A} agonist.[12] Compounds **3a** and **3b** were also compared in the drug discrimination paradigm with the hallucinogenic phenethylamine ligand (±)-2,5-dimethoxy-4-iodoamphetamine, DOI.

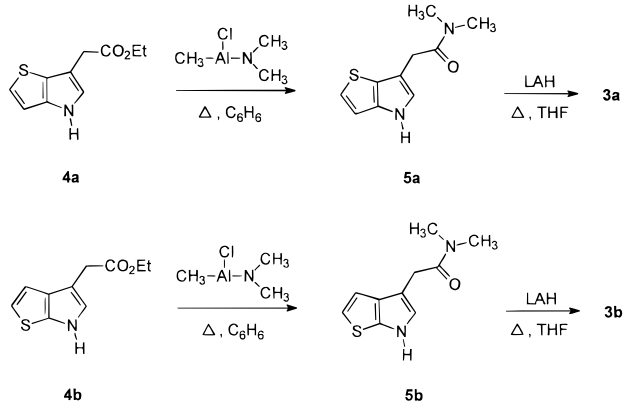


3a **3b**

Chemistry

Several recent literature precedents allowed reasonably facile synthetic approaches to each of the two target compounds. Following the general procedure of Wensbo et al.,[11] both **4a** and **4b** (Scheme 1) were obtained in excellent yields. As outlined in Scheme 1 these esters were then converted into the corresponding dimethylamides **5a** and **5b** by treatment with methylchloroaluminum dimethylamide,[13] prepared from commercially available trimethylaluminum and dimethylamine hydrochloride, to afford yields of 95% and 89%, respectively. Reduction of the amides was carried out with $LiAlH_4$ in THF at reflux, leading to isolation of **3a** and **3b** in yields of 97% and 93%, respectively. These two products proved to be surprisingly stable, as long as acidic conditions were avoided. The benzoate salts of both isomers were prepared by reaction with exactly 1 equiv of benzoic acid and were obtained as clear colorless crystals following recrystallization from ethyl acetate.

Scheme 1



4a **5a** **4b** **5b**

Table 1. Drug Discrimination Experiments for **3a** and **3b** in LSD-Trained Rats

	dose					ED_{50} (95% CL)	
drug	mg/kg	μmol/kg	*N*	%D	%SDL	μmol/kg	mg/kg
LSD	0.01	0.023	13	0	31		
	0.02	0.046	14	7	62	0.037	0.016
	0.04	0.093	15	0	81	(0.023−0.057)	(0.01−0.025)
	0.08	0.186	16	0	100		
1a	1.0	3.33	8	0	37		
	2.0	6.66	11	18	22	10.16	3.24
	4.0	13.32	13	23	40	(4.7−21.9)	(1.5−7.0)
	8.0	26.64	19	37	83		
3a	2.0	6.32	8	25	0		
	4.0	12.6	9	22	43	NS	NS
	8.0	25.3	11	36	12.5		
	16.0	50.6	8	62.5	0		
3b	0.5	1.58	9	22	14		
	1.0	3.16	13	0	7.7		
	2.0	6.32	11	0	9	NS	NS
	4.0	12.6	12	0	16.7		
	8.0	25.3	11	0	9		
	16.0	50.6	12	50	16.7		

[a] *N*, total number of animals tested; %D, percentage of animals failing to emit 50 lever presses within 15 min; %SDL, percentage of animals emitting >80% of their lever presses on the LSD-appropriate lever; NS, no substitution.

Pharmacology

Both **3a** and **3b** benzoates were compared with **1a** fumarate in two-lever drug discrimination (DD) paradigms in rats trained to discriminate the hallucinogen LSD from saline and LY293284 (a potent 5-HT_{1A} agonist, (−)-(4*R*)-6-acetyl-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz[*c*,*d*]indole)[12] from saline. Compounds **3a** and **3b** were also compared in rats trained to discriminate DOI (a 5-HT_2 agonist and hallucinogen) from saline. In addition, **3a** and **3b** were compared with **1a** for affinity at the rat brain 5-HT_{1A} receptor labeled with [^{3}H]-8-OH-DPAT and at the cloned human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors.

Results and Discussion

In the drug discrimination studies, neither **3a** nor **3b** substituted for LSD or DOI up to doses of 50 μmol/kg (Tables 1 and 2). By comparison, the known hallucinogen **1a** fully substituted in LSD-trained rats. These data indicate that **3a** and **3b** lack recognizable LSD-like behavioral effects in rats. They also suggest

Table 2. Drug Discrimination Experiments for **3a** and **3b** in DOI-Trained Rats

	dose						
						ED_{50} (95% CL)	
drug	mg/kg	μmol/kg	*N*	%D	%SDL	μmol/kg	mg/kg
DOI	0.1	0.28	9	0	56	0.30	0.11
	0.2	0.56	10	10	89	(0.19−0.47)	(0.07−0.17)
	0.4	1.12	10	0	100		
3a	4.0	12.6	9	11	37.5		
	8.0	25.3	9	78	0	NS	NS
	16.0	50.6	8	62.5	0		
3b	2.0	6.32	6	0	0		
	4.0	12.6	8	0	0		
	8.0	25.3	8	12.5	0	NS	NS
	16.0	50.6	8	50	0		

[a] *N*, total number of animals tested; %D, percentage of animals failing to emit 50 lever presses within 15 min; %SDL, percentage of animals emitting >80% of their lever presses on the DOI-appropriate lever; NS, no substitution.

that **3a** and **3b** lack full efficacy at the 5-HT_{2A} receptor, since agonists at that site generally substitute in LSD-trained rats.

The radioligand competition results (Table 4) show that both **3a** and **3b** had lower affinity than did **1a** at the agonist-labeled cloned 5-HT_{2A} human receptor. The affinities of **1a** and **3b** at that site are not significantly different, indicating perhaps the greatest degree of similarity between **1a** and **3b** at the 5-HT_{2A} receptor. At the two other cloned receptors **3b** had significantly lower affinity than **3a**. It would appear that the recognition features for the aromatic system of ligands at the 5-HT_{2B} and 5-HT_{2C} sites are more similar and differ somewhat from those at the 5-HT_{2A} receptor. Thus, while thiophene may serve as a useful bioisostere in many molecular systems, it seems to lack equivalence as a replacement for the phenyl ring in the indole nucleus of tryptamines that bind to 5-HT_2 receptor isoforms.

On the other hand, **3a** and **3b** did fully substitute for the 5-HT_{1A} agonist LY293284, with **3a** having about twice the potency of **3b** (Table 3). Both **3a** and **3b** induced a brief "serotonin syndrome" (e.g., flat body posture and lower lip retraction) and salivation at doses of 25−50 and 50 μmol/kg, respectively, an indication of 5-HT_{1A} receptor activation.

Whereas both of the thienopyrroles had lower affinity than **1a** at 5-HT_2 receptors, both **3a** and **3b** had significantly *greater* affinity at the 5-HT_{1A} receptor, with **3a** having a nearly 4-fold higher affinity than **1a**. The relative affinities of **3a** and **3b** at this receptor parallel their potencies in the drug discrimination results (Table 3). These results were somewhat surprising. In contrast to the 5-HT_{2A} receptor, thienopyrrole does appear to serve as a suitable bioisostere for the indole nucleus in compounds that bind to the serotonin 5-HT_{1A} receptor. These differences in biological activity suggest that serotonin receptor isoforms are sensitive to subtle changes in the electronic character of the aromatic systems of indole compounds.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded using either a 500-MHz Varian VXR-500S or 300-MHz Bruker ARX-300 NMR spectrometer. Chemical shifts are reported in δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference (0.03% v/v). Abbreviations used in NMR analyses are as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, q = quartet, p = pentet, m = multiplet, b = broad, Ar = aromatic. Chemical ionization mass spectra (CIMS) using methane as the carrier gas were obtained with a Finnigan 4000 spectrometer. IR measurements were taken with a Perkin-Elmer 1600 series FT-IR spectrophotometer. Elemental analyses were performed by the Purdue University Microanalysis Laboratory and were within ±0.4% of the calculated values unless otherwise noted. Thin-layer chromatography (TLC) was typically performed using Baker-flex silica gel IB2-F, plastic-backed plates with fluorescent indicator (2.5 × 7.5 cm; J. T. Baker), eluting with CH_2Cl_2, and visualizing with UV light at 254 nm and/or I_2 vapor unless otherwise noted. Plates used for radial centrifugal chromatography (Chromatotron, Harrison Research, Palo Alto, CA) were prepared from silica gel 60 PF2-54 containing gypsum. Reactions were carried out under an atmosphere of dry nitrogen.

***N,N*-Dimethyl-4*H*-thieno[3,2-*b*]pyrrole-6-acetamide, 5a.** Methyl chloroaluminum dimethylamide[13] (50.8 mL, 0.67 M solution in benzene/toluene) was added in one portion to a solution of ethyl (4*H*-thieno[3,2-*b*]pyrrolyl)-6-acetate[11] (3.56 g, 17.0 mmol; **4a**) in dry benzene (178 mL). The solution was heated at reflux for 2 h, cooled to room temperature, and then carefully quenched with water (CAUTION: vigorous gas evolution!). The organic layer was separated, and the aqueous layer was extracted with 3 × 150 mL of ethyl acetate. The organic extracts were combined, dried with $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by column chromatography over silica gel with 50% EtOAc/CH_2Cl_2 as eluent to afford, after solvent removal, amide **5a** (3.35 g, 95%): mp 128 °C; ^{1}H NMR δ 8.36 (br s, 1, NH), 7.08 (d, 1, ArH, *J* = 5.0 Hz), 6.91 (d, 1, ArH, *J* = 5.1 Hz), 6.88 (s, 1, ArH), 3.71 (s, 2, CH_2), 3.07 (s, 3, NCH_3), 3.00 (s, 3, NCH_3); CIMS 209 (M + H^+). Anal. ($C_{10}H_{12}N_2OS$) C, H, N.

***N,N*-Dimethyl-6*H*-thieno[2,3-*b*]pyrrole-4-acetamide, 5b.** This amide was obtained from (6*H*-thieno[2,3-*b*]pyrrolyl)-4-acetate[11] (**4b**) and methylchloroaluminum dimethylamide[13] by the same procedure used for the preparation of amide **5a**. Reflux time was 20 min to afford **5b** in 89% yield after isolation and purification: mp 107 °C; ^{1}H NMR δ 8.54 (br s, 1, NH), 7.00 (d, 1, ArH, *J* = 5.3 Hz), 6.82 (s, 1, ArH), 6.80 (d, 1, ArH, *J* = 5.3 Hz), 3.72 (s, 2, CH_2), 3.04 (s, 3, NCH_3), 2.97 (s, 3, NCH_3); CIMS 209 (MH^+). Anal. ($C_{10}H_{12}N_2OS$) C, H, N.

***N,N*-Dimethyl-2-(4*H*-thieno[3,2-*b*]pyrrol-6-yl)ethanamine, 3a.** The amide **5a** (2.89 g, 13.9 mmol) in THF was added dropwise into a mixture of $LiAlH_4$ (1.58 g, 41.6 mmol) in dry THF (330 mL). The reaction mixture was allowed to reflux for 2 h, then cooled to room temperature, and quenched with water. The mixture was filtered through Celite and the filtrate concentrated under reduced pressure. The resulting residue was dissolved in 30 mL of ether, dried with Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by centrifugal radial chromatography (Chromatotron, Harrison Research, Palo Alto, CA), in two portions on 4-mm silica gel plates, eluting with 5% MeOH/CH_2Cl_2 under an atmosphere of nitrogen and ammonia to afford **3a** (2.61 g, 97%) as an off-white solid: mp 43−44 °C; ^{1}H NMR δ 8.31 (br s, 1, NH), 7.08 (d, 1, ArH, *J* = 5.2 Hz), 6.91 (d, 1, ArH, *J* = 5.2 Hz), 6.80 (s, 1, ArH), 2.83 (t, 2, CH_2, *J* = 8.3 Hz), 2.66 (t, 2, CH_2, *J* = 8.3 Hz), 2.33 (s, 6, NCH_3); CIMS 195 (M + H^+). Anal. ($C_{10}H_{14}N_2S$) C, H, N. Prepared as the benzoate salt (recrystallized from EtOAc): mp 178 °C; ^{1}H NMR (DMSO-d_6) δ 10.82 (br s, 1, NH), 7.95 (m, 2, benzoate-H), 7.56 (t, 1, benzoate-H, *J* = 7.5 Hz), 7.46 (t, 2, benzoate-H, *J* = 7.2 Hz), 7.12 (d, 1, ArH, *J* = 5.3 Hz), 6.94 (d, 1, ArH, *J* = 5.3 Hz), 6.86 (s, 1, ArH), 2.71 (t, 2, CH_2, *J* = 8.0 Hz), 2.60 (t, 2, CH_2, *J* = 8.0 Hz), 2.27 (s, 6, NCH_3); CIMS 195 (M + H^+). Anal. ($C_{17}H_{20}N_2O_2S$) C, H, N.

***N,N*-Dimethyl-2-(6*H*-thieno[2,3-*b*]pyrrol-4-yl)ethanamine, 3b.** This amine was obtained in 93% yield as an off-white solid by the same procedure used to prepare amine **3a**: mp 38−39 °C; ^{1}H NMR δ 8.28 (br s, 1, NH), 7.00 (d, 1, ArH *J*

Table 3. Drug Discrimination Experiments for **3a** and **3b** in LY293284-Trained Rats[a]

drug	dose mg/kg	dose μmol/kg	*N*	%D	%SDL	ED_{50} (95% CL) μmol/kg	ED_{50} (95% CL) mg/kg
LY293284	0.005	0.015	12	8	9	0.031	0.01
	0.01	0.030	11	0	45	(0.02−0.05)	(0.007−0.02)
	0.02	0.060	12	0	83		
	0.027	0.080	11	27	100		
1a	0.5	1.67	8	0	0		
	1.0	3.33	9	11	20		
	2.0	6.66	10	20	37.5	PS	PS
	4.0	13.32	10	20	62.5		
	8.0	26.64	10	80	100		
3a	2.0	6.32	11	8	50		
	4.0	12.6	10	30	57	7.3	2.32
	8.0	25.3	9	22	71	(2.3−23.3)	(0.73−7.38)
	16.0	50.6	6	0	83		
3b	2.0	6.32	10	0	10		
	4.0	12.6	9	0	67	16.8	5.31
	8.0	25.3	7	0	58	(9.62−29.3)	(3.05−9.27)
	16.0	50.6	6	0	83		

[a] *N*, total number of animals tested; %D, percentage of animals failing to emit 50 lever presses within 15 min; %SDL, percentage of animals emitting >80% of their lever presses on the LY293284-appropriate lever; PS, partial substitution. Test for parallelism: The slopes of the **3a** and **3b** cues are not significantly different (*T*-value = 1.28; table value = 2.78). The slopes of the LY293284 and **3b** cues also are not significantly different (*T*-value = 2.54; table value = 2.78). The slopes of the **3a** and LY293284 cues, however, differ significantly from parallelism (*T*-value = 7.41; table value = 2.78).

Table 4. Receptor Affinity Measurements at the Rat Brain 5-HT_{1A} Receptor and the Cloned Human 5-HT_2 Receptor Family[a]

compd	[^{3}H]-8-OH-DPAT 5-HT_{1A}	[^{125}I]DOI 5-HT_{2A}	[^{3}H]-5-HT 5-HT_{2B}	[^{125}I]DOI 5-HT_{2C}
1a	259 ± 8 (3)	65 ± 8 (4)	101 (73.5, 128)*	33 ± 7 (4)
3a	76 ± 7 (3)	276 ± 53 (3)	214 ± 44 (3)	64 ± 9 (3)
3b	184 ± 13 (3)	106 ± 20 (3)	483 ± 24 (3)	102 ± 9 (3)

[a] Mean K_i (nM) ± SEM for the number of separate experiments given in parentheses, except as noted. *Value is the mean from two separate experiments, with individual values given in parentheses. A one-way ANOVA, followed by a post hoc Tukey test, showed that **3a** and **3b** were significantly different from each other at all four sites (at least $p < 0.05$). There was no significant difference between the affinity of **1a** and **3b** at the 5-HT_{2A} site and no significant difference between **1a** and **3a** at both the 5-HT_{2B} and 5-HT_{2C} isoforms ($p > 0.05$).

= 5.2 Hz), 6.82 (d, 1, ArH, *J* = 5.2 Hz), 6.81 (s, 1, ArH), 2.85 (t, 2, CH_2, *J* = 7.3 Hz), 2.62 (t, 2, CH_2, *J* = 7.1 Hz), 2.33 (s, 6, NCH_3); CIMS 195 ($M + H^+$). Anal. ($C_{10}H_{14}N_2S$) C, H, N. Prepared as the benzoate salt (recrystallized from EtOAc): mp 136 °C; ^{1}H NMR (DMSO-d_6) δ 10.95 (br s, 1, NH), 7.93 (d, 2, benzoate-H, *J* = 7.9 Hz), 7.56 (t, 1, benzoate-H, *J* = 7.2 Hz), 7.45 (t, 2, benzoate-H, *J* = 7.7 Hz), 6.97 (d, 1, ArH, *J* = 5.2 Hz), 6.86 (d, 1, ArH, *J* = 5.0 Hz), 6.83 (s, 1, ArH), 2.74 (t, 2, CH_2), 2.62 (t, 2, CH_2), 2.30 (s, 6, NCH_3); CIMS 195 ($M + H^+$). Anal. ($C_{17}H_{20}N_2O_2S$) C, H, N.

Pharmacological Methods. 1. Drug Discrimination Studies. The procedures for the drug discrimination assays were essentially as described in previous reports.[14,15] Male Sprague−Dawley rats (Harlan Laboratories, Indianapolis, IN), weighing 200−220 g at the beginning of the study, were trained to discriminate LSD tartrate, DOI hydrochloride, or LY293284 (free base) from saline. None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages, and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions to maintain approximately 80% of free-feeding weight. Lights were on from 0700 to 1900. The laboratory and animal facility temperature was 22−24 °C, and the relative humidity was 40−50%. Experiments were performed between 0830 and 1700 each day, Monday through Friday.

A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. To avoid positional preference, half of the rats were trained on drug-L (left), saline-R (right) and the other half on drug-R, saline-L. Training sessions lasted 15 min and were conducted at the same time each day. Response levers were cleaned with 10% ethanol solution between animals to avoid olfactory cues. Presses on the incorrect lever had no programmed consequences.

Test sessions were interspersed between training sessions, either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. Test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min, the session was ended and scored as a disruption. Treatments were randomized at the beginning of the study.

The training drugs were (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg, 0.186 μmol/kg; NIDA), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI; 0.4 mg/kg, 1.12 μmol/kg; NIDA), and LY293284[12] (0.025 mg/kg, 0.075 μmol/kg; a gift of Eli Lilly and Co.) All drugs were dissolved in 0.9% saline and were injected intraperitoneally in a volume of 1 mL/kg, 30 min before the sessions. Data from the drug discrimination study were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (%SDL) for each dose of test compound was determined. The degree of substitution was determined by the maximum %SDL for all doses of the test drug. "No substitution" was defined as 59% SDL or less, and "partial" substitution was 60−79% SDL. If the drug was one that completely substituted for the training drug (at least one dose resulted in a %SDL = 80% or higher), the method of Litchfield and Wilcoxon[16] was used to determine the ED_{50} (log−probit analysis as the dose producing 50% drug−lever responding) and 95% confidence interval (95% CI). This method also allowed for tests of parallelism between dose−response curves of the drug and the training drug. If 50% or more of the animals tested were disrupted at a dose, even if the nondisrupted rats gave 80% SDL, no ED_{50} was calculated.

2. 5-HT_{1A} Radioreceptor Assays Using Rat Brain Homogenate. Male Sprague−Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 175−199 g were used. The animals were kept in groups of 5 rats/cage with free access to food and water. [^{3}H]-8-OH-DPAT was purchased from New England

Nuclear (Boston, MA) at a specific activity of 135.5−216.0 Ci/mmol. 5-HT was purchased from Sigma Chemical Co. (St. Louis, MO).

The procedures of Johnson et al.[17] were employed. Briefly, the hippocampal brain regions from 20−40 rats were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in 8 volumes of 0.32 M sucrose. The homogenate was centrifuged at 36000*g* for 10 min, and the resulting pellet was resuspended in the same volume of sucrose. Separate aliquots of tissue suspension were then frozen at −70 °C until assay. For each separate experiment, a tissue aliquot was thawed slowly and diluted 1:25 with 50 mM Tris HCl (pH 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 36500*g* for 20 min with an intermittent wash. The resulting pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na_2EDTA, 10 mM $MgCl_2$, 0.1% Na ascorbate, and 10 mM pargyline HCl (pH 7.4). A second preincubation for 10 min at 37 °C was conducted, and the tissues were then cooled in an ice bath.

All experiments were performed in triplicate in glass tubes containing buffer to which 200−400 μg of protein was added, giving a final volume of 1 mL. The tubes were allowed to equilibrate for 15 min at 37 °C before filtering through Whatman GF/C filters using a cell harvester (Brandel, Gaithersburg, MD) followed by two 5-mL washes using ice-cold Tris buffer. Specific binding was defined using 10 μM 5-HT. Filters were air-dried, placed into scintillation vials with 10 mL of Ecolite scintillation cocktail, and allowed to sit overnight before counting for tritium. Under these conditions, the K_D of 8-OH-DPAT was 2.03 ± 0.04 nM, while that of LY293284 was 0.052 ± 0.005 nM.

3. Radioligand Competition Experiments Using Cloned Human 5-HT$_2$ Receptors. All chemicals were obtained from the sources previously described.[18] [^{3}H]-5-HT was purchased from DuPont-NEN (Wilmington, DE) or Amersham Corp. (Arlington Heights, IL) at 22.8−26.7 or 81−91 Ci/mmol, respectively, and [^{125}I]DOI (2200 Ci/mmol) was purchased from DuPont-NEN (Wilmington, DE).

Membranes were prepared essentially as previously described using AV12 cell lines (Syrian hamster fibroblast, ATCC no. CRL 9595) stably transformed with the human 5-HT$_{2A}$, 5-HT$_{2B}$, or 5-HT$_{2C}$ receptor.[18] In brief, cells expressing the receptor of interest were grown in suspension and harvested by centrifugation. The cell pellets were then resuspended in a minimal volume of a hypotonic buffer, 50 mM Tris-HCl, pH 7.4, and frozen at −70 °C until needed. On the day of the assay, the membrane suspension was thawed and diluted to 35 mL per 0.5 × 10^9 cells with 50 mM Tris-HCl, pH 7.4. The combination of hypotonic buffer and vortexing was sufficient to lyse the cells for the membrane preparation. After vortexing, the preparation was centrifuged at 39000*g* for 10 min at 4 °C, and the resulting membrane pellet was resuspended and incubated at 37 °C for 10 min and then centrifuged at 39000*g* for 10 min at 4 °C. This pellet was resuspended and centrifuged one more time, and the final membrane pellet was resuspended (using a Tissumizer, setting 65 for 15 s) in Tris-HCl, pH 7.4, for cells expressing the human 5-HT$_{2B}$ receptor and in Tris-HCl, pH 7.4, containing $MgCl_2$ and EDTA for [^{125}I]-DOI binding to the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptor.

4. 5-HT$_{2B}$ [^{3}H]-5-HT Binding Studies. Human 5-HT$_{2B}$ receptor binding assays using [^{3}H]-5-HT were performed as previously described.[18] The assay was automated using a Biomek 1000 (Beckman Instruments, Fullerton, CA). [^{3}H]-5-HT in Tris-HCl containing $CaCl_2$, pargyline, and L-ascorbic acid, adjusted to pH 7.4, was added to drug dilutions, spanning 6 log units, in water. Then 200 μL of membrane resuspension (approximately 100−150 μg of protein) was added with mixing followed by incubation for 15 min at 37 °C. The total incubation volume was 800 μL, and all incubations were performed in triplicate. The final concentration of $CaCl_2$, pargyline, Tris, and L-ascorbic acid was 3 mM, 10 μM, 50 mM, and 0.1%, respectively. The assay was terminated by vacuum filtration through Whatman GF/B filters that had been presoaked with 0.5% poly(ethylenimine) (w/v) and precooled with 4 mL of ice-cold wash buffer (50 mM Tris-HCl, pH 7.4), using a Brandel cell harvester (model MB-48R, Brandel, Gaithersburg, MD). The filters then were washed rapidly four times with 1 mL of ice-cold wash buffer. The amount of [^{3}H]-5-HT trapped on the filters was determined by liquid scintillation spectrometry (Ready Protein, LS 6000IC, Beckman Instruments, Fullerton, CA). The final [^{3}H]-5-HT concentration for competition studies was approximately 2 nM (range = 1.7−2.5 nM). The actual free radioligand concentration was determined by sampling the supernatant of identical tubes where bound ligand was removed by centrifugation. Nonspecific binding was defined with 10 μM 5-HT or 10 μM 1-naphthylpiperazine (1-NP). The amount of protein was determined by the method of Bradford, with bovine serum albumin as the standard.[19]

5. 5-HT$_{2A/2C}$ [^{125}I]DOI Binding Studies. Human 5-HT$_{2A}$ or 5-HT$_{2C}$ binding studies were performed essentially as described for [^{3}H]-5-HT binding to the 5-HT$_{2B}$ receptor with the following exceptions. The assay buffer contained, in a final concentration, 10 μM pargyline, 9.75 mM $MgCl_2$, 0.5 mM Na_2-EDTA, 0.1% sodium ascorbate, and 50 mM Tris-HCl, pH 7.4. Incubations were performed at 37 °C for 30 min with approximately 40 and 30 μg of protein for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively, followed by filtration and washing as described above. The amount of [^{125}I]DOI trapped on the filters was determined using a gamma counter. Nonspecific binding was determined with 10 μM mianserin for 5-HT$_{2C}$ and 1 μM ketanserin for 5-HT$_{2A}$ receptors. The final concentration of [^{125}I]DOI was approximately 0.07−0.15 nM.

6. Data Analysis. The resulting data from each competition assay were analyzed by nonlinear regression using the model for sigmoid curves in the curve-fitting program Prism (Graphpad Inc., San Francisco, CA). This program generated IC_{50} values and a Hill coefficient for each curve. The apparent affinities were calculated by the Cheng−Prusoff equation.[20] The ANOVA on the receptor affinity data was performed using Instat for Windows (Graphpad Inc.).

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