Synthesis and Serotonin Receptor Affinities of a Series of *trans*-2-(Indol-3-yl)cyclopropylamine Derivatives

Suwanna Vangveravong, Arthi Kanthasamy, Virginia L. Lucaites,‡ David L. Nelson,‡ and David E. Nichols*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

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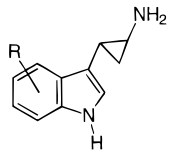
A series of four racemic ring-substituted *trans*-2-(indol-3-yl)cyclopropylamine derivatives was synthesized and tested for affinity at the 5-HT_{1A} receptor, by competition with [^{3}H]-8-OH-DPAT in rat hippocampal homogenates, and for affinity at the agonist-labeled cloned human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor subtypes. None of the compounds had high affinity for the 5-HT_{1A} receptor, with the 5-methoxy substitution being most potent (40 nM). At the 5-HT_{2A} and 5-HT_{2B} receptor isoforms, most of the compounds lacked high affinity. At the 5-HT_{2C} receptor, however, affinities were considerably higher. The 5-fluoro-substituted compound was most potent, with a K_i at the 5-HT_{2C} receptor of 1.9 nM. In addition, the 1*R*,2*S*-(−) and 1*S*,2*R*-(+) enantiomers of the unsubstituted compound were also evaluated at the 5-HT_2 isoforms. While the 1*R*,2*S* enantiomer had higher affinity at the 5-HT_{2A} and 5-HT_{2B} sites, the 1*S*,2*R* isomer had highest affinity at the 5-HT_{2C} receptor. This reversal of stereoselectivity may offer leads to the development of a selective 5-HT_{2C} receptor agonist. The cyclopropylamine moiety therefore appears to be a good strategy for rigidification of the ethylamine side chain only for tryptamines that bind to the 5-HT_{2C} receptor isoform.

Introduction

A number of tryptamine derivatives have been isolated from natural sources or prepared by chemical synthesis and evaluated for their biological activity.[1−6] One of the more important and well-studied of these, 5-hydroxytryptamine (serotonin, 5-HT), is a neurotransmitter with numerous physiological functions in the central and peripheral nervous systems. Many tryptamines, such as *N*,*N*-dimethyl- and *N*,*N*-diethyltryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine, and 4-hydroxy-*N*,*N*-dimethyltryptamine (psilocin), have psychoactive properties similar to those of LSD.[7] Others, such as α-methyl- and α-ethyltryptamine, have various pharmacological properties, for example, as inhibitors of 5-HT reuptake.[3]

Identification of the conformations of drugs at their biological targets remains an important topic of research. Little is known regarding the mutual conformational changes induced in a ligand and its receptor during their interaction. Although it is sometimes appealing to speculate that the molecule may bind in its preferred solution or solid-state conformation, the structural demands of the receptor may lead to binding of the molecule in a conformation that would not be favored in its nonbound state. One way to circumvent this uncertainty is to evaluate the pharmacological potencies of conformationally rigid analogues. With respect to tryptamines, which are representatives of the arylethylamine class of neurotransmitters, the conformational flexibility of the side chain makes it difficult to identify the binding conformation. Although many types of strategies have been employed in designing conformationally rigid molecules,[8−14] probably one of the simplest is to incorporate an ethylamine side chain into a cyclopropyl ring. This approach introduces only minimal additional molecular bulk, while the degrees of conformational freedom are effectively reduced. Cyclopropane analogues have been used for many years and have proven useful in obtaining information regarding the active conformations of various compounds.[8−10,13,15] Thus, this report describes the synthesis of *trans*-2-(indol-3-yl)cyclopropylamines **1a**−**d**. These compounds were evaluated for affinity at the rat brain 5-HT_{1A} receptor, as well as at the cloned human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, known to be important targets for a number of tryptamine agonists.



1a R = H
b R = 4-OCH_3
c R = 5-OCH_3
d R = 5-F

Chemistry

Although cyclopropyl analogues of tryptamines have been obvious targets for structure−activity relationship studies for many years, the unique chemistry of indoles has precluded many of the usual chemical approaches employed with more stable aromatic templates. Fur-

* Address correspondence to: Dr. David E. Nichols at Purdue University. Phone: (765)-494-1461. Fax: (765)-494-1414. E-mail: drdave@pharmacy.purdue.edu.
‡ Eli Lilly and Co.

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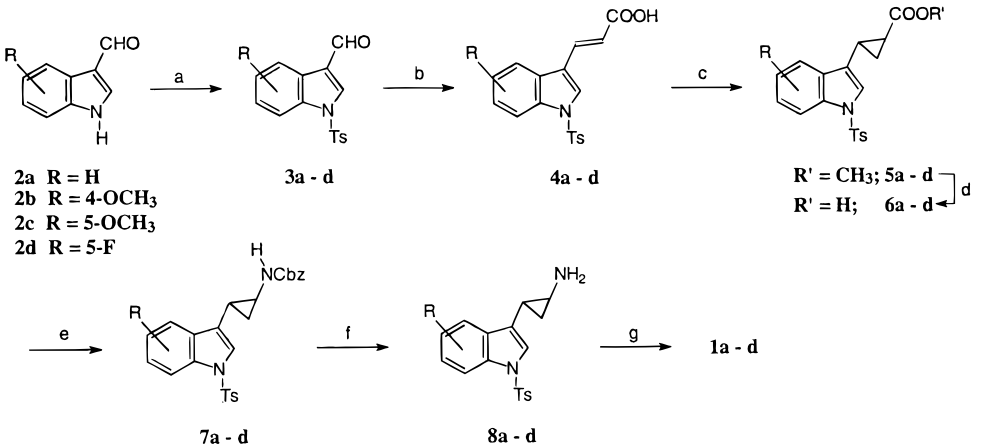
Scheme 1[a]



[a] Reagents: (a) *p*-toluenesulfonyl chloride, Et_3N; (b) malonic acid, pyridine, piperidine; (c) i. CH_2N_2, ii. CH_2N_2/$Pd(OAc)_2$; (d) NaOH/MeOH, then HCl; (e) i. Et_3N, $ClCOOC_2H_5$, NaN_3, ii. benzyl alcohol, heat; (f) H_2, Pd−C, EtOH; (g) Na−Hg, Na_2HPO_4, MeOH.

thermore, arylcyclopropylamines with electron-rich aromatic systems tend to be chemically unstable. These, and other obstacles, impeded our work on indolylcyclopropylamines until we noted the synthesis of phenylcyclopropylamines published by Arvidsson et al.[13] It was reasoned that their general approach offered a relatively facile entry into the desired indole compounds. Indeed, this proved to be the case, and we initially used that precedent as a basis for an asymmetric synthesis of the two enantiomers of **1a**.[16]

As shown in Scheme 1, indole-3-carboxaldehydes **2a**−**d** were first protected as the *N*(1)-*p*-toluenesulfonyl derivatives **3a**−**d**. This protection was *key* to the success of the synthesis, not only because it enhanced reactivity of the carboxaldehyde but also because attempts to deprotect prior to the final step led to intermediates that proved so unstable they could not be isolated and purified. The protected indolecarboxaldehydes were condensed with malonic acid to afford the *trans*-*β*-indol-3-ylacrylic acids **4a**−**d**. The acids were converted to their methyl esters, and cyclopropanation with retention of trans stereochemistry was then accomplished using ethereal diazomethane in the presence of catalytic palladium diacetate.[13] Hydrolysis of the cyclopropyl methyl esters provided acids **6a**−**d**, which were subjected to conditions of the Curtius rearrangement. The intermediate isocyanates were trapped with benzyl alcohol, followed by catalytic hydrogenation to give the amines **8a**−**d.** *N*-Detosylation as the final step was accomplished using sodium amalgam under buffered conditions to afford the desired indolecyclopropylamines **1a**−**d**.

Radioligand Competition Studies

Serotonin receptor affinity data were obtained for the four target compounds in rat brain homogenate at the 5-HT_{1A} receptor labeled with [^{3}H]-8-OH-DPAT. In addition, affinities were measured for the new compounds, as well as the enantiomers of **1a**, at the cloned human 5-HT_2 receptor isoforms.

Results and Discussion

As seen in Table 1, none of the compounds had particularly high affinity at the 5-HT_{1A} receptor. The 5-methoxy compound **1c** had highest affinity at this site, but the value obtained here is considerably lower than the reported affinity for the flexible congener 5-methoxytryptamine.[17] The order of affinity is consistent, however, with the conclusion that 5-substituted tryptamines are more potent at 5-HT_{1A} receptors.[18]

Table 1. Affinities of Compounds **1a**−**d** at 5-HT_{1A} Receptors in Rat Hippocampal Homogenate Labeled with [^{3}H]-8-OH-DPAT[a]

compound	K_i (nM) 5-HT_{1A}
(1*R*,2*S*)-(−)-**1a**[16]	258 ± 23
(1*S*,2*R*)-(+)-**1a**[16]	810 ± 52
(±)-**1b**	1528 ± 61
(±)-**1c**	40.1 ± 2.4
(±)-**1d**	258 ± 15
8-OH-DPAT	2.1 ± 0.1

[a] Data are expressed as mean K_i values (nM) ± SEM for 3−4 separate experiments.

Table 2. Affinities of Compounds **1a**−**d** at Cloned Human 5-HT_2 Receptors[a]

compound	[^{125}I]DOI 5-HT_{2A}	[3H]-5-HT 5-HT_{2B}	[^{125}I]DOI 5-HT_{2C}
(±)-**1a**	164 ± 37	58.3 ± 18.2	29.9 ± 3.3
(1*R*,2*S*)-(−)-**1a**[16]	98.2 ± 7. 5	58.5 ± 4.0	44.1 ± 5.5
(1*S*,2*R*)-(+)-**1a**[16]	132 ± 3	93.5 ± 19.6	21.3 ± 4.0
(±)-**1b**	45.9 ± 7.6	64.9 ± 5.7	11.8 ± 1.9
(±)-**1c**	30.9 ± 2.5	38.1 ± 5.1	17.8 ± 1.2
(±)-**1d**	10.2 ± 3.2	5.7 ± 0.3	1.9 ± 0.5
α-methyltryptamine	41.0 ± 13.8	87.0 ± 32.2	9.3 ± 3.4
5-methoxy-α-methyltryptamine	2.2 ± 0.3	8.3 ± 0.5	1.0 ± 0.1

[a] Data are expressed as mean K_i values (nM) ± SEM for 3−4 separate experiments.

Radioreceptor competition data at the agonist-labeled cloned human 5-HT_2 receptors are reported in Table 2. While in general the affinities were low for these sites, all the compounds had highest affinity at the 5-HT_{2C} receptor. The affinities of **1a**,**c** at the 5-HT_{2A} receptor are significantly lower than for their flexible analogues, α-methyltryptamine and 5-methoxy-α-methyltryptamine. This result contrasts sharply with the affinity at the 5-HT_{2A} site of a ring-substituted phenylcyclopropylamine, when compared to its α-methylphenethylamine congener. For example, we have previously shown that *trans*-2,5-dimethoxy-4-methylphenylcyclopropylamine has very high affinity for 5-HT_{2A} sites.[19] It would appear therefore, at least in the present tryptamine series, that

the cyclopropylamine fails to provide a satisfactory replacement for the ethylamine side chain at the 5-HT_{2A} receptor.

The 5-fluoro compound **1d** had high affinity at all three 5-HT_2 sites and was extremely potent at the 5-HT_{2C} receptor. The recent report that fluorinated tryptamines and isotryptamines are potent and selective ligands for 5-HT_{2C} receptors[20] presents a parallel to compound **1d**. The present results reinforce the conclusion of Bös et al.[20] that halogenated tryptamines have higher affinities for 5-HT_{2C} than for 5-HT_{2A} receptors.

In addition, Bös et al.[20] note that compounds with the *S* absolute configuration have higher affinity at the 5-HT_{2C} receptor. Our previous study of enantiomeric α-methyltryptamines showed that the *S*-(+) enantiomers of 5-oxygenated compounds had higher affinity than their *R* isomers at the 5-HT_{2A} site, while the order of affinity was reversed for the unsubstituted and 4-hydroxy compounds.[21] In that same study, we also found that at the 5-HT_{1B} receptor, *R* enantiomers generally had higher affinity than their *S* isomers. The order of affinities obtained at the 5-HT_{2A} receptor for the (+) and (−) isomers of **1a** in the present study is in agreement with our earlier study of the enantiomers of unsubstituted α-methyltryptamine (1*R*,2*S* > 1*S*,2*R*; *R* > *S*), but *at the 5-HT_{2C} receptor the stereoselectivity is reversed*.

This observation clearly merits further investigation with other optically active compounds. The high degree (79%) of structural homology between the transmembrane regions of the 5-HT_{2A} and 5-HT_{2C} receptors has made it difficult to design agonist molecular probes specific for one site or the other. To our knowledge however, no one has so far exploited a possible reversal of binding stereoselectivity to search for 5-HT_{2C} ligands among the various molecules known to possess high affinity for both the 5-HT_{2A} and 5-HT_{2C} receptors. These data, combined with our earlier studies,[19,21] also suggest the possibility of differences in binding orientations between the phenethylamines and the tryptamines at various serotonin receptor isoforms.

In summary, constraining the ethylamine side chain of tryptamines into a cyclopropyl ring does not provide rigid analogues that are good models of the binding conformations of flexible tryptamines, except perhaps at the 5-HT_{2C} receptor. This may not simply be the consequence of increased steric demand and limited conformational flexibility, but it could also be related to amine pK_a, which is lower for cyclopropylamines. The binding data provide further support for the idea that tryptamines bind to serotonin receptors in conformations that may differ from the binding conformations of phenethylamine-derived molecules.

Experimental Section

Chemistry. Melting points, determined with a Thomas-Hoover Meltemp apparatus, are uncorrected, except where indicated. ^{1}H NMR spectra were recorded on a Varian VXR-500S 500-MHz instrument. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane (TMS) in $CDCl_3$, except where noted. Abbreviations used in NMR analysis are as follows: br s = broad singlet, d = doublet, dd = doublet of doublets, m = multiplet, q = quartet, s = singlet, t = triplet, td = triplet of doublets. Analytical thin-layer chromatography (TLC) was performed on Baker-flex silica gel 1B2-F plastic plates. The chemical ionization mass spectra (CIMS) were determined on the Purdue University Department of Medicinal Chemistry's Finnegan 4000 quadrupole mass spectrometer, using isobutane as the reagent gas and are reported as *m*/*z* (relative intensity). Fast-atom bombardment mass spectra (FABMS) were obtained on a Kratos MS-50 spectrometer. Elemental analyses were obtained from Purdue Microanalytical Laboratory and were within 0.4% of the calculated values, unless otherwise noted. A Parr apparatus was used for hydrogenations.

General Procedure for the Preparation of 1-(*p*-Tolylsulfonyl)indole-3-carboxaldehydes 3a−d. A mixture of the appropriate indole-3-carboxaldehyde (10.3 mmol) and *p*-toluenesulfonyl chloride (15.7 mmol) in triethylamine (25 mL) was heated at 90−95 °C for 1 h. The reaction mixture was poured into ice-cold water (35 mL), stored in a refrigerator for 1 h, and then filtered. The insoluble solid was washed with water and air-dried. Recrystallization from ethyl acetate gave the respective pure 1-(*p*-tolylsulfonyl)indole-3-carboxaldehydes.

1-(*p*-Tolylsulfonyl)indole-3-carboxaldehyde, 3a. This material was obtained in 80% yield as a white powder: mp 148−150 °C, lit.[22] mp 148−150 °C; ^{1}H NMR δ 10.09 (s, 1H, CHO), 8.26 (m, 1H, ArH), 8.22 (s, 1H, ArH), 7.96 (m, 1H, ArH), 7.85 (d, 2H, ArH, *J* = 8.6 Hz), 7.38 (m, 2H, ArH), 7.30 (d, 2H, ArH, *J* = 8.6 Hz), 2.38 (s, 3H, $ArCH_3$); CIMS 300 (MH^+).

4-Methoxy-1-(*p*-tolylsulfonyl)indole-3-carboxaldehyde, 3b. This material was obtained in 90% yield as off-white crystals: mp 172−173 °C; ^{1}H NMR δ 10.49 (s, 1H, CHO), 8.23 (s, 1H, ArH), 7.83 (d, 2H, ArH, *J* = 8.0 Hz), 7.61 (d, 1H, ArH, *J* = 8.0 Hz), 7.31 (t, 1H, ArH, *J* = 8.0 Hz), 7.27 (d, 2H, ArH, *J* = 8.0 Hz), 6.78 (d, 1H, ArH, *J* = 8.0 Hz), 3.95 (s, 3H, $ArOCH_3$), 2.36 (s, 3H, $ArCH_3$); CIMS 330 (MH^+). Anal. ($C_{17}H_{15}NO_4S$) C, H, N.

5-Methoxy-1-(*p*-tolylsulfonyl)indole-3-carboxaldehyde, 3c. This material was obtained in 90% yield as an off-white powder: mp 124−126 °C, lit.[23] mp 128−129 °C; ^{1}H NMR δ 10.06 (s, 1H, CHO), 8.17 (s, 1H, ArH), 7.82 (d, 3H, ArH, *J* = 8.8 Hz), 7.70 (d, 1H, ArH, *J* = 2.3 Hz), 7.28 (d, 2H, ArH, *J* = 8.8 Hz), 7.00 (dd, 1H, ArH, *J* = 9.0 and 2.6 Hz), 3.85 (s, 3H, $ArOCH_3$), 2.37 (s, 3H, $ArCH_3$); CIMS 330 (MH^+).

5-Fluoro-1-(*p*-tolylsulfonyl)indole-3-carboxaldehyde, 3d. This material was obtained in 82% yield as a white powder: mp 226−227 °C; ^{1}H NMR δ 10.06 (s, 1H, CHO), 8.25 (s, 1H, ArH), 7.93 (dd, 1H, ArH, *J* = 8.7 and 2.5 Hz), 7.90 (dd, 1H, ArH, *J* = 9.1 and 4.3 Hz), 7.84 (d, 2H, ArH, *J* = 8.2 Hz), 7.32 (d, 2H, ArH, *J* = 8.2 Hz), 7.14 (td, 1H, ArH, *J* = 9.0 and 2.6 Hz), 2.39 (s, 3H, $ArCH_3$); CIMS 318 (MH^+). Anal. ($C_{16}H_{12}FNO_3S$) C, H, N.

General Procedure for the Preparation of *trans*-β-(1-(*p*-Tolylsulfonyl)indol-3-yl)acrylic Acids 4a−d. Following the method of Moffatt,[24] a solution of the appropriate 1-(*p*-tolylsulfonyl)indole-3-carboxaldehyde (6.68 mmol) and malonic acid (21.62 mmol) in pyridine (8 mL) containing piperidine (8 drops) was heated at 75−80 °C under nitrogen for 3 h. The solution was poured into ice-cold water (50 mL) and acidified with 5 N hydrochloric acid. After the mixture had been stored in the refrigerator overnight, the precipitate was filtered and washed with water. Recrystallization from methanol gave the respective *trans*-β-(1-(*p*-tolylsulfonyl)indol-3-yl)acrylic acids.

***trans*-β-(1-(*p*-Tolylsulfonyl)indol-3-yl)acrylic Acid, 4a.** This compound was obtained in 87% yield as a white powder: mp 243−244 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, 1H, COOH), 8.43 (s, 1H, ArH), 7.96 (d, 1H, ArH, *J* = 8.3 Hz), 7.92 (d, 1H, ArH, *J* = 8.3 Hz), 7.90 (d, 2H, ArH, *J* = 8.4 Hz), 7.74 (d, 1H, β-H, *J* = 16.2 Hz), 7.41 (m, 1H, ArH), 7.39 (d, 2H, ArH, *J* = 8.4 Hz), 7.34 (m, 1H, ArH), 6.57 (d, 1H, α-H, *J* = 16.2 Hz), 2.30 (s, 3H, $ArCH_3$); CIMS 342 (MH^+). Anal. ($C_{18}H_{15}NO_4S$) C, H, N.

***trans*-β-(4-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)acrylic Acid, 4b.** This compound was obtained in 89% yield as an off-white solid: mp 255−256 °C; ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H, ArH), 8.00 (d, 1H, β-H, *J* = 16.0 Hz), 7.91 (d, 2H, ArH, *J* = 8.0 Hz), 7.54 (d, 1H, ArH, *J* = 8.0 Hz), 7.40 (d, 2H, ArH, *J* = 8.0 Hz), 7.30 (t, 1H, ArH, *J* = 8.0 Hz), 6.86 (d, 1H, ArH,

J = 8 Hz), 6.63 (d, 1H, α-H, J = 16.0 Hz), 3.88 (s, 3H, $ArOCH_3$), 2.31 (s, 3H, $ArCH_3$); CIMS 372 (MH^+). Anal. ($C_{19}H_{17}NO_5S$) C, H, N.

***trans*-β-(5-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)acrylic Acid, 4c.** This compound was obtained in 76% yield as off-white crystals: mp 244−245 °C; ^{1}H NMR (DMSO-d_6) δ 8.85 (br s, 1H, COOH), 8.38 (s, 1H, ArH), 7.86 (d, 2H, ArH, J = 8.5 Hz), 7.84 (d, 1H, ArH, J = 9.2 Hz), 7.73 (d, 1H, β-H, J = 16.2 Hz), 7.38 (d, 2H, ArH, J = 8.5 Hz), 7.32 (d, 1H, ArH, J = 2.5 Hz), 7.00 (dd, 1H, ArH, J = 9.2 and 2.5 Hz), 6.57 (d, 1H, α-H, J = 16.2 Hz), 3.80 (s, 3H, $ArOCH_3$), 2.30 (s, 3H, $ArCH_3$); CIMS 372 (MH^+). Anal. ($C_{19}H_{17}NO_5S$) C, H, N.

***trans*-β-(5-Fluoro-1-(*p*-tolylsulfonyl)indol-3-yl)acrylic Acid, 4d.** This compound was obtained in 65% yield as white needles: mp 254−255 °C; ^{1}H NMR (DMSO-d_6) δ 12.35 (br s, 1H, COOH), 8.51 (s, 1H, ArH), 7.97 (dd, 1H, ArH, J = 9.1 and 4.4 Hz), 7.91 (d, 2H, ArH, J = 8.4 Hz), 7.78 (dd, 1H, ArH, J = 9.4 and 2.4 Hz), 7.71 (d, 1H, β-H, J = 16.2 Hz), 7.40 (d, 2H, ArH, J = 8.4 Hz), 7.26 (td, 1H, ArH, J = 9.1 and 2.4 Hz), 6.58 (d, 1H, α-H, J = 16.2 Hz), 2.31 (s, 3H, $ArCH_3$); CIMS 360 (MH^+); Anal. ($C_{18}H_{14}FNO_4S$) C, H, N.

General Procedure for the Preparation of *trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic Acid Methyl Esters 5a−d.[13] Diazomethane was prepared as previously described.[25] A solution of 4.07 g (19.0 mmol) of *N*-nitroso-*N*-methyl-4-toluenesulfonamide in ether (50 mL) was slowly added to a heated mixture of potassium hydroxide (1.23 g, 21.9 mmol), ether (3 mL), water (3 mL), and 2-(2-ethoxyethoxy)ethane (6 mL). The ether solution of diazomethane thus formed was continuously distilled into a cold (ice−salt bath) stirring solution of the appropriate *trans*-β-(1-(*p*-tolylsulfonyl)indol-3-yl)acrylic acid (4.39 mmol) in ether (15 mL) and dichloromethane (2 mL). The reaction mixture was kept at −5 to 0 °C (bath temperature) until all diazomethane had been distilled; stirring was continued for an additional 1 h. Palladium diacetate (10 mg) in a small amount of tetrahydrofuran was added, and an azeotrope of a second portion of diazomethane and ether (prepared from the same amount of reagents) was again distilled into the reaction mixture. After all diazomethane had been distilled, the reaction mixture was stirred at room temperature overnight and then filtered. The solid on the filter was washed with dichloromethane. The filtrate and washing were evaporated to give the crude product which was then further purified by column chromatography (silica gel, 5% ethyl acetate in methylene chloride).

Methyl *trans*-2-(1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylate, 5a. This compound was obtained in 70% yield as a yellow oil that precipitated upon addition of methanol: mp 115−116 °C; ^{1}H NMR δ 7.96 (d, 1H, ArH, J = 8.3 Hz), 7.74 (d, 2H, ArH, J = 8.4 Hz), 7.56 (d, 1H, ArH, J = 7.8 Hz), 7.32 (m, 1H, ArH), 7.26 (s, 1H, ArH,), 7.25 (m, 1H, ArH,), 7.22 (d, 2H, ArH, J = 8.4 Hz), 3.74 (s, 3H, $COOCH_3$), 2.50 (m, 1H, cyclopropyl H), 2.34 (s, 3H, $ArCH_3$), 1.88 (m, 1H, cyclopropyl H), 1.59 (m, 1H, cyclopropyl H), 1.29 (m, 1H, cyclopropyl H); CIMS 370 (MH^+). Anal. ($C_{20}H_{19}NO_4S$) C, H, N.

Methyl *trans*-2-(4-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylate, 5b. This compound was obtained in 65% yield as a yellow oil that precipitated upon addition of methanol: mp 146−147 °C; ^{1}H NMR δ 7.72 (d, 2H, ArH, J = 8.4 Hz), 7.54 (d, 1H, ArH, J = 7.8 Hz), 7.21 (d, 2H, ArH, J = 8.2 Hz), 7.20 (t, 1H, ArH, J = 7.8 Hz), 7.10 (s, 1H, ArH), 6.62 (d, 1H, ArH, J = 7.8 Hz), 3.82 (s, 3H, ArOCH3), 3.74 (s, 3H, COOCH3), 2.80 (m, 1H, cyclopropyl H), 2.34 (s, 3H, ArCH3), 1.75 (m, 1H, cyclopropyl H), 1.56 (m, 1H, cyclopropyl H), 1.28 (m, 1H, cyclopropyl H); CIMS 400 (MH^+). Anal. ($C_{21}H_{21}N_5S$) C, H, N.

Methyl *trans*-2-(5-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylate, 5c. This compound was obtained in 60% yield as a pale yellow oil that solidified upon addition of methanol: mp 137−139 °C; ^{1}H NMR δ 7.85 (d, 1H, ArH, J = 9.0 Hz), 7.70 (d, 2H, ArH, J = 8.4 Hz), 7.21 (d, 2H, ArH, J = 8.4 Hz), 7.20 (s, 1H, ArH), 6.97 (d, 1H, ArH, J = 2.5 Hz), 6.93 (dd, 1H, ArH, J = 9.0 and 2.5 Hz), 3.83 (s, 3H, $ArOCH_3$), 3.75 (s, 3H, $COOCH_3$), 2.44 (m, 1H, cyclopropyl H), 2.34 (s, 3H, $ArCH_3$), 1.87 (m, 1H, cyclopropyl H), 1.58 (m, 1H, cyclopropyl H), 1.26 (m, 1H, cyclopropyl H); CIMS 400 (MH^+). Anal. ($C_{21}H_{21}NO_5S$) C, H, N.

Methyl *trans*-2-(5-Fluoro-1-(*p*-tolylsulfonyl)indol-3-yl)-cyclopropanecarboxylate, 5d. This compound was obtained in 79% yield as a yellow oil that precipitated upon addition of methanol: mp 134−135 °C; ^{1}H NMR δ 7.90 (dd, 1H, ArH, J = 9.1 and 4.4 Hz), 7.72 (d, 2H, ArH, J = 8.4 Hz), 7.29 (s, 1H, ArH), 7.23 (d, 2H, ArH, J = 8.4 Hz), 7.20 (dd, 1H, ArH, J = 8.7 and 2.3 Hz), 7.05 (td, 1H, ArH, J = 9.0 and 2.3 Hz), 3.75 (s, 3H, $COOCH_3$), 2.45 (m, 1H, cyclopropyl H), 2.35 (s, 3H, $ArCH_3$), 1.85 (m, 1H, cyclopropyl H), 1.59 (m, 1H, cyclopropyl H), 1.27 (m, 1H, cyclopropyl H); CIMS 388 (MH^+, 73.6), 356 (100). Anal. ($C_{20}H_{18}FNO_4S$) C, H, N.

***trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic Acids 6a−d. General Procedure.** The appropriate methyl *trans*-2-(1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylates (2.40 mmol) were subjected to hydrolysis by stirring for 5 h in 2 mL of a 50% sodium hydroxide solution in methanol at room temperature. The reaction mixture was poured into a stirred mixture of water (10 mL), concentrated HCl (2.3 mL), and ice. Extraction with dichloromethane (3 × 10 mL), washing, drying ($MgSO_4$), and evaporation gave the respective *trans*-2-(1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic acids which were used directly without further purification.

***trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic Acid, 6a.** This compound was obtained in 85% yield as a brown oil: ^{1}H NMR δ 7.97 (d, 1H, ArH, J = 7.3 Hz), 7.75 (d, 2H, ArH, J = 8.5 Hz), 7.58 (d, 1H, ArH, J = 7.0 Hz), 7.29 (m, 3H, ArH), 7.27 (d, 2H, ArH, J = 8.5 Hz), 2.58 (m, 1H, cyclopropyl H), 2.34 (s, 3H, $ArCH_3$), 1.88 (m, 1H, cyclopropyl H), 1.67 (m, 1H, cyclopropyl H), 1.39 (m, 1H, cyclopropyl H); CIMS 356 (MH^+).

***trans*-2-(4-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic Acid, 6b.** This compound was obtained in 82% yield as a brown oil: ^{1}H NMR δ 7.72 (d, 2H, ArH, J = 8.0 Hz), 7.54 (d, 1H, ArH, J = 8.0 Hz), 7.21 (t, 1H, ArH, J = 8.0 Hz), 7.20 (d, 2H, ArH, J = 8.0 Hz), 7.12 (s, 1H, ArH), 6.63 (d, 1H, ArH, J = 8.0 Hz), 3.84 (s, 3H, $ArOCH_3$), 2.85 (m, 1H, cyclopropyl H), 2.34 (s, 3H, $ArCH_3$), 1.75 (m, 1H, cyclopropyl H), 1.63 (m, 1H, cyclopropyl H), 1.37 (m, 1H, cyclopropyl H); CIMS 386 (MH^+).

***trans*-2-(5-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic Acid, 6c.** This compound was obtained in 83% yield as a brown oil: ^{1}H NMR δ 7.86 (d, 1H, ArH, J = 9.0 Hz), 7.71 (d, 2H, ArH, J = 8.4 Hz), 7.24 (s, 1H, ArH), 7.21 (d, 2H, ArH, J = 8.0 Hz), 6.99 (d, 1H, ArH, J = 2.3 Hz), 6.94 (dd, 1H, ArH, J = 9.0 and 2.4 Hz), 3.83 (s, 3H, $ArOCH_3$), 2.51 (m, 1H, cyclopropyl H), 2.34 (s, 3H, $ArCH_3$), 1.87 (m, 1H, cyclopropyl H), 1.65 (m, 1H, cyclopropyl H), 1.35 (m, 1H, cyclopropyl H); CIMS 386 (MH^+).

***trans*-2-(5-Fluoro-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic Acid, 6d.** This compound was obtained in 87% yield as a brown oil: ^{1}H NMR δ 7.91 (dd, 1H, ArH, J = 9.1 and 4.0 Hz), 7.72 (d, 2H, ArH, J = 8.4 Hz), 7.32 (s, 1H, ArH), 7.24 (d, 2H, ArH, J = 8.4 Hz), 7.22 (dd, 1H, ArH, J = 8.6 and 2.3 Hz), 7.06 (td, 1H, ArH, J = 9.0 and 2.3 Hz), 2.51 (m, 1H, cyclopropyl H), 2.36 (s, 3H, $ArCH_3$), 1.86 (m, 1H, cyclopropyl H), 1.66 (m, 1H, cyclopropyl H), 1.36 (m, 1H, cyclopropyl H); CIMS 374 (MH^+).

General Procedure for the Preparation of *trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)carbobenzoxamidocyclopropanes 7a−d. Following the method of Weinstock,[25] the appropriate *trans*-2-(1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropanecarboxylic acid (3.38 mmol) was suspended in 0.5 mL of water, and sufficient acetone was added to complete the solution. The solution was cooled to 0 °C (ice−salt bath), and a solution of triethylamine (4.30 mmol) in acetone (5 mL) was added. While the temperature was maintained at 0 °C, a solution of ethyl chloroformate (5.23 mmol) in acetone (2 mL) was added slowly. The mixture was stirred for 1 h at this temperature. A solution of sodium azide (12.3 mmol) in water (3 mL) was

added dropwise, and the mixture was stirred for 2 h. The reaction mixture was poured into ice water (15 mL) and extracted with toluene (4 × 10 mL). The toluene solution was dried ($MgSO_4$) and filtered. The filtrate was heated to gentle reflux with dry benzyl alcohol (24.0 mmol) for 10 h. The solvent and excess benzyl alcohol were removed in vacuo. Purification of the crude product by column chromatography (silica gel, 1% ethyl acetate in methylene chloride) provided the respective *N*-carbobenzoxy derivatives.

***trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)carbobenzoxamidocyclopropane, 7a.** This was obtained in 70% yield as a yellow oil that solidified upon standing: mp 86−88 °C; ^{1}H NMR δ 7.95 (d, 1H, ArH, J = 8.5 Hz), 7.72 (d, 2H, ArH, J = 8.4 Hz), 7.32 (m, 9H, ArH), 7.20 (d, 2H, ArH, J = 8.1 Hz), 5.15 (s, 2H, $ArCH_2O$), 5.13 (br s, 1H, NH), 2.73 (m, 1H, cyclopropyl H), 2.33 (s, 3H, $ArCH_3$), 2.06 (m, 1H, cyclopropyl H), 1.18 (m, 2H, cyclopropyl CH_2); CIMS 461 (MH^+). Anal. ($C_{26}H_{24}N_2O_4S$) C, H, N.

***trans*-2-(4-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)carbobenzoxamidocyclopropane, 7b.** This was obtained in 65% yield as a yellow oil that solidified upon standing: mp 55−57 °C; ^{1}H NMR δ 7.70 (d, 2H, ArH, J = 8.4 Hz), 7.56 (d, 1H, ArH, J = 8.1 Hz), 7.36 (m, 5H, ArH), 7.21 (d, 2H, ArH, J = 8.4 Hz), 7.20 (t, 1H, ArH, J = 8.1 Hz), 7.04 (s, 1H, ArH), 6.63 (d, 1H, ArH, J = 8.1 Hz), 5.12 (s, 2H, $ArCH_2O$), 5.08 (br s, 1H, NH), 3.85 (s, 3H, $ArOCH_3$), 2.65 (m, 1H, cyclopropyl H), 2.35 (m, 1H, cyclopropyl H), 1.20 (m, 2H, cyclopropyl CH_2); CIMS 491 (MH^+). Anal. ($C_{27}H_{26}N_2O_5S$) C, H, N.

***trans*-2-(5-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)carbobenzoxamidocyclopropane, 7c.** This was obtained in 60% yield as a yellow oil that solidified upon standing: mp 54−55 °C; ^{1}H NMR δ 7.82 (d, 1H, ArH, J = 9.0 Hz), 7.68 (d, 2H, ArH, J = 8.2 Hz), 7.35 (m, 6H, ArH), 7.18 (d, 2H, ArH, J = 8.0 Hz), 7.17 (s, 1H, ArH), 6.92 (dd, 1H, ArH, J = 9.0 and 2.5 Hz), 5.13 (s, 2H, $ArCH_2O$), 5.09 (br s, 1H, NH), 3.86 (s, 3H, $ArOCH_3$), 2.64 (m, 1H, cyclopropyl H), 2.32 (s, 3H, $ArCH_3$), 2.00 (m, 1H, cyclopropyl H), 1.14 (m, 2H, cyclopropyl CH_2); CIMS 491 (MH^+). Anal. ($C_{27}H_{26}N_2O_5S$) C, H, N.

***trans*-2-(5-Fluoro-1-(*p*-tolylsulfonyl)indol-3-yl)carbobenzoxamidocyclopropane, 7d.** This was obtained in 70% yield as a yellow oil that solidified upon standing: mp 119−120 °C; ^{1}H NMR δ 7.88 (dd, 1H, ArH, J = 9.0 and 4.0 Hz), 7.70 (d, 2H, ArH, J = 8.2 Hz), 7.36 (m, 7H, ArH), 7.21 (d, 2H, ArH, J = 8.2 Hz), 7.04 (td, 1H, ArH, J = 9.0 and 2.6 Hz), 5.15 (s, 2H, $ArCH_2O$), 5.08 (br s, 1H, NH), 2.67 (m, 1H, cyclopropyl H), 2.35 (s, 3H, $ArCH_3$), 2.00 (m, 1H, cyclopropyl H), 1.15 (m, 2H, cyclopropyl CH_2); CIMS 479 (MH^+). Anal. ($C_{26}H_{23}FN_2O_4S$) C, H, N.

General Procedure for the Preparation of *trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)cyclopropylamines 8a−d.[27] A solution of the appropriate *N*-carbobenzoxy derivative (2.39 mmol) in absolute ethanol (50 mL) containing 10% palladium on charcoal (250 mg) was shaken on a Parr apparatus for 7 h under 50 psi of hydrogen. The catalyst was removed by filtration and washed with ethanol. The combined filtrate and washing were evaporated to obtain a residue, which was purified via centrifugal rotary chromatography (Chromatotron) to give the respective amines.

***trans*-2-(1-(*p*-Tolylsulfonyl)indol-3-yl)cyclopropylamine, 8a.** This compound was obtained in 53% yield as a yellow oil: ^{1}H NMR δ 7.95 (d, 1H, ArH, J = 7.5 Hz), 7.72 (d, 2H, ArH, J = 8.3 Hz), 7.59 (d, 1H, ArH, J = 7.7 Hz), 7.31 (m, 1H, ArH), 7.25 (m, 1H, ArH), 7.20 (d, 2H, ArH, J = 8.3 Hz), 7.11 (s, 1H, ArH), 2.51 (m, 1H, cyclopropyl H), 2.33 (s, 3H, $ArCH_3$), 1.85 (br s, 3H, NH_2 and cyclopropyl H), 1.03 (m, 1H, cyclopropyl H), 0.95 (m, 1H, cyclopropyl H); CIMS 327 (MH^+). Anal. ($C_{18}H_{18}N_2O_2S$) C, H, N.

***trans*-2-(4-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropylamine, 8b.** This compound was obtained in 50% yield as a yellow oil: ^{1}H NMR δ 7.68 (d, 2H, ArH, J = 8.5 Hz), 7.53 (d, 1H, ArH, J = 8.0 Hz), 7.30 (m, 3H, ArH), 6.94 (s, 1H, ArH), 6.60 (d, 1H, ArH, J = 8.0 Hz), 3.86 (s, 3H, $ArOCH_3$), 2.31 (s, 3H, $ArCH_3$), 2.24 (m, 1H, cyclopropyl H), 1.80 (br s, 2H, NH_2), 1.70 (m, 1H, cyclopropyl H), 0.92 (m, 2H, cyclopropyl CH_2); CIMS 357 (MH^+). Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N.

***trans*-2-(5-Methoxy-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropylamine, 8c.** This compound was obtained in 50% yield as a yellow oil: ^{1}H NMR δ 7.84 (d, 1H, ArH, J = 9.0 Hz), 7.68 (d, 2H, ArH, J = 8.5 Hz), 7.19 (d, 2H, ArH, J = 8.5 Hz), 7.05 (s, 1H, ArH), 7.01 (d, 1H, ArH, J = 2.3 Hz), 6.92 (dd, 1H, ArH, J = 9.0 and 2.3 Hz), 3.83 (s, 3H, $ArOCH_3$), 2.49 (m, 1H, cyclopropyl H), 2.33 (s, 3H, $ArCH_3$), 1.78 (m, 1H, cyclopropyl H), 1.70 (br s, 2H, NH_2), 1.02 (m, 1H, cyclopropyl H), 0.93 (m, 1H, cyclopropyl H); CIMS 357 (MH^+). Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N.

***trans*-(5-Fluoro-1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropylamine, 8d.** This compound was obtained in 50% yield as a yellow oil: ^{1}H NMR δ 7.89 (dd, 1H, ArH, J = 9.1 and 4.4 Hz), 7.69 (d, 2H, ArH, J = 8.3 Hz), 7.22 (dd, 1H, ArH, J = 8.8 and 2.6 Hz), 7.21 (d, 2H, ArH, J = 8.3 Hz), 7.14 (s, 1H, ArH), 7.03 (td, 1H, ArH, J = 9.1 and 2.6 Hz), 2.49 (m, 1H, cyclopropyl H), 2.34 (s, 3H, $ArCH_3$), 1.85 (br s, 2H, NH_2), 1.76 (m, 1H, cyclopropyl H), 1.02 (m, 1H, cyclopropyl H), 0.93 (m, 1H, cyclopropyl H); CIMS 345 (MH^+). Anal. ($C_{18}H_{17}FN_2O_2S$) C, H, N.

General Procedure for the *N*(1)-Detosylation, 1a−d.[28] The appropriate *trans*-2-(1-(*p*-tolylsulfonyl)indol-3-yl)cyclopropylamine **8a−d** (0.45 mmol) and anhydrous disodium hydrogen phosphate (1.87 mmol) were stirred magnetically in dry methanol (8 mL) at room temperature under nitrogen. To the suspension was added pulverized 6% sodium amalgam (904 mg). Stirring was continued until the reaction was complete as indicated by TLC (about 45 min). The mixture was poured into water (10 mL), extracted with ether (4 × 10 mL), dried ($MgSO_4$), filtered, and concentrated to afford a yellow oil that was converted to its oxalate salt and recrystallized from ethanol−petroleum ether.

***trans*-2-(Indol-3-yl)cyclopropylamine Oxalate, 1a.** This was obtained in 60% yield as an off-white powder: mp 153−154 °C dec; ^{1}H NMR (CD_3OD) δ 7.66 (td, 1H, ArH, J = 7.9 and 0.9 Hz), 7.32 (td, 1H, ArH, J = 8.1 and 0.8 Hz), 7.10 (m, 1H, ArH), 7.03 (m, 1H, ArH), 7.02 (s, 1H, ArH), 2.73 (m, 1H, cyclopropyl H), 2.40 (m, 1H, cyclopropyl H), 1.35 (m, 1H, cyclopropyl H), 1.26 (m, 1H, cyclopropyl H); FABMS 173 (MH^+); high-resolution FABMS calcd for $C_{11}H_{12}N_2$ 173.1079, found 173.1080.

***trans*-2-(4-Methoxyindol-3-yl)cyclopropylamine Oxalate, 1b.** This was obtained in 60% yield as a yellow-brown powder: mp >200 °C dec; ^{1}H NMR (CD_3OD) δ 7.00 (t, 1H, ArH, J = 8.0 Hz), 6.92 (d, 1H, ArH, J = 8.1 Hz), 6.82 (s, 1H, ArH), 6.49 (d, 1H, ArH, J = 7.6 Hz), 3.93 (s, 3H, $ArOCH_3$), 2.76 (m, 1H, cyclopropyl H), 2.64 (m, 1H, cyclopropyl H), 1.25 (m, 2H, cyclopropyl CH_2); FABMS 203 (MH^+); high-resolution FABMS calcd for $C_{12}H_{14}N_2O$ 203.1184, found 203.1186.

***trans*-2-(5-Methoxyindol-3-yl)cyclopropylamine Oxalate, 1c.** This was obtained in 60% yield as a yellow-brown powder: mp >200 °C dec; ^{1}H NMR (CD_3OD) δ 7.21 (d, 1H, ArH, J = 8.8 Hz), 7.15 (d, 1H, ArH, J = 2.5 Hz), 6.98 (s, 1H, ArH), 6.77 (dd, 1H, ArH, J = 8.8 and 2.5 Hz), 3.83 (s, 3H, $ArOCH_3$), 2.70 (m, 1H, cyclopropyl H), 2.36 (m, 1H, cyclopropyl H), 0.97 (m, 1H, cyclopropyl H), 0.92 (m, 1H, cyclopropyl H); FABMS 203 (MH^+). Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

***trans*-2-(5-Fluoroindol-3-yl)cyclopropylamine Oxalate, 1d.** This was obtained in 50% yield as a yellow-brown powder: mp 141−144 °C; ^{1}H NMR (CD_3OD) δ 7.35 (dd, 1H, ArH, J = 9.8 and 2.5 Hz), 7.28 (dd, 1H, ArH, J = 9.0 and 4.4 Hz), 7.09 (s, 1H, ArH), 6.88 (td, 1H, ArH, J = 9.1 and 2.5 Hz), 2.71 (m, 1H, cyclopropyl H), 2.33 (m, 1H, cyclopropyl H), 1.34 (m, 1H, cyclopropyl H), 1.24 (m, 1H, cyclopropyl H); FABMS 191 (MH^+); high-resolution FABMS calcd for $C_{11}H_{11}FN_2$ 191.0985, found 191.0977.

Pharmacology Methods. Radioreceptor Competition Assays in 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, at the same conditions described above but 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 216 Ci/mmol. 5-HT was purchased from Sigma (St. Louis, MO).

The procedures of Huang et al.[29] 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, 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 with triplicate determinations using 200−400 μg of protein, in a final incubation 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 as that displaceable with 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 at an efficiency of 37% for tritium.

Radioligand Competition Experiments Using Cloned Human 5-HT$_2$ Receptors. All chemicals were obtained from the sources previously described.[30] [^{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$_{2C}$ receptors.[30] 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/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, 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 resupended (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 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors.

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.[30] 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 concentrations of $CaCl_2$, pargyline, Tris, and L-ascorbic acid were 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.[31]

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 final concentration, 10 μM pargyline, 9.75 mM $MgCl_2$, 0.5 mM disodium 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$_{2C}$ receptors and 1 μM ketanserin for 5-HT$_{2A}$ receptors. The final concentration of [^{125}I]DOI was approximately 0.07−0.15 nM.

Data Analysis. Radioligand competition data for the 5-HT$_{1A}$ receptor were analyzed using the computer programs EBDA and Ligand as described by McPherson.[32] The values from three separate experiments were combined.

For the competition curves run against the 5-HT$_2$ family of receptors, the IC_{50} values were determined by nonlinear regression analysis using a four-parameter logistic equation described by De Lean et al.[33] After verification that the curves best fit a one-site model, the IC_{50} values were then converted to K_i values using the Cheng−Prusoff equation.[34]

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