1-(2,5-Dimethoxy-4-(trifluoromethyl)phenyl)-2-aminopropane: A Potent Serotonin 5-HT$_{2A/2C}$ Agonist

David E. Nichols,*,† Stewart Frescas,† Danuta Marona-Lewicka,† Xuemei Huang,† Bryan L. Roth,‡ Gary A. Gudelsky,‡ and J. Frank Nash‡

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907-1333, and Department of Psychiatry, School of Medicine, Case Western Reserve University, 2040 Abington Road, Cleveland, Ohio 44106-5000

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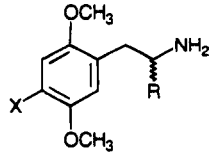
A method was found to synthesize 1-(2,5-dimethoxy-4-(trifluoromethyl)phenyl)-2-aminopropane, **5**, and its des-α-methyl congener 2-(2,5-dimethoxy-4-(trifluoromethyl)phenyl)aminoethane, **6**, the trifluoromethyl analogs of substituted hallucinogenic phenethylamine derivatives such as 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (**3**, DOI) that are potent 5-HT$_{2A/2C}$ agonists. In our hands, **5** and **6** have proven to have affinity for [^{3}H]ketanserin or [^{125}I]-**3**-labeled 5-HT$_{2A/2C}$ sites in rat cortex comparable to or higher than the analogous bromo or iodo analogs. Similarly, **5** and **6** had potency comparable to or slightly greater than that of their bromo or iodo congeners in the two-lever drug discrimination assay in rats trained to discriminate saline from LSD tartrate. The agonist properties of **5** and **6** were evaluated by measuring the accumulation of [^{3}H]inositol monophosphate in cultured cells selectively expressing either 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors. In comparison to serotonin (5-HT), compounds **3** (DOI), **5**, and **6** were equally efficacious and full agonists at the 5-HT$_{2C}$ receptor. Similarly, **3** and **5** produced equivalent responses at the 5-HT$_{2A}$ receptor as compared to 5-HT. In contrast, **6**, the α-desmethyl analog of **5**, was only half as potent at stimulating inositol monophosphate accumulation at the 5-HT$_{2A}$ receptor. In conclusion, the title compound **5** and its α-desmethyl congener **6** appear to be the most potent of the so-called hallucinogenic amphetamine 5-HT agonists reported to date. Further, the reduced efficacy of **6** at the 5-HT$_{2A}$ receptor may offer at least a partial explanation for the observed higher *in vivo* potencies of α-methyl-substituted compounds in this series.

Introduction

The 2,5-dimethoxy-substituted phenethylamine and phenylisopropylamine derivatives comprise a potent class of serotonergic compounds which possess hallucinogenic effects in humans. Recently, molecules of this type have been used as selective agonists for the serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes.[1] For example, compounds **1**–**3** are potent hallucinogens with high affinity for 5-HT$_2$ sites. Compound **2** (DOB) is slightly more potent than **1**, and in its tritiated form has been used as a radioligand to label the serotonin 5-HT$_2$ receptor.[2] Compound **3** (DOI) is comparable to **2** in potency, and as its ^{125}I-labeled congener has also been used to label the 5-HT$_2$ receptor, with the advantage that it has extremely high specific activity.[3] Additionally, **4** has high affinity for the 5-HT$_2$ receptor and has been used as a radiolabel for this site.[4]



1 X = R = CH_3

2 X = Br, R = CH_3 (DOB)

3 X = I, R = CH_3 (DOI)

4 X = I, R = H

5 X = CF_3, R = CH_3

6 X = CF_3, R = H

These examples illustrate the interesting pharmacological properties of these molecules. A great variety of similar molecules have been synthesized and tested, where the only structural variation has been the nature of the 4-substituent. These include 4-alkoxy, 4-alkyl, 4-alkylthio, 4-halogeno, and even 4-nitro compounds.[5] Absent from this listing to the present time has been the 4-trifluoromethyl analog **5**.

Table 1. Comparison of Physicochemical Properties of Aromatic Substituents

atom/ group	electro-negativity[a]	hydro-phobicity[b]	effective van der Waals radius (Å)[c]	molar refraction[d]
CH_3	2.3[7]	0.51	1.80	5.65
Br	2.8[6]	0.94	1.86	8.88
I	2.5[6]	1.15	1.97	13.94
CF_3	3.5[7]	1.07	2.20	5.02
OCH_3	2.7[7]	0.12	1.52	7.87

[a] Pauling scale.[7,8] [b] As measured by the relative partition coefficients of meta-substituted 3-phenoxyacetic acids between 1-octanol and water.[9] [c] Effective van der Waals radii derived from rotational barriers in 6-aryl-1,1,5-trimethylindans.[10] [d] Molar refraction is an approximate measure of steric bulk commonly utilized in QSAR studies.[11]

The trifluoromethyl group has physicochemical properties that are similar to other moieties that give high activity in this series.[6-11] Table 1 contains physicochemical data for various atoms/groups that have been substituted in the 4-position. The hydrophobicity and effective van der Waals radii of the trifluoromethyl group compare favorably with the para substituents of compounds **2** and **3**, the most potent drugs in this series. The high electronegativity of the trifluoromethyl group, combined with a molar refraction index value close to that of a methyl group, could therefore be expected to confer properties to the molecule similar to those of **2** and **3**. In addition, enhanced resistance to metabolism often results after incorporation of a trifluoromethyl group into a biologically active molecule.[12-14]

* Address all correspondence to: Dr. David E. Nichols, Department of Medicinal Chemistry and Pharmacognosy, Robert Heine Pharmacy Building, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907-1333. (317) 494-1461 (office); (317) 494-6790 (FAX). Internet: drdave@mace.cc.purdue.edu.

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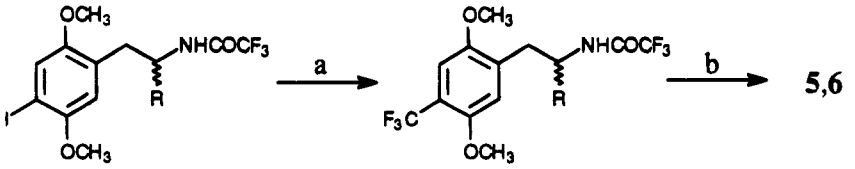
Scheme 1



7 R = CH_3 **8** R = CH_3

9 R = H **10** R = H

[a] (a) $ClCF_2COOCH_3$, CuI, KF, DMF; (b) KOH, iPrOH.

Table 2. K_I Values for Radioligand Competition Experiments[a]

	[^{3}H]ketanserin			[^{125}I]-(±)-**3**			[^{3}H]-8-OHDPAT		
compd	K_I (nM)	Hill	*n*	K_I (nM)	Hill	*n*	K_I (nM)	Hill	*n*
4	80.9 ± 10.4	0.74	4	1.9 ± 0.2	0.76	4	ND[b]	–	
5	33.0 ± 5.0	0.76	4	1.5 ± 0.2	0.89	3	2225 ± 343	1.1	4
6	74.5 ± 10.4	0.69	5	1.1 ± 0.0	0.94	3	3422 ± 516	0.90	3

[a] The K_D for ketanserin was 1.37 ± 0.21 nM, for DOI was 1.88 ± 0.2, and for 8-OH-DPAT was 2.5 ± 0.2 nM. [b] Not determined.

Table 3. Drug Discrimination Data

drug	dose (μmol/kg)	*N*	%*D*	%SDL	ED_{50} (μmol/kg)[a]
2	0.80	7	0	14	1.12
	1.21	5	0	60	(0.86–1.46)
	1.61	10	20	87.5	
3	0.14	14	14	34	0.97
	0.28	17	18	35	(0.60–1.70)
	0.56	18	44	80	
	1.12	18	44	90	
4					1.04
					(0.69–1.57)[b]
5	0.67	8	0	62.5	ND[c]
	1.33	10	50	80	
	2.00	11	72	100	
6	0.44	10	40	0	0.82
	0.63	6	16	20	(0.65–1.04)
	0.88	11	45	83	
	1.00	8	37.5	60	
	1.25	12	17	80	
	1.75	6	16	100	

[a] Number in parentheses is the 95% confidence interval. [b] Taken from ref 24. [c] Not determined; although full substitution occurred, greater than 50% of the animals were disrupted at these doses.

The reason that this molecule has not been previously synthesized lies in the general absence of effective synthetic strategies for introduction of the trifluoromethyl moiety into an appropriate synthon. For several years we have followed the literature and run pilot reactions utilizing a variety of procedures in attempts to prepare this molecule in an efficient manner. Recently, however, Su et al.[15] reported a method to introduce the trifluoromethyl group by replacement of an aromatic halogen under conditions deemed compatible with the functional groups necessary to prepare **5**. Therefore, the present report describes the synthesis and biological evaluation of **5**, as well as its α-desmethyl congener **6**.

Chemistry

The *N*-trifluoroacetamido-2,5-dimethoxy-4-iodophenyl compounds **7**[3] and **9** were ring trifluoromethylated in good yield according to the method of Su et al.[15] using methyl chlorodifluoroacetate in the presence of KF and CuI in DMF (Scheme 1). Nitrogen deprotection was accomplished using alcoholic KOH[3] to afford the phenylaminopropane **5** and phenylaminoethane **6**, which were characterized as their hydrochloride salts. Initial attempts to utilize the 4-bromo analog of **9** resulted only in recovery of starting material. Furthermore, nitrogen protection with an acetyl group in place of the trifluoroacetyl resulted in low yields.

Pharmacology

Compounds **5** and **6** were tested in several biological assays. Their affinity for 5-HT$_{2A/2C}$ receptors was assessed using displacement of [^{125}I]-(±)-**3** (to label the agonist high-affinity state) and [^{3}H]ketanserin (to label the agonist low affinity state) in rat frontal cortex homogenates. Compounds **5** and **6** were also examined for their ability to displace [^{3}H]-8-OH-DPAT from 5-HT$_{1A}$ sites in rat hippocampus. To assess functional efficacy at 5-HT$_2$ receptors, these same two compounds were compared, along with **3**, for their ability to stimulate phosphotidyl inositol turnover in cultured cells expressing 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors. Finally, compounds **5** and **6** were tested using the two-lever drug discrimination paradigm in rats trained to discriminate ip injections of LSD tartrate (0.08 mg/kg; 183 nmol/kg) from saline.

Results

Radioligand binding data are presented in Table 2. Both **5** and **6** showed low affinity for the site labeled by the 5-HT$_{1A}$ agonist 8-OH DPAT. For competition at the site labeled by the 5-HT$_{2A/2C}$ antagonist ketanserin, **6** was not significantly different from **4**, and had approximately two-fold lower affinity than **5**. In previous studies, **4** has been shown to have affinity in this assay comparable to **3**.[4]

Both **4** and **5** bound to sites labeled by the 5-HT$_{2A/2C}$ agonist [^{125}I]-(±)-**3** with affinities that were not significantly different. It is also important to note that **5** is a racemic compound and previous studies have shown that higher affinity resides in the *R* enantiomer within this class of compounds.[18] The α-desmethyl analog **6** had a slight but significantly enhanced affinity for this site compared with **4**.

The results of the drug discrimination assay are presented in Table 3. The ED_{50} value for **6** was 20% lower than that of the iodo analog **4**, although this difference was not statistically significant. The slopes of the dose–response curves for **6** and the training drug LSD did not differ significantly.

Compound **5** fully substituted for LSD in the drug discrimination assay but simultaneously produced dis-

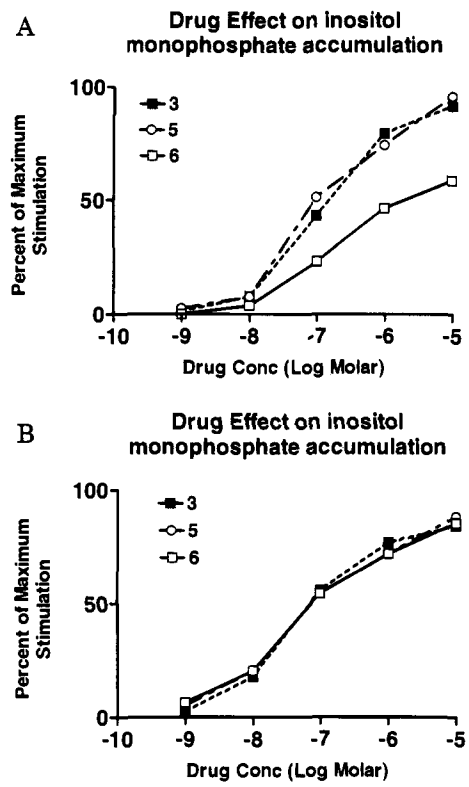


Figure 1. (A) Effects of **3**, **5**, and **6** on the percent maximum accumulation of [^{3}H]inositol monophosphate in 3T3 cells expressing 5-HT$_{2A}$ receptors. The values at each concentration of drug are the percent of the maximum response produced by 5-HT (10^{-5} M) after subtraction of the control (no drug) value. (B) Effects of **3**, **5**, and **6** on the percent maximum accumulation of [^{3}H]inositol monophosphate in A9 cells expressing 5-HT$_{2C}$ receptors. The values at each concentration are the percent of the maximum response produced by 5-HT (10^{-5} M) after subtraction of the control (no drug) value.

ruption rates greater than 50%. According to the stringent testing criterion employed in our laboratory, an ED_{50} value was therefore not calculated. Disruption can occur in this assay any time that a drug produces overt behavioral changes such as hyperlocomotion, stereotypy, elements of the serotonin syndrome, etc. (e.g. amphetamine, clonidine, apomorphine). Compounds with a strong sedative or cataleptogenic effect such as haloperidol or chloropromazine will also cause behavioral disruption. If, however, one considers data only from the nondisrupted animals, it might be hypothesized that **5** is equipotent to **6** in a subpopulation of the rat colony. Nevertheless, **5** must possess additional non-LSD like pharmacological properties that are responsible for the high percentage of disruption.

The effect of **3**, **5**, and **6** on the percent maximum stimulation of [^{3}H]inositol monophosphate accumulation in cells expressing the 5-HT$_{2A}$ receptor is illustrated in Figure 1A. Both **3** and **5** produced equivalent concentration response curves. The maximum responses (V_{max}) were nearly the same as those produced by 5-HT (10^{-5} M) (Table 4). In contrast, the response to **6** was approximately half of that produced by 5-HT or the other two compounds tested.

The effect of **3**, **5**, and **6** on the percent maximum stimulation of [^{3}H]inositol monophosphate accumulation in cells expressing the 5-HT$_{2C}$ receptor is illustrated in Figure 1B. All three compounds were found to be equipotent in this assay. The maximum response for **5** and **6** (and **3**) was nearly the same as that produced by 5-HT (10^{-5} M). Each of these compounds has a V_{max} value of approximately 80% of that for 5-HT, suggesting that these agents are all partial agonists at this receptor (Table 4).

Table 4. Estimates of the V_{max} and K_{aff} for the Percent of Maximum Stimulation of Phosphotidylinositol Turnover Produced by **3**, **5**, and **6** at Cloned 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors

	5-HT$_{2A}$ receptor		5-HT$_{2C}$ receptor	
compd	K_{aff} (nM)	V_{max}	K_{aff} (nM)	V_{max}
3	119 ± 3	94 ± 3	43 ± 6	82 ± 2
5	86 ± 3	91 ± 9	43 ± 15	82 ± 5
6	165 ± 27	58 ± 2	32 ± 12	80 ± 5

[a] Each value is the mean ±SE calculated from triplicate determinations in three separate experiments. The V_{max} and K_{aff} values were calculated using nonlinear regression analysis.

Discussion

The structure–activity relationships for numerous phenylalkylamines have been studied extensively.[19] Generally, addition of a methyl group to the α-carbon of 2,4,5-trisubstituted phenethylamines increases the *in vivo* activity of these compounds.[20] For instance, if R = H in structures **1**, **2**, or **3**, activity in humans is reduced by approximately 1 order of magnitude.[21] In an extreme case, 2,4,5-trimethoxy-β-phenethylamine is inactive in humans,[22] whereas its corresponding α-methylated congener is 17 times more potent than mescaline, with an effective oral dosage in humans of 20–40 mg.[23] Previous studies, based only on affinity measurements from radioligand competition experiments, had failed to reveal an explanation for these differences. For example, in an examination of several phenylalkylamines reported by Glennon et al.,[1] addition of an α-methyl group had little influence on affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in rat brain homogenate. Similarly, Johnson et al.[4] reported that the affinity of **4** and (−)-**3** for the 5-HT$_2$ receptor was comparable (although the affinity of the behaviorally more potent (−)-**3** was greater than (+)-**3**). In the absence of any basis for explaining the *in vivo* activity difference based on receptor affinities, it has been speculated that the α-methyl group might enhance potency by conferring resistance to metabolic side chain deamination and would also generally increase the lipophilicity of the molecule, allowing better CNS penetration.[19]

In the present study, the incorporation of an α-methyl group led to increased accumulation of [^{3}H]inositol monophosphate in cells expressing the 5-HT$_{2A}$ but not 5-HT$_{2C}$ receptors. The difference in efficacy between **5** and **6** now suggests, *for the first time*, a pharmacological mechanism that may contribute to differences in the clinical potencies of hallucinogenic phenethylamines and their α-methyl homologues. That is, the α-methyl compounds may be more active, at least in part, because they possess increased intrinsic activity at the 5-HT$_{2A}$ receptor. These data would also be consistent with the notion that the 5-HT$_{2A}$ receptor may play a more prominent role than the 5-HT$_{2C}$ receptor in mediating the hallucinogenic effects of these types of compounds.

The substitution of the trifluoromethyl group at the 4-position of the 2,5-dimethoxy amines as in **5** and **6** enhances the *in vivo* potency of the compounds relative to the 4-halo-substituted analogs without apparent

alteration of the basic receptor affinity profile for this class of compounds. The 5-HT$_2$ binding sites appear to be tolerant of the highly electronegative yet hydrophobic trifluoromethyl group. This characteristic may suggest that the 4-position substituent interacts with a complementary portion of the receptor through a charge-transfer complex. Although several reviews of fluorine biochemistry suggest the possibility of fluorine atoms functioning as H-bond acceptors in a manner analogous to oxygen atoms,[14] this ability is not shared by the other hydrophobic groups (e.g., iodo) that confer high potency in this series. The enhancements in potency are more evident in the *in vivo* versus the *in vitro* pharmacology, suggesting that increased metabolic stability and changes in pharmacokinetics may contribute to the high potency of **5** and **6**.

Experimental Section

Chemistry. Materials and Methods. Melting points were determined on a Thomas-Hoover Meltemp apparatus and are uncorrected except where indicated. ^{1}H NMR spectra were recorded on a Chemagnetics 200-MHz or 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 in $CDCl_3$, except where noted. Abbreviations used in NMR analysis are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; dd, doublet of doublets; dt, doublet of triplets. Analytical thin-layer chromatography (TLC) was performed on Baker-flex silica gel IB2-F plastic plates with fluoroscent indicator. Microanalyses were obtained from the Purdue Microanalytical Laboratory and from Galbraith Laboratories, Inc. (Knoxville, TN). The chemical ionization mass spectra (CIMS) were determined on a Finnigan 4000 quadrupole spectrometer using ammonia or isobutane as the reagent gas and are reported as *m/e* (relative intensity). Solvents and reagents were used as purchased, except where noted. Chloroform was washed with distilled water, to remove ethanol, and then dried over $MgSO_4$.

1-(2,5-Dimethoxy-4-(trifluoromethyl)phenyl)-2-(trifluoroacetamido)propane (8). In an oven-dried, 50 mL, 2-neck flask fitted with a magnetic stirrer and reflux condenser and blanketed with N_2 were placed purified CuI (2.09 g, 11 mmol), dry KF (0.64 g, 11 mmol), and 1-(2,5-dimethoxy-4-iodophenyl)-2-(trifluoroacetamido)propane (**7**)[3] (3.0 g, 7.2 mmol). Anhydrous DMF (15 mL) was added via syringe, and the reaction flask was heated in a 120 °C oil bath. Methyl chlorodifluoroacetate (2.17 g, 15 mmol) was added via syringe pump over 3 h. (Note: The success of this reaction depends on slow addition so that concentrations of the reagent remain low.) After addition was complete, the mixture was stirred at 120 °C for 4 h. The reaction mixture was poured with stirring into 250 mL of H_2O. The fluffy tan precipitate that formed was collected by vacuum filtration. The solid filtercake was suspended in 75 mL of $CHCl_3$ and vacuum filtered through Celite to remove Cu salts. The organic solution was then washed with 2 N HCl (2 × 50 mL), H_2O (2 × 50 mL), and brine (75 mL) and was dried over $MgSO_4$. Filtration and concentration by rotary evaporation gave a tan solid that was recrystallized from 2-propanol/ethyl acetate to afford 1.95 g (75.4%) of an off-white crystalline solid: mp 157–158 °C; CIMS, *m/e* 360 (M + 1); ^{1}H NMR ($CDCl_3$) δ 7.08 (s, 1, ArH), 6.80 (s, 1, ArH), 6.20 (bs, 1, NH), 4.20 (m, 1, $ArCH_2CH$), 3.86, 3.82 (2s, 6, OCH_3), 2.92 (dd, 1, $ArCH_2$), 2.87 (dd, 1, $ArCH_2$), 1.29 (d, 3, CH_3). Anal. ($C_{14}H_{15}F_6NO_3$) C, H, N.

1-(2,5-Dimethoxy-4-iodophenyl)-2-(trifluoroacetamido)ethane (9). Following a procedure similar to that described for the preparation of **7**,[3] 1-(2,5-dimethoxy-4-iodophenyl)-2-aminoethane[4] (1.34 g, 4.3 mmol) was dissolved in 20 mL of toluene in a 100 mL 2-neck flask equipped with magnetic stirring, N_2, and an addition funnel. The solution was chilled in an ice water bath to 15 °C, and then trifluoroacetic anhydride (15 mL, 0.106 m) was added dropwise. The two-phase mixture was vigorously stirred at room temperature for 2.5 h. TLC analysis showed that the reaction had gone to completion. Volatile materials were removed by rotary evaporation followed by drying under high vacuum overnight. The essentially pure fluffy off-white crystals weighing 1.7 g were recrystallized from 2-propanol/ethyl acetate to yield 1.6 g (92.1%): mp 137 °C. CIMS *m/e* 405 (M + 1); ^{1}H NMR ($CDCl_3$) δ 7.11 (bs, 1, NH), 7.09 (s, 1, ArH), 6.80 (s, l, ArH), 3.89 (2s, 6, OCH_3), 3.84 (m, 2, $ArCH_2CH_2$), 2.91 (m, 2, $ArCH_2$). Anal. $C_{12}H_{13}F_3INO_3$ C, H, N.

1-(2,5-Dimethoxy-4-(trifluoromethyl)phenyl)-2-(trifluoroacetamido)ethane (10). Using a procedure similar to that for synthesis of **8** 1-(2,5-dimethoxy-4-iodophenyl)-2-(trifluoroacetamido)ethane **9** (2.0 g, 5 mmol), KF (0.49 g, 8.5 mmol), and CuI (1.43 g, 7.5 mmol) were combined in 15 mL of DMF and heated to 120 °C. Methyl chlorodifluoroacetate (2.17 g, 15 mmol) was added over 3 h, and heating was continued for 8 h. Workup as described for **8** gave a yellow solid which, after recrystallization from 2-propanol/ethyl acetate, produced off-white crystals: 1.23 g (71.5%); mp 152–153 °C; CIMS *m/e* 346 (M + 1); ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 1, ArH), 6.91 (s, 1, ArH), 6.10 (bs, 1, NH), 3.91, 3.83 (2s, 6, OCH_3), 3.77 (m, 2, $ArCH_2CH_2$), 3.06 (m, 2, $ArCH_2$). Anal. ($C_{13}H_{13}F_6NO_3$) C, H, N.

1-(2,5-Dimethoxy-4-(trifluoromethyl)phenyl)-2-aminopropane Hydrochloride (5·HCl). To a solution of **8** (1.0 g, 2.9 mmol) in 75 mL of 2-propanol was added 5 mL of 2 N KOH. The solution was stirred at room temperature for 3 h. The solvent was removed by rotary evaporation, and the residue was dissolved in Et_2O (75 mL). The solution was washed with H_2O (25 mL), followed by extraction with 3 N HCl (2 × 50 mL). The pooled acidic extracts were combined and made strongly basic with the addition of 5 N NaOH. The aqueous solution was extracted with $CHCl_3$ (3 × 50 mL), the pooled extracts were dried ($MgSO_4$) and filtered, and the $CHCl_3$ was removed by rotary evaporation. The residual oil was dissolved in EtOH and acidified with 1 N ethanolic HCl. The solvent was removed, and the white crystalline residue was recrystallized from EtOH/ethyl acetate to yield 0.7 g (84%) of a white crystalline solid: mp 210 °C; CIMS *m/e* 264 (M + 1); ^{1}H NMR (D_2O) δ 7.08 (s, 1, ArH), 6.92 (s, 1, ArH), 3.70, 3.65 (2s, 6, OCH_3), 3.50 (m, 1, $ArCH_2CH$), 2.80 (d, 2, $ArCH_2$), 1.11 (d, 3, CH_3). Anal. ($C_{12}H_{17}ClF_3NO_2$) C, H, N.

1-(2,5-Dimethoxy-4-(trifluoromethyl)phenyl)-2-aminoethane hydrochloride (6·HCl). In a procedure similar to the synthesis of **5**·HCl, **10** (3.0 g, 8.6 mmol) was dissolved in 225 mL of 2-propanol, and 15 mL of 2 N KOH was added. The solution was stirred at room temperature for 3 h. Workup as before yielded a waxy solid which was dissolved in EtOH and acidified with 1 N ethanolic HCl. The solvent was removed by rotary evaporation, and the white crystalline residue was recrystallized from EtOH/ethyl acetate to yield 2.26 g (91.0%) of white crystalline solid: mp 260 °C; CIMS *m/e* 250 (M + 1); ^{1}H NMR (D_2O) δ 7.07 (s, 1, ArH), 6.94 (s, 1, ArH), 3.69, 3.67 (2s, 6, OCH_3), 3.07 (m, 2, CH_2), 2.85 (m, 2, $ArCH_2$). Anal. ($C_{11}H_{15}ClF_3NO_2$) C, H, N.

Pharmacology Methods. Drug Discrimination. Twenty male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200–220 g at the beginning of the drug discrimination study were used as subjects trained to discriminate LSD tartrate 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 hours. The laboratory and animal facility temperature was 22–24 °C, and the relative humidity was 40–50%. Experiments were performed between 0830 and 1700 hours each day, Monday–Friday.

Six standard operant chambers (model E10-10RF, Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a food hopper (combination dipper pellet trough, model E14-

06, module size 1/2), all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, interfaced through a Med. Associates interface to a 486-based microcomputer, controlled reinforcement and data acquisition with a locally-written program.

A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. The drug discrimination procedure details have been described elsewhere.[25,26] After habituation to the experimental conditions (1 week after isolation in the individual home-cages and at the beginning of the food deprivation), the rat's initial shaping was started. During the first 2–3 sessions, rats were trained only to associate a characteristic noise (click) after lever pressing with a delivered food pellet (without drug injections). Initially, rats were shaped to lever press on an FR1 schedule so that one food pellet was dispensed for each press. Half of the rats were trained on drug-L (left), saline-R (right) and the other half on drug-R, saline-L to avoid positional preference. Training sessions lasted 15 min and were conducted at the same time each day. Levers were cleaned between animals with 10% ethanol solution to avoid olfactory cues. Only one appropriate lever was present during the first 10 sessions of initial learning (after beginning to administer saline or training drug, ip 30 min before sessions). Afterward both levers were present during all following phases of training, but reinforcements were delivered only after responses on the appropriate lever. Presses on the incorrect lever had no programmed consequences. As responding rates stabilized (during the next 15 sessions), the schedule of reinforcement was gradually increased to an FR50. Once at the FR50, training continued until an accuracy of at least 85% [(number of correct presses × 100)/number of total presses] was attained for 8 of 10 consecutive sessions (ca. 40–60 sessions). Once criterion performance was attained, 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 drugs were administered ip 30 min prior to the sessions; 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 drug was (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg, 186 nmol/kg, NIDA). 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" is defined as 59% SDL or less, and "partial" substitution is 60–79% SDL. If the drug was one which completely substituted for the training drug (at least one dose resulted in a %SDL = 80% or higher) the method of Litchfield and Wilcoxon[27] 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 where the nondisrupted rats gave 80% SDL, no ED_{50} was calculated.

Radioligand Binding Assays. Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 175–199 g were used. The animals were kept in groups of five rats per cage, at the same conditions described above, but with free access to food and water.

[^{125}I]-(±)-DOI, [^{3}H]ketanserin, and [^{3}H]-8-OH-DPAT were purchased from New England Nuclear (Boston, MA) at specific activities of 2200, 61, and 135.5–216 Ci/mmol, respectively. (+)-LSD tartrate was obtained from the National Institute on Drug Abuse. Cinanserin was a gift from the SQUIBB Institute for Medical Research and 5-HT was purchased from Sigma (St. Louis, MO).

The procedure of Johnson et al.[4] was employed. Briefly, the frontal cortex or hippocampal brain regions from 20–40 rats were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in 4 or 8 volumes of 0.32 M sucrose for frontal cortex or hippocampus, respectively. The homogenates were centrifuged at 36000*g* for 10 min, and the resulting pellets were 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 to 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). In experiments with [^{125}]DOI and [^{3}H]ketanserin, either 10 mM $MgCl_2$ or 5.7 mM $CaCl_2$ were included, respectively. 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 the appropriate 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 as that displaceable with 10 μM cinanserin in both the [^{3}H]ketanserin and [^{125}I]DOI binding study and with 10 μM 5-HT in the [^{3}H]-8-OH-DPAT binding study. Filters were air-dried and 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 and directly counted in a γ counter for [^{125}I]-labeled ligand at an efficiency of 79.4%.

[^{125}I]DOI saturation experiments were carried out by varying the concentration of unlabeled ligand with a constant amount (0.1 nM) of radioligand, according to the procedure described previously.[18,28,29] This method is accurate when the unlabeled and labeled ligands have identical physiochemical properties.[30] Under the above experimental conditions, [^{125}I]-DOI was found to bind to a single site (Hill coefficient of 0.91 ± 0.03) with a B_{max} of 44.0 ± 5.7 fmol/mg protein and a K_D of 1.88 ± 0.24 nM. The ability of 8–10 concentrations of test drug to compete for 0.1 nM [^{125}I]DOI binding was measured in drug competition studies. Four to five concentrations of radioligand were used in both [^{3}H]ketanserin and [^{3}H]-8-OH-DPAT saturation experiments. [^{3}H]Ketanserin bound to a single site (Hill coefficient 1.08 ± 0.06) with B_{max} of 297.3 ± 39.6 fmol/mg protein and a K_D of 1.37 ± 0.20 nM. [^{3}H]-8-OH-DPAT bound to a single site (Hill coefficient 1.00 ± 0.01) with a B_{max} of 119 ± 8 fmol/mg protein and a K_D of 2.49 ± 0.23 nM. The ability of 8–10 concentrations of test drug to compete for 0.75 nM [^{3}H]ketanserin or [^{3}H]-8-OH-DPAT binding was determined in drug competition studies.

Phosphoinositide Hydrolysis. Studies were conducted in A9 cells expressing the cloned 5-HT_{2C} receptor and in 3T3 cells expressing the cloned 5-HT_{2A} receptor and have been described in detail elsewhere.[31–33] For each experiment the cells were harvested by trypsinization and split into 24- or 48-well plates with complete medium. The cells were washed with inositol-free DMEM 24 h later and then incubated for an additional 18 h with inositol-free DMEM containing 1 μCi/mL [^{3}H]inositol and 10% dialyzed fetal calf serum. After this incubation, the cells were rinsed three times with a Krebs bicarbonate buffer composed of (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 25; and glucose, 11. The buffer was equilibrated with 95%O_2/5% CO_2, prior to use. The cells were incubated for 60 min with the test compounds together with 10 mM LiCl in Krebs bicarbonate buffer. The reaction was terminated by the addition of 1.2 mL of methanol/water/HCl (25:25:0.1). The cells were harvested into glass tubes, and 0.6 mL of chloroform was added, followed by

vigorous vortexing. After phase separation, an aliquot (0.9 mL) of the upper aqueous phase was removed and added to 1 mL of Dowex AG 1 × 8 (formate form) resin packed in a disposable column. The [^{3}H]inositol monophosphate was eluted as described elsewhere.[34]

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