1-Aminomethylbenzocycloalkanes: Conformationally Restricted Hallucinogenic Phenethylamine Analogues as Functionally Selective 5-HT$_{2A}$ Receptor Agonists[1]

Thomas H. McLean, Jason C. Parrish, Michael R. Braden, Danuta Marona-Lewicka, Alejandra Gallardo-Godoy, and David E. Nichols*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana 47907-1333

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A series of conformationally restricted analogues of the hallucinogenic phenethylamine **1** (2,5-dimethoxy-4-bromophenethylamine, 2C-B) was synthesized to test several hypotheses concerning the bioactive conformation of phenethylamine ligands upon binding to the 5-HT$_{2A}$ receptor. These benzocycloalkane analogues were assayed for their receptor binding affinity and ability to activate downstream signaling pathways, and one exceptional compound was selected for testing in an in vivo drug discrimination model of hallucinogenesis. All compounds were examined in silico by virtual docking into a homology model of the 5-HT$_{2A}$ receptor. On the basis of these docking experiments, it was predicted that the *R* enantiomer of benzocyclobutene analogue **2** would be the most potent. Subsequent chemical resolution and X-ray crystallography confirmed this prediction, as (*R*)-**2** proved to be equipotent to LSD in rats trained to discriminate LSD from saline. Thus, we propose that the conformation of **2** mimics the active binding conformation of the more flexible phenethylamine type hallucinogens. In addition, (*R*)-**2** is one of the most potent and selective compounds yet discovered in the in vivo drug discrimination assay. Further, **2** was found to be a functionally selective agonist at the 5-HT$_{2A}$ receptor, having 65-fold greater potency in stimulating phosphoinositide turnover than in producing arachidonic acid release. If hallucinogenic effects are correlated with arachidonic acid production, such functionally selective 5-HT$_{2A}$ receptor agonists may lack the intoxicating properties of hallucinogens such as LSD.

Introduction

The phenethylamine pharmacophore is one of the most "privileged" substructures found in biologically active molecules. It has been extensively modified, resulting in potent ligands for a wide range of biological targets. Of particular interest to our ongoing research focus on the 5-HT$_2$ receptor are the hallucinogenic phenethylamines,[2] chemically related to the naturally occurring cactus alkaloid, mescaline. Extensive modifications to the core aromatic ring have resulted in compounds with greatly increased affinity and potency.[3,4] Nevertheless, despite long-standing efforts in our laboratory to map the active side chain conformation,[5−9] those attempts have failed to provide conclusive evidence for the bioactive conformation of the aminoalkyl side chain.

Many of our earlier attempts to produce a rigid analogue that would mimic the bioactive conformation of phenethylamines have been based on the idea that the side chain might adopt a conformation where it was coplanar with the aromatic ring, as exemplified in the ergolines (e.g., see Monte et al.[8]). Yet extremely potent analogues have been synthesized where the aromatic methoxy groups of the phenethylamines are constrained into a conformation coplanar with the aromatic ring.[4,10] These benzodifuran analogues, through peri interactions between the additional methylene groups and the side chain, show an energetic penalty for the side chain to adopt an in-plane orientation. Thus, it seems more reasonable to assume that the side chain might lie in a plane perpendicular to the aromatic ring plane. Consistent with that reasoning, NMR spectroscopic data have shown that the aqueous solvated conformation of amphetamine is antiperiplanar.[11] This finding strengthens the argument for an out-of-plane orientation but is somewhat confounded by nonbonded steric interactions provided by the methoxy groups.

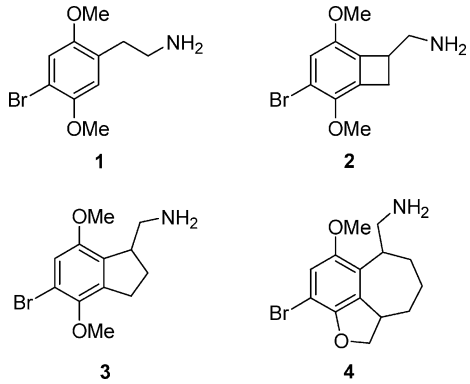

Figure 1. Flexible parent ("2C-B") **1** and analogues studied in this work.

Serotonergic hallucinogens are believed to act as agonists at the serotonin 5-HT$_{2A}$ receptor.[12] Therefore, our SAR efforts have largely focused on the interaction of phenethylamines with this receptor. Recently, we used a homology model of the 5-HT$_{2A}$ receptor to design a conformationally constrained 1-aminomethylindan analogue of mescaline.[13] In that work, the rigid analogue placed the ethylamine side chain into an out-of-plane conformation. The methoxy groups in mescaline, however, are not adjacent to the side chain, and issues of nonbonded interactions between the side chain and the methoxy groups are not relevant.

The current series of analogues **2**−**4** (Figure 1) was designed to explore the bioactive conformation of the side chain in 2,5-dimethoxy-substituted phenethylamines, using benzocycloalkane

* To whom correspondence should be addressed. Phone: (765) 494-1461. Fax: (765) 494-1414. E-mail: drdave@pharmacy.purdue.edu.

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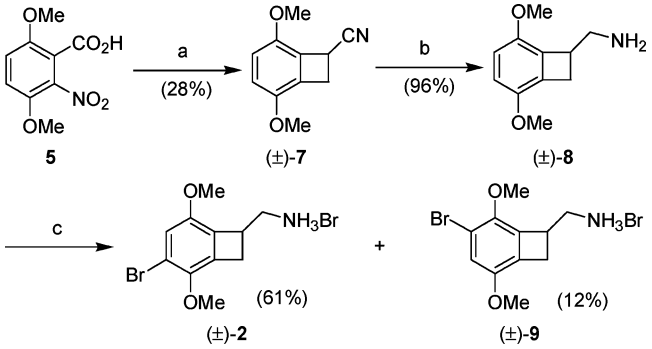
Scheme 1. Racemic Synthesis of Benzocyclobutene Analogue **2**[a]



[a] Reagents: (a) (i) H_2, Pd/C, EtOH; (ii) AmONO, HCl, EtOH; (iii) acrylonitrile, propylene oxide, DCE; (b) BH_3·THF; (c) Br_2, glacial AcOH.

templates of different sizes. Although **4** incorporates a dihydrofuran ring to replace one of the methoxy groups, this modification is essential to retain high activity when a nonbonded interaction with the tethering ring moiety might perturb the essential in-plane conformation of an adjacent methoxy group. This effect is minimized when the cycloalkane ring is less than six carbons, as in **2** and **3**, and we have previously reported on the six carbon cycloalkane analogue of **4**.[6] These analogues also incorporate a chiral center at the benzylic position. We reasoned that knowledge of the absolute configuration of the more active isomer in this series would be essential to our proposed virtual docking studies using a homology model of the human 5-HT_{2A} receptor.[14] Thus, the most potent of the analogues was resolved into its enantiomers, which were assayed individually and compared to the racemate.

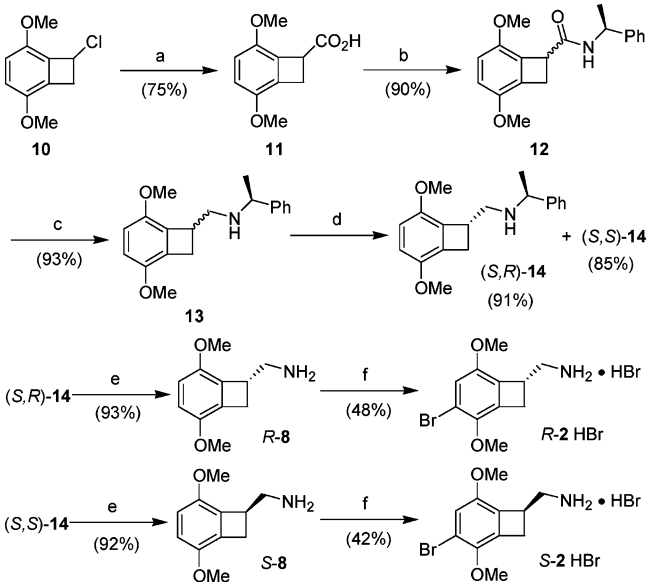
An additional goal of this work was to determine whether conformational restriction of a flexible 5-HT_{2A} agonist might lead to some degree of functional selectivity.[15−17] This idea is based on evidence that different classes of ligands (agonists, antagonists, inverse agonists, etc.) cause spectroscopically discernable differences in receptor morphology.[18] If a flexible ligand activates multiple downstream pathways, it seems possible that through conformational restriction, some of the ligand−receptor microstates[19] might be rendered inaccessible. Thus, conformational restriction might be a strategy to activate a specific second messenger pathway, to the exclusion of others that also are potentially coupled to that receptor.

Chemistry

All three ligands under investigation were synthesized in racemic form as shown in Schemes 1−4. The synthesis of racemic benzocyclobutene analogue **2** (Scheme 1) began with 3,6-dimethoxy-2-nitrobenzoic acid **5**, prepared by the procedure of Azadi-Ardakani and Wallace.[20] Catalytic hydrogenation of the nitro group, followed by diazotization of the resulting anthranilic acid **6**, provided 3,6-dimethoxybenzenediazonium 2-carboxylate. Thermal decomposition to the dimethoxybenzyne and subsequent 2 + 2 cycloaddition with acrylonitrile provided the key 3,6-dimethoxybenzocyclobutanecarbonitrile **7**. The nitrile was reduced to the 1-aminomethyl compound by hydrogenation over Raney nickel. Bromination of the free base of **8** using Br_2 in acetic acid afforded (±)-**2** as a 5:1 mixture with its undesired 3-bromo regioisomer **9**.

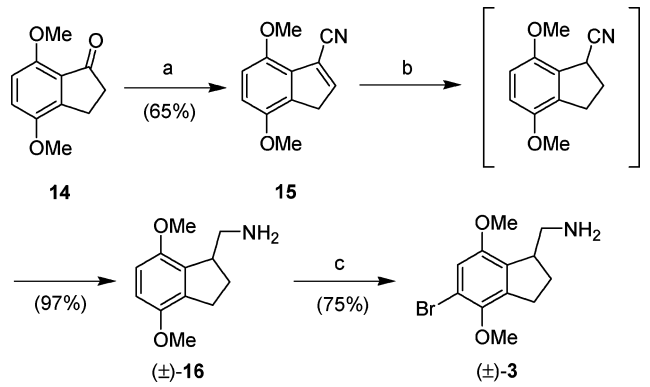
Attempts to resolve the enantiomers of **2** by fractional crystallization of various diastereomeric salts or by chromatographic separation proved to be ineffective, so an earlier intermediate was resolved instead. The Grignard reagent formed from 1-chloro-3,6-dimethoxybenzocyclobutene (±)-**10**[20] was

Scheme 2. Synthesis of the Enantiomers of **2**[a]



[a] Reagents: (a) (i) Mg, THF; (ii) CO_2, THF; (b) (i) oxalyl chloride, EtOAc, DMF; (ii) (*S*)-1-phenethylamine, 1 N NaOH, EtOAc; (c) BH_3·THF; (d) radial chromatography, silica/gypsum, EtOAc/hexanes (2:3); (e) H_2, $Pd(OH)_2$/C, MeOH, H_2O; (f) Br_2, glacial AcOH.

Scheme 3. Synthesis of Indan Analogue (±)-**3**[a]



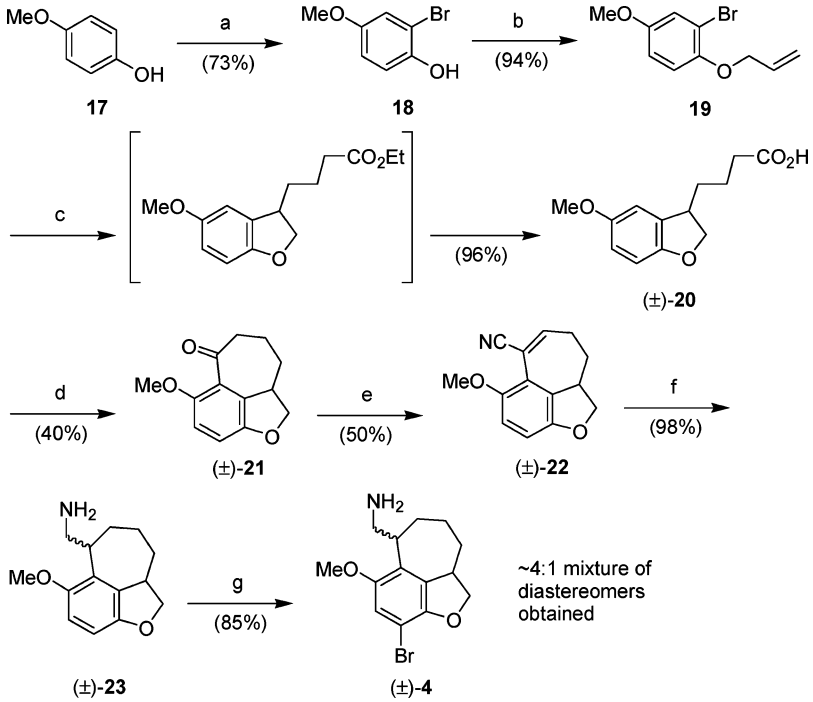
[a] Reagents: (a) (i) TMSCN, ZnI_2, CH_2Cl_2; (ii) Amberlyst-15, benzene; (b) (i) H_2, Pd/C, EtOH; (ii) H_2, Raney nickel, NH_3, EtOH; (c) Br_2, AcOH.

quenched with CO_2 to provide the benzocyclobutene-1-carboxylic acid (±)-**11** (Scheme 2). Condensation of the racemic acid with (*S*)-1-phenethylamine gave a mixture of diastereomeric amides (±)-**12** that was reduced by borane in THF to afford the corresponding diastereomeric *N*-1-phenethylamines (*S*,*R*) and (*S*,*S*)-**13**. These amines were separable by radial chromatography (Chromatotron), and catalytic debenzylation of each secondary amine provided the enantiopure primary amines (*R*)-**8** and (*S*)-**8**. Bromination, as before, gave a 5:1 mixture of each desired 4-bromo compound **2** and its undesired 3-bromo regioisomer.

The stereochemistry of the final compounds was determined by HPLC separation of the diastereomeric amides (*R*,*S*)- and (*S*,*S*)-**12** and X-ray crystallography of the (*S*,*S*) amide. Borane reduction of each amide diastereomer and comparison of those products to the diastereomeric amines resolved by radial chromatography allowed the unambiguous assignment of the absolute configuration of the amines.

The synthesis of (±)-**3** began with formation of 4,7-dimethoxyindene-1-carbonitrile **15** from 4,7-dimethoxy-1-indanone **14**[21] by treatment with trimethylsilylcyanide and elimination of the trimethylsilyloxyl group catalyzed by Amberlyst-15 acidic resin (Scheme 3). The olefin and nitrile were reduced

Scheme 4. Synthesis of Tricyclic Analogue (±)-**4**[a]



[a] Reagents: (a) Br_2, MeOH; (b) allyl bromide, K_2CO_3, acetone; (c) (i) ethyl acrylate, AIBN, Bu_3SnH, benzene; (ii) KOH, H_2O, MeOH; (d) TFAA, TFA; (e) (i) TMSCN, ZnI_2, CH_2Cl_2; (ii) Amberlyst-15, toluene; (f) H_2, Raney nickel, NH_3, EtOH; (g) Br_2, MeOH.

in a two-step hydrogenation sequence, first catalyzed by Pd/C to reduce the olefin and then by Raney nickel to reduce the nitrile to the 1-aminomethylindan (±)-**16**. Bromination using Br_2 in acetic acid gave the final product (±)-**3** as the sole regioisomer.

Tricyclic benzofuran compound (±)-**4** was synthesized in a seven-step sequence starting from 4-methoxyphenol **17**, as shown in Scheme 4. Ortho bromination with Br_2 followed by O-alkylation of **18** with allyl bromide afforded 3-bromo-4-allyloxyanisole **19**.[22,23] Radical-promoted cyclization and in situ alkylation using ethyl acrylate followed a published methodology.[24,25] Alkaline hydrolysis of the ester provided excellent yields of the benzofuranylbutanoic acid (±)-**20**. This material was reliably converted in 40% yield to tricyclic ketone (±)-**21** by treatment with a mixture of trifluoroacetic acid and trifluoroacetic anhydride; other cyclization methods rarely gave yields greater than 20%. Ketone (±)-**21** was treated with trimethylsilylcyanide and catalytic zinc iodide to form the intermediate trimethylsilylcyanohydrin, which was then dehydrated using Amberlyst-15 acidic resin to provide unsaturated nitrile (±)-**22**. One-step catalytic hydrogenation of both the olefin and nitrile groups over Raney nickel gave a 4:1 mixture of diastereomeric amines (±)-*syn*-**23** and (±)-*anti*-**23**. The mixture of brominated diastereomers could not be completely separated by radial chromatography, and NMR analysis of the minor component using NOESY and COSY showed it to be the anti diastereomer of (±)-**4**.

Results

Each of the compounds was assessed in a competition binding assay for affinity at both the rat and human 5-HT_{2A} receptor using displacement of the agonist radioligand (±)-[^{125}I]DOI. The results are summarized in Table 1.

Compound **3** and both isomers of **4** showed decreased affinity relative to **1**. Compound **2**, as the racemate, has affinity equal to **1**, and the *R* isomer has approximately twice the affinity.

Table 1. Radioligand Competition Binding Data at Cloned Rat and Human 5-HT_{2A} Receptors

	$K_i \pm$ SEM (nM)	
compd	rat 5-HT_{2A}	human 5-HT_{2A}
(±)-**2**	0.73 ± 0.12	0.75 ± 0.09
(*R*)-**2**	0.35 ± 0.01	0.26 ± 0.01
(*S*)-**2**	15 ± 2.7	42 ± 0.6
(±)-**3**	53 ± 2.9	47 ± 6.5
(±)-anti-**4**	200 ± 16	170 ± 11
(±)-syn-**4**	170 ± 26	74 ± 4.7
1 ("2C−B")	0.66 ± 0.12	0.88 ± 0.04

Table 2. Functional Data in NIH3T3 Cells Stably Expressing the Rat 5-HT_{2A} Receptor

	IP_3 accumulation		AA release	2-AG production[b]
compd	$EC_{50} \pm$ SEM (nM)	IA[a] ± SEM (%)	$EC_{50} \pm$ SEM (nM)	$EC_{50} \pm$ SEM (nM)
(±)-**2**	36 ± 3.6	94 ± 6.8	nd[c]	nd[c]
(*R*)-**2**	18 ± 2.8	97 ± 2.3	1180 ± 180	1120 ± 210
(*S*)-**2**	460 ± 53	89 ± 1.3	>10000	>10000
1 ("2C-B")	27 ± 3.1	82 ± 1.2	nd[c]	nd[c]

[a] IA = intrinsic activity, the percent maximal stimulation relative to 10 μM 5-HT. [b] Parrish et al.[26] [c] nd = not determined.

The *S* isomer shows about a 22-fold lower affinity than **2**, leading to a eudismic ratio of 43:1.

In vitro functional assays were performed with compound **2** to measure the ability of this ligand to activate downstream signaling pathways. The racemate and both of the enantiomers were first examined for their ability to stimulate phospholipase C mediated production of inositol phosphates (IP_3). Each enantiomer was then assessed for the ability to stimulate arachidonic acid (AA) release and 2-arachidonylglycerol (2-AG) production. The results from these assays are summarized in Table 2.

In addition to the in vitro assays, the enantiomers of **2** were tested in a two-lever drug discrimination procedure in rats. This

Table 3. In Vivo Two-Lever Drug Discrimination Assay in Male Sprague-Dawley Rats

	LSD-trained rats		DOI-trained rats	
compd	ED_{50} (nmol/kg)	95% CI[a] (nmol/kg)	ED_{50} (nmol/kg)	95% CI[a] (nmol/kg)
LSD[b]	38	22−57	15	5−44
DOI[b]	270	160−470	320	230−440
(*R*)-**2**	24	14−39	24	11−50
(*S*)-**2**	ns[c]	ns[c]	ns[c]	ns[c]

[a] 95% confidence interval. [b] Data from Parker et al.[4] [c] No substitution at 250 nmol/kg.

method tests whether a training drug cue substitutes for the test drug. In this assay rats are trained to discriminate saline from either LSD or the phenethylamine hallucinogen DOI. The results of these assays are shown in Table 3.

Results of the drug discrimination assay indicate that (*R*)-**2** is equipotent to both LSD and the heretofore most potent phenethylamine hallucinogen ever reported.[4] Corroborating results obtained from the in vitro assays, (*R*)-**2** is 11 times more potent than the unconstrained phenethylamine DOI in LSD-trained rats and 13 times more potent than DOI in DOI-trained rats, indicating that conformational restriction is capable of inducing dramatic changes in agonist potency. (*R*)-**2** is also significantly more potent than (*S*)-**2**, the latter failing to produce responding in more than 20% of the animals tested at a dose of 250 nmol/kg.

All of the compounds were docked using GOLD 2.2[27] into an in silico activated homology model of the 5-HT_{2A} receptor based on the crystal structure of bovine rhodopsin.[14] Notable observations from these virtual assays include the correlation of actual pharmacological potency with the docking results obtained for these analogues. The top 10 docked orientations returned for (*R*)-**2** were essentially identical, and less potent compounds returned a wider variety of orientations, including some that were inconsistent with prior knowledge of binding orientation obtained from mutagenesis studies.[28−30] In accordance with data from previous benzodifuranphenethylamine analogues,[10] the current docking studies suggest that the aromatic methoxy groups assume a coplanar orientation in the most potent analogue (*R*)-**2**, whereas in (*S*)-**2**, in order to adopt a reasonable pose, the methoxy groups appear to be forced out-of-plane. A stereoview of the enantiomers of **2** docked into the putative 5-HT_{2A} binding site is shown in Figure 2.

Discussion

The results clearly show that, of the three series examined here, **2** provides the highest affinity ligand for the human 5-HT_{2A} receptor and is essentially a full agonist in stimulating IP accumulation. These results also can be compared with six-membered ring homologues, which had much higher affinity than **3** or **4** but which were only weak partial agonists in stimulating PI turnover.[6] Further, the six-membered ring homologue that was the most potent, in vitro, failed to produce substitution in the drug discrimination test in rats trained to discriminate LSD. Thus, **2** represents a conformationally constrained analogue of the phenethylamine type 5-HT_{2A} agonists that possesses exceptional in vitro and in vivo potency in assays predictive of hallucinogenic activity in man.

Therefore, we conclude that when phenethylamine type agonists bind to the 5-HT_{2A} receptor, specifically in the complex that leads to IP accumulation, they likely adopt a conformation where the side chain lies in a plane approximately perpendicular to the aromatic ring plane, as inferred from Figure 2. These results further reinforce our conclusion that there is no structural relationship between the phenethylamine hallucinogens and the ergolines.[6] These ideas are driving our further efforts to understand the structure and function of the 5-HT_{2A} receptor.

We emphasize the fact that **2** is a functionally selective agonist at the 5-HT_{2A} receptor, having about 65-fold selectivity for the activation of the PLC signaling pathway over arachidonic acid release or 2-arachidonylglycerol (2-AG) production (Table 2). Although it is generally assumed that all 5-HT_{2A} agonists will possess hallucinogenic properties, a belief that has led to their neglect by the pharmaceutical industry, we challenge that assumption. We have previously shown that hallucinogenic activity is better correlated with production of arachidonic acid than with activation of PLC.[16] If hallucinogenic properties are in fact associated with the production of arachidonic acid, or other eicosanoids, and not phosphoinositide turnover, it seems entirely possible that functionally selective 5-HT_{2A} agonists such as **2** might represent new therapies. In particular, on the basis of the high expression of 5-HT_{2A} receptors on cortical pyramidal cells, where agonists at this receptor depolarize the cell membrane (see review by Nichols[12]), one could speculate that functionally selective 5-HT_{2A} agonists might be useful in treating cognitive and memory deficits.

Thus, the present results are important not only because they identify the binding orientation of agonists at this receptor but because they also potentially provide a lead compound for discovery of novel therapies for various types of cognitive dysfunction. Finally, these results also provide a compelling case for conformational restriction as a powerful tool for the design of functionally selective analogues from nonselective flexible agonists.

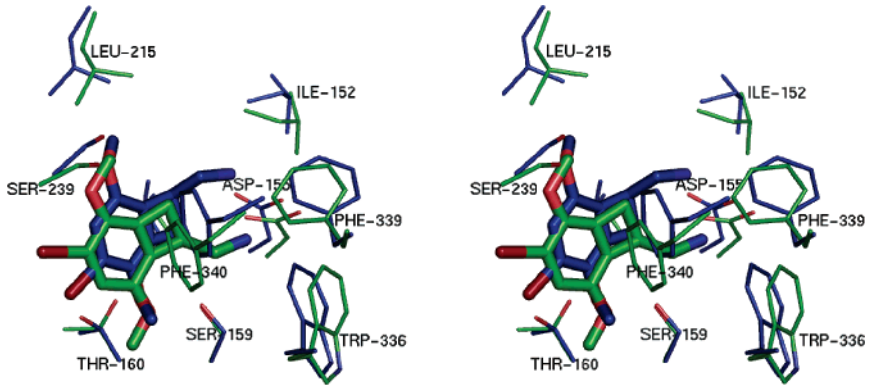


Figure 2. Cross-eyed stereo-overlay of (*R*)-**2** (green) and (*S*)-**2** (blue) docked into the putative 5-HT_{2A} binding domain, illustrating differences in binding orientation. The view is within the membrane, with transmembrane helix 6 toward the front, the transmembrane helices oriented approximately vertically, and the extracellular face of the receptor toward the top of the figure.

Experimental Section

General. All reagents were commercially available and were used without further purification unless otherwise indicated. Dry THF and diethyl ether were obtained by distillation from benzophenone sodium under nitrogen immediately before use. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded using either a 500 MHz Bruker DRX-500 or a 300 MHz Bruker ARX-300 NMR spectrometer. Chemical shifts are reported in δ values (ppm) relative to an internal reference (0.03%, v/v) of tetramethylsilane (TMS) in $CDCl_3$ except where noted. Abbreviations used to report NMR peaks are as follows: bs = broad singlet, d = doublet, dd = doublet of doublets, dq = doublet of quartets, dt = doublet of triplets, m = multiplet, q = quartet, s = singlet, t = triplet, td = triplet of doublets. Chemical ionization mass spectra (CIMS), using isobutane as the carrier gas, were obtained with a Finnigan 4000 spectrometer. Elemental analyses were performed by the Purdue University Microanalysis Laboratory, and the results are within ±0.4% of the calculated values. Thin-layer chromatography was performed using J. T. Baker flex silica gel IB2-F, plastic-backed sheets with fluorescent indicator, visualizing with UV light at 254 nm and eluting with 4:1 hexanes/ethyl acetate unless otherwise noted. Column chromatography was carried out using silica gel 60, 230−400 mesh (J. T. Baker). All reactions were carried out under an inert atmosphere of argon unless otherwise indicated.

1-Cyano-3,6-dimethoxybenzocyclobutene ((±)-7). A solution of 3,6-dimethoxy-2-nitrobenzoic acid **5**[20] (1.0 g, 4.4 mmol) in 200 mL of absolute EtOH was placed into a hydrogenation flask containing 0.5 g of 5% Pd/C and shaken under 20 psi of H_2 for 30 min. The catalyst was removed by filtration through diatomaceous earth, and the volume of filtrate was reduced to about 20 mL by rotary evaporation. The solution of crude 3,6-dimethoxyanthranilic acid was cooled to 0 °C in an ice bath, and 0.5 mL of concentrated HCl was added dropwise with magnetic stirring, followed by the dropwise addition of isoamyl nitrite (1.3 mL, 10 mmol). A color change to dark brown was immediately observed, and the solution was left stirring for 10 min. Diethyl ether (20 mL) was added, and the mixture was stirred for another 5 min. The precipitated 3,6-dimethoxybenzenediazonium carboxylate hydrochloride was collected by suction on a fritted funnel and washed with 10 mL of Et_2O, taking care not to allow the filter cake to dry completely because of the possible explosion hazard. The brown, powdery diazonium salt was transferred to a round-bottomed flask and suspended in a solution of 1,2-dichloroethane (15 mL), propylene oxide (1 mL), and acrylonitrile (2 mL). A condenser was attached, and the suspension was heated at reflux for 2 h, during which time significant gas evolution was observed. The hot solution was filtered through a pad of diatomaceous earth, and the filtrate was concentrated under reduced pressure. The dark-brown residue containing the crude product was redissolved in Et_2O and washed sequentially with 1 N HCl, saturated $NaHCO_3$, and brine, then dried over anhydrous Na_2SO_4. Column chromatography over silica (4:1, hexanes/EtOAc) gave the title compound (±)-**7** (231 mg, 28% based on nitro acid) as a white solid. Repeated runs from 4 to 20 mmol gave yields ranging from 15% to 28%: mp 85−86 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (d, J = 9 Hz, 1 H, Ar*H*), 6.70 (d, J = 9 Hz, 1H, Ar*H*), 4.27 (q, J = 2 Hz, 3 Hz, 1 H, ArC*H*), 3.87 (s, 1 H, OCH_3), 3.80 (s, 1 H, OCH_3), 3.75 (dd, J = 9 Hz, 5 Hz, 1 H, ArCH_2), 3.59 (dd, J = 11 Hz, 2 Hz, 1 H, ArCH_2); low-resolution CIMS, *m*/*z* (rel intensity) 190 (M + H, 100). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

(±)-(3,6-Dimethoxybenzocyclobuten-1-yl)methylamine ((±)-8). Cyanobenzocyclobutene (±)-**7** (100 mg, 1.28 mmol) was dissolved in anhydrous THF (10 mL) under argon in a flame-dried flask. A solution of 2.56 mL (2.56 mmol) of a 1 M BH_3·THF complex was added at room temperature, and the mixture was stirred at 50 °C for 6 h. Once the mixture had cooled to room temperature, 1.2 mL of 1 N methanolic HCl was carefully added, and the mixture was heated at 40 °C for 25 min to hydrolyze the intermediate boramine complex. The solvent was removed by rotary evaporation, and the residue was redissolved in 15 mL of 1 N aqueous NaOH and extracted with 3 × 15 mL of CH_2Cl_2. The organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated to give **8** free base as a clear oil (98 mg, 96%). Amine (±)-**8** was converted to its hydrochloride salt for analysis by dissolving the free base in 1 N methanolic HCl and evaporating the solvent. The salt was purified by recrystallization from EtOH−Et_2O to afford white crystals: mp 183−185 °C. ^{1}H NMR (300 MHz, CD_3OD, HCl salt) δ 6.67 (d, J = 9 Hz, 1 H, Ar*H*), 6.63 (d, J = 9 Hz, 1 H, Ar*H*), 3.72 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.67 (m, 1 H, ArC*H*), 3.42 (dd, J = 8 Hz, 5 Hz, 1 H, ArCH_2), 3.18 (d, J = 7 Hz, 2 H, CH_2NH$_2$), 2.96 (dd, J = 12 Hz, 2 Hz, 1 H, ArCH_2); low-resolution ESIMS, *m*/*z* (rel intensity) 194 (MH^+, 100). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

(±)-(4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine Hydrobromide ((±)-2·HBr). The free base of (±)-**8** (260 mg, 1.34 mmol) was dissolved in 5 mL of glacial AcOH in a foil-wrapped flask with magnetic stirrer. Br_2 (215 mg, 1.34 mmol) in 1 mL of glacial AcOH was added dropwise, allowing the orange color to partially discharge before adding the next drop. The mixture was stirred protected from light for 20 min at room temperature, eventually forming a precipitate. Et_2O (15 mL) was added, and the solids formed were collected by suction filtration and washed with Et_2O. The product was obtained along with its 5-bromo regioisomer in a 5:1 ratio, as determined by ^{1}H NMR. Recrystallization of the pink solid from EtOH yielded pure (±)-**2** (289 mg, 61%) as fine white needles, free of the 5-bromo isomer as determined by TLC and ^{1}H NMR: mp 258−259 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (br s, 3 H, NH_3), 7.01 (s, 1 H, Ar*H*), 3.68 (s, 3 H, OCH_3), 3.73 (s, 3 H, OCH_3), 3.67 (m, 1 H, ArC*H*), 3.57 (dd, J = 9 Hz, 5 Hz, 1 H, CH_2NH$_3$), 3.31−3.19 (m, 2 H, CH_2NH$_2$, ArCH_2), 3.14 (dd, J = 9 Hz, 11 Hz, 1 H, ArCH_2); irradiation at 3.73 ppm produces a positive ^{1}H NOE with 7.01 and 7.92 ppm; low-resolution ESIMS, *m*/*z* (rel intensity) 272 (MH^+, 100), 274 (MH^+, 97).

3,6-Dimethoxybenzocyclobutene-1-carboxylic Acid ((±)-11). Following the procedure of Schiess et al.,[31] chlorobenzocyclobutene (±)-**10**[20] (0.29 g, 1.28 mmol) was dissolved in anhydrous THF (30 mL) under argon in a flame-dried round-bottomed flask containing powdered magnesium (0.16 g, 6.58 mmol). A reflux condenser was attached, and the mixture was heated to reflux. After several minutes the solution darkened and heating was continued for a further 10 min to allow complete formation of the Grignard species. Solid CO_2 was placed into a stoppered Erlenmeyer flask fitted with a cannula. The cannula was inserted into the reaction flask, and CO_2 gas was bubbled through the stirring Grignard solution for 30 min at room temperature as product formation was monitored by TLC. The mixture was poured into water and acidified to pH 1 with 6 N HCl and extracted with 3 × 50 mL of CH_2Cl_2. The combined organic extract was washed with brine, then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield (±)-**11** (0.26 g, 75%) as a white solid: mp 100−101 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.60 (s, 1 H, CO_2H), 6.73 (s, 2 H, 2 × Ar*H*), 4.34 (dd, 1 H, ArC*H*), 3.81 (s, 6 H, 2 × OCH_3), 3.60 (dd, 1H, ArCH_2), 3.54 (dd, 1H, ArCH_2); low-resolution EIMS, *m*/*z* 208 (M^+). Anal. ($C_{11}H_{12}O_4$) C, H.

3,6-Dimethoxy-*N*-((*S*)-1-phenylethyl)benzocyclobutene-1-carboxamide ((±)-12). Benzocyclobutene carboxylic acid (±)-**11** (3.4 g, 16.3 mmol) was dissolved in 60 mL of EtOAc in a 100 mL conical vial. A drop of DMF was added, oxalyl chloride (2.9 g, 22.9 mmol) was added dropwise, and the solution was stirred at room temperature for 30 min. Approximately half of the solvent volume was removed by rotary evaporation to azeotrope excess oxalyl chloride. Excess (*S*)-1-phenethylamine (4.0 g, 32.6 mmol) dissolved in 20 mL of EtOAc was then added, followed by 60 mL of aqueous 2 N NaOH. The vial was immediately agitated on a Vortex mixer for 30 s, and the phases were separated. The aqueous phase was extracted with 2 × 20 mL of EtOAc, and the organic extracts were combined and washed with 1 N HCl, then dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude mixture of diastereomeric amides was purified by gravity column chromatography (silica gel, EtOAc:hexanes, 2:3) to yield the mixture of (*R*,*S*)-

and (*S*,*S*)-**12** (4.6 g, 90%) as a white solid: mp 174−176 °C. Low-resolution CIMS, *m*/*z* (rel intensity) 312 (MH^+, 100). Anal. ($C_{19}H_{21}NO_3$) C, H, N.

HPLC Separation of the Diastereomers of 12 for X-ray Structural Determination. The mixture of diastereomeric amides **12** was dissolved in acetonitrile and injected onto a semipreparative reversed-phase C8 silica column (4.6 mm × 150 mm) with a 250 μL injection loop, UV (254 nm) detection, and automated fraction collector. Gradient elution over 25 min (35:65 to 50:50 acetonitrile/H_2O containing 0.1% trifluoroacetic acid) with a flow rate of 3 mL/min provided partial resolution of the diastereomers. Fractions containing a single diastereomer were combined and lyophilized, and fractions containing a mixture were combined, concentrated, and reinjected. Repetition of this process with 45 mg of mixture at a time ultimately afforded approximately 350 mg of each pure diastereomer. X-ray quality crystals of each diastereomer were obtained by slow vapor diffusion of hexanes into a concentrated solution of each amide in EtOAc. Crystallographic data for the *S*,*S* diastereomer are reported in the Supporting Information.

(*S*)-3,6-Dimethoxy-*N*-((*S*)-1-phenethyl)benzocyclobutene-1-carboxamide ((*S*,*S*)-12). HPLC retention time, 12.15 min; mp 179−180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30−7.13 (m, 4 H, 4 × Ar*H*), 6.94 (d, *J* = 6.4 Hz, 1 H, Ar*H*), 6.58 (s, 2 H, Ar*H*), 5.01 (m, 1 H, C*H*N), 4.22 (t, *J* = 4.2 Hz, 1 H, ArC*H*), 3.73 (s, 3 H, OCH_3), 3.63 (s, 3 H, OCH_3), 3.49 (d, *J* = 6 Hz, 2 H, ArCH_2), 1.45 (d, *J* = 6.8 Hz, 3 H, CH_3); low-resolution ESIMS, *m*/*z* (rel intensity) 334 (M + Na, 100); $[\alpha]^{20}_D$ +21.79° (*c* 1.00, $CHCl_3$).

(*R*)-3,6-Dimethoxy-*N*-((*S*)-1-phenethyl)benzocyclobutene-1-carboxamide ((*R*,*S*)-12). HPLC retention time, 12.89 min; mp 176−177 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.19 (m, 4 H, 4 × Ar*H*), 6.92 (d, *J* = 7.3 Hz, 1 H, Ar*H*), 6.63 (s, 2 H, 2 × Ar*H*), 5.02 (m, 1 H, C*H*N), 4.18 (t, *J* = 4.3 Hz, 1 H, ArC*H*), 3.76 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 3.50 (d, *J* = 4.4 Hz, 2 H, ArCH_2), 1.36 (d, *J* = 6.9 Hz, 3 H, CH_3); low-resolution ESIMS, *m*/*z* (rel intensity) 334 (M + Na, 100); $[\alpha]^{20}_D$ −57.43° (*c* 1.00, $CHCl_3$).

Diastereomers of (*S*)-*N*-(3,6-Dimethoxybenzocyclobuten-1-yl)-methyl)-1-phenethylamine (13). The unresolved mixture of amide diastereomers **12** (0.15 g, 0.48 mmol) was dissolved in anhydrous THF (10 mL) under argon in a flame-dried flask. A solution of 4 mL of 1 M BH_3·THF complex was added in one portion by syringe, and the reaction was monitored by TLC. Upon complete consumption of starting material (approximately 1 h), 1 N methanolic HCl (3 mL) was very carefully added dropwise to quench excess borane reagent. Additional MeOH (3 mL) was added, and the solution was heated at reflux for 2 h to decompose the intermediate boramine complex. The solvent was completely removed by rotary evaporation, and the oily residue was redissolved in MeOH and evaporated again to azeotropically remove residual trimethyl borate. The residual mixture of hydrochloride salts was neutralized with 2 N NaOH, and the free bases were extracted into 3 × 10 mL of CH_2Cl_2. The organic extracts were combined, dried over anhydrous Na_2SO_4, and evaporated to yield the ∼1:1 mixture of diastereomeric free bases of **13** (0.13 g, 93%). Radial chromatography on an oven-dried 4 mm silica/gypsum rotor, eluting with 2:3 EtOAc/hexanes under a N_2/NH_3 atmosphere, gave nearly complete separation of the diastereomers with the *S*,*R* isomer eluting first. Fractions containing a mixture of diastereomers were combined, evaporated, and rechromatographed on another oven-dried rotor. Fractions containing only a single diastereomeric free base were combined and evaporated. Hydrochlorides were prepared by neutralizing with 1 N methanolic HCl, evaporating the solvent, and recrystallizing from EtOH/EtOAc. Their characterization follows.

(*S*)-*N*-(((*R*)-3,6-Dimethoxybenzocyclobuten-1-yl)methyl)-1-phenethylamine ((*S*,*R*)-13). This product was obtained as a clear oil (65 mg, 91%): mp 220−221 °C (HCl salt). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.21 (m, 5 H, Ar*H*), 6.56 (s, 1 H, ArH), 3.77 (q, *J* = 6 Hz, 6 Hz, 1 H, C*H*NH), 3.72 (s, 3 H, OCH_3), 3.67 (s, 3 H, OCH_3), 3.63 (m, 1 H, CH_2NH), 3.29 (dd, *J* = 5 Hz, 8 Hz, 1 H, CH_2NH), 2.74 (m, 3 H, ArC*H*CH_2), 1.35 (d, *J* = 6 Hz, 3 H, CH_3); low-resolution ESIMS, *m*/*z* (rel intensity) 298 (MH^+, 100); $[\alpha]^{20}_D$ −8.36° (*c* 1.00, MeOH, HCl salt). Anal. ($C_{19}H_{23}NO_2$) C, H, N.

(*S*)-*N*-(((*S*)-3,6-Dimethoxybenzocyclobuten-1-yl)methyl)-1-phenethylamine ((*S*,*S*)-13). This product was obtained as a clear oil (61 mg, 85%): mp 225−227 °C (HCl salt). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.21 (m, 5 H, Ar*H*), 6.56 (s, 2 H, ArH), 3.86 (q, *J* = 6 Hz, 6 Hz, 1 H, C*H*NH), 3.72 (s, 3 H, OCH_3), 3.66 (s, 3 H, OCH_3), 3.29 (dd, *J* = 5 Hz, 8 Hz, 1 H, CH_2NH), 2.91 (q, 6 Hz, 6 Hz, 1 H, ArC*H*CH_2), 2.76 (dd, *J* = 12 Hz, 2 Hz, 1 H, ArCH_2), 2.64 (dd, *J* = 9 Hz, 2 Hz, 1 H, ArCH_2), 1.39 (d, *J* = 6 Hz, 3 H, CH_3); low-resolution ESIMS, *m*/*z* (rel intensity) 298 (MH^+, 100); $[\alpha]^{20}_D$ −61.96° (*c* 1.00, MeOH, HCl salt). Anal. ($C_{19}H_{23}NO_2$) C, H, N.

((*R*)-3,6-Dimethoxybenzocyclobuten-1-yl)-methylamine ((*R*)-8). The free base of (*S*,*R*)-**13** (50.0 mg, 0.17 mmol) was dissolved in 10 mL of MeOH and added to a 25 mL glass Ace hydrogenation flask containing a suspension of 20% $Pd(OH)_2$ (100 mg) in 2 mL of MeOH with a drop of H_2O. The flask was pressurized to 60 psi with H_2 and stirred at room temperature for 16 h. Following complete consumption of starting material, as monitored by TLC, the mixture was filtered through diatomaceous earth to remove the catalyst (Warning! $Pd(OH)_2$ and MeOH spontaneously ignite in air!), and the MeOH was evaporated to yield 30.2 mg (93%) of (*R*)-**7** free base as a clear oil, analytically identical to the racemate by TLC, ^{1}H NMR, and ESIMS. The hydrochloride was prepared for analysis by dissolving the free amine in 1 N methanolic HCl and evaporating the solvent, followed by recrystallization from EtOH/EtOAc: mp 190−191 °C (HCl salt); $[\alpha]^{20}_D$ +16.2° (*c* 1.00, MeOH).

((*S*)-3,6-Dimethoxybenzocyclobuten-1-yl)methylamine ((*S*)-8). The free base of (*S*,*S*)-**13** (50.0 mg, 0.17 mmol) was dissolved in 5 mL of MeOH and added to a 25 mL glass Ace hydrogenation flask containing a suspension of 20% $Pd(OH)_2$ (75 mg) in 2 mL of MeOH with a drop of H_2O. The flask was pressurized to 40 psi with H_2 and stirred at room temperature for 6 h. Following complete consumption of starting material, as monitored by TLC, the mixture was filtered through diatomaceous earth to remove the catalyst (Warning! $Pd(OH)_2$ and MeOH spontaneously ignite in air!), and the MeOH was evaporated to yield 30.0 mg (92%) of (*S*)-**7** free base as a clear oil, analytically identical to the racemate by TLC, ^{1}H NMR, and ESIMS. The hydrochloride was prepared for analysis by dissolving the free amine in 1 N methanolic HCl and evaporating the solvent, followed by recrystallization from EtOH/EtOAc: mp 186−189 °C (HCl salt); $[\alpha]^{20}_D$ −16.1° (*c* 1.00, MeOH).

((*R*)-4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine Hydrobromide ((*R*)-2·HBr). (*R*)-**8** free base (370 mg, 1.91 mmol) was treated with Br_2 (306 mg, 1.91 mg) by the same procedure used for the racemate to obtain (*R*)-**2** (322 mg, 48%) after recrystallization from EtOH. The product was analytically identical to the racemate by TLC, ESIMS, and ^{1}H NMR: mp 261−262 °C; $[\alpha]^{20}_D$ +15.9° (*c* 1.00, MeOH). Anal. ($C_{11}H_{15}Br_2NO_2$) C, H, N.

((*S*)-4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine Hydrobromide ((*S*)-2·HBr). (*S*)-**8** free base (390 mg, 2.01 mmol) was treated with Br_2 (322 mg, 2.01 mg) by the same procedure used for the racemate, to obtain (*S*)-**2** (322 mg, 42%) after recrystallization from EtOH. The product was analytically identical to the racemate by TLC, ESIMS, and ^{1}H NMR: mp 265−266 °C; $[\alpha]^{20}_D$ −15.6° (*c* 1.00, MeOH). Anal. ($C_{11}H_{15}Br_2NO_2$) C, H, N.

4,7-Dimethoxy-3*H*-indene-1-carbonitrile (15). To a solution of indanone **14**[21] (0.5 g, 2.60 mmol) in CH_2Cl_2 (30 mL) under argon was added ZnI_2 (20 mg) and trimethylsilyl cyanide (0.46 mL, 3.5 mmol). The mixture was heated at reflux for 5 h, following which the solvent was evaporated. The resulting oil was redissolved in 30 mL of benzene, and 0.5 g of Amberlyst-15 resin was added. The flask was fitted with a Dean−Stark trap. The solution was heated at reflux for 3 h until no more water was collected. The dark solution was filtered through diatomaceous earth to remove the resin, the benzene evaporated, and the residue was purified by gravity column chromatography over silica (EtOAc/hexanes, 30:20) to provide **15** (0.34 g, 65%) as a yellow solid. An analytical sample was recrystallized from absolute EtOH to give the product

as fine yellow needles: mp 105−107 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (t, J = 2 Hz, 1 H, ArCC*H*), 6.74 (d, J = 9 Hz, 1 H, Ar*H*), 6.69 (d, J = 9 Hz, 1 H, Ar*H*), 3.83 (s, 3 H, OCH_3), 3.78 (s, 1 H, OCH_3), 3.47 (d, J = 2 Hz, 2 H, ArC*H*2); low-resolution CIMS, *m*/*z* (rel intensity) 202 (M + H, 100). Anal. ($C_{12}H_{11}NO_2$) C, H, N.

***C*-(4,7-Dimethoxyindan-1-yl)methylamine Hydrochloride ((±)-16).** Unsaturated nitrile **15** (0.25 g, 1.24 mmol) dissolved in absolute EtOH (250 mL) was placed in a 500 mL glass Parr hydrogenation flask containing 200 mg of 10% Pd/C and shaken for 20 min under 20 psi of H_2. The solution was filtered through diatomaceous earth to remove the catalyst and then placed back into the hydrogenation flask along with 1 g of activated Raney nickel 2800. Ammonia gas was bubbled through the solution for 1 min, and the flask was then shaken under 45 psi of H_2 for 6 h. The catalyst was removed by filtration through diatomaceous earth and the solvent was evaporated to yield a clear oil that was acidified with 1 N methanolic HCl and evaporated to yield (±)-**16** hydrochloride (0.29 g, 97%) as a white solid. An analytical sample was recrystallized from 95% EtOH: mp 144−146 °C. ^{1}H NMR (free base, 300 MHz, $CDCl_3$) δ 6.53 (s, 2 H, 2 × Ar*H*), 3.68 (s, 3 H, OCH_3), 3.66 (s, 3 H, OCH_3), 3.32−3.24 (m, 1 H, ArC*H*), 2.95−2.72 (m, 4 H, CH_2NH$_2$ and ArCH_2), 2.17−2.06 (m, 1 H, ArCHCH_2CH$_2$), 1.90−1.81 (m, 1 H, ArCHCH_2CH$_2$); low-resolution CIMS, *m*/*z* (rel intensity) 208 (M + H, 100). Anal. ($C_{12}H_{18}ClNO_2 \cdot {}^1/_3H_2O$) C, H, N.

***C*-(5-Bromo-4,7-dimethoxyindan-1-yl)methylamine Hydrochloride ((±)-3).** Amine free base (±)-**16** (0.29 g, 0.85 mmol) was dissolved in glacial AcOH (15 mL) with stirring. Br_2 (0.14 g, 0.87 mmol) dissolved in 3 mL of glacial AcOH was added dropwise over 1 min, and the mixture was stirred in the dark for 20 min. Et_2O (40 mL) was added, and the resulting precipitate was collected by suction filtration. This solid was dissolved in a minimum of H_2O and basified to pH 11 with 2 N aqueous NaOH. The mixture was extracted with 3 × 30 mL of CH_2Cl_2, and the organic extracts were combined and evaporated to afford an oil. Neutralization of the free base with 1 N methanolic HCl and evaporation gave (±)-**3** hydrochloride (0.21 g, 75%) as an off-white powder. The salt was recrystallized from MeOH/EtOAc to afford a white powder: mp 231−233 °C. ^{1}H NMR (300 MHz, D_2O) δ 7.11 (s, 1 H), 3.75 (s, 3 H), 3.74 (s, 3 H), 3.67 (m, 1 H), 3.21 (dd, J = 6 Hz, 1 H), 3.07 (dd, J = 7 Hz, 1 H), 2.75 (t, J = 7 Hz, 2 H), 2.30 (m, 1 H), 1.89 (m, 1 H); low-resolution ESIMS, *m*/*z* (rel intensity) 286/288 (M + H, 100). Anal. ($C_{11}H_{15}Br_2NO_2$) C, H, N.

2-Bromo-4-methoxyphenol (18). Following the procedure of Chambers,[6] *p*-methoxyphenol **17** (200 g, 1.61 mol) was dissolved in 1 L of MeOH in a 2 L flask fitted with an addition funnel and magnetic stirrer. The flask was cooled to 0 °C, and Br_2 (284 g, 1.78 mol) was added dropwise with stirring. Upon complete addition of the bromine, the mixture was allowed to warm to room temp and was stirred overnight. The MeOH was evaporated under reduced pressure, and the residue was purified by short path distillation at water aspirator pressure. The fraction distilling at 125−135 °C was collected and allowed to solidify, providing a solid mass of needle-like crystals (241 g, 73%): bp 130 °C (25 mmHg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (d, J = 3 Hz, 1 H, Ar*H*), 6.92 (d, J = 8.9 Hz, 1 H, Ar*H*), 6.78 (dd, J = 8.9, 3.0 Hz, 1 H, Ar*H*), 5.18 (bs, 1 H, O*H*), 3.72 (s, 3 H, OMe); low-resolution CIMS, *m*/*z* (rel intensity) 202/204 (MH^+, 51/49).

4-Allyloxy-3-bromoanisole (19). Following the procedure of Green,[23] 2-bromo-4-methoxyphenol **18** (60 g, 295 mmol) and allyl bromide (40 g, 310 mmol) were dissolved in 600 mL of acetone in a mechanically stirred round-bottomed flask containing K_2CO_3 (200 g, 1.44 mol). The mixture was heated at reflux overnight, allowed to cool, and filtered to remove solids. The acetone was removed by rotary evaporation, and the residue was dissolved in 500 mL of Et_2O and washed with 2 × 150 mL each of 2 N aqueous NaOH and brine. The ether layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield a brown oil. Kugelrohr distillation provided a water white oil (67.69 g, 94%): bp 90 °C (0.05 Torr). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (s, 1 H, Ar*H*), 6.70 (dd, 2 H, Ar*H* × 2), 5.96 (m, 1 H, vinyl C*H*), 5.35 (d, 1H, vinyl CH_2), 5.25 (d, 1 H, vinyl CH_2), 4.45 (d, 2 H, OCH_2CH), 3.63 (s, 3 H, OMe); low-resolution CIMS, *m*/*z* (rel intensity) 252/254 (MH^+, 51/49).

4-(5-Methoxy-2,3-dihydrobenzofuran-3-yl)butanoic Acid ((±)-20). The procedure of Togo et al.[24] was employed. In a 2 L, four-necked resin kettle with mechanical stirrer, addition funnel, and reflux condenser with argon inlet was placed **19** (50 g, 205 mmol) dissolved in 800 mL of benzene. The solution was purged with argon for 15 min and then heated to reflux. A solution of tributyltin hydride (75 g, 257 mmol) dissolved in 200 mL of dry benzene was sparged with argon and then placed into the addition funnel. Ethyl acrylate was added by syringe in one portion to the boiling solution. AIBN (3 g, 18 mmol) dissolved in 10 mL of benzene was then added dropwise in four portions over 1 h, while the tributyltin hydride solution was added dropwise over the same time period. Upon complete addition of the tributyltin hydride, the mixture was heated at reflux for an additional hour and then allowed to cool to room temperature. The solution was transferred to a 1000 mL round-bottomed flask, and the benzene was removed under reduced pressure to yield a clear oil. This oil was dissolved in 600 mL of MeOH, and 200 mL of 3 N aqueous KOH was added. The mixture was left stirring overnight at room temperature. The MeOH was removed under reduced pressure, and 300 mL of H_2O was added. The alkaline aqueous solution was washed with 2 × 100 mL of Et_2O, then acidified to pH 1 with concentrated H_2SO_4, resulting in the formation of a cloudy suspension. The mixture was extracted with 3 × 100 mL of Et_2O, and the ether extracts were washed with 1 N HCl and brine, then dried over anhydrous Na_2SO_4. Evaporation of the ether afforded 4-(5-methoxy-2,3-dihydrobenzofuran-3-yl)butanoic acid (41.4 g, 85%) as a viscous off-white oil that solidified overnight under vacuum. An analytical sample was recrystallized from EtOAc/hexanes (mp 70−71 °C). Smaller runs provided yields up to 96%: mp 70−71 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71 (s, 1 H, Ar*H*), 6.56 (d, J = 2 Hz, 2 H, Ar*H* × 2), 4.47 (t, J = 9 Hz, 1 H, OCH_2), 4.03 (dd, J = 7 Hz, 2 Hz, 1 H, OCH_2), 3.58 (s, 3 H, OMe), 3.29 (m, 1 H, ArC*H*), 2.15 (t, J = 9 Hz, 2 H, CH_2CO$_2$H), 1.61 (m, 1 H, CHCH_2), 1.43 (m, 3 H, CHCH_2CH_2); low-resolution CIMS, *m*/*z* (rel intensity) 237 (MH^+, 100). Anal. ($C_{13}H_{16}O_4$) C, H.

5-Methoxy-7,8,9,9a-tetrahydro-1*H*-2-oxabenzo[*cd*]azulen-6-one ((±)-21). Following the procedure of Hellwinkel and Kosack,[32] carboxylic acid (±)-**20** (22.5 g, 95.2 mmol) was dissolved in trifluoroacetic acid (350 mL) with stirring at room temperature. Trifluoroacetic anhydride (60 mL) was then added, and the solution was stirred for another hour at room temperature, followed by 6 h at reflux. After allowing the solution to cool to room temperature, it was poured onto crushed ice (500 g) and the aqueous mixture was extracted with 3 × 150 mL of CH_2Cl_2. The organic extracts were washed with 1 N aqueous NaOH solution until the washes became basic, and then the organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated to give the product as a brown solid. Recrystallization twice from hexanes gave the product (±)-**21** (7.1 g, 34%) as a light-yellow solid: mp 62−63 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (d, J = 9 Hz, 1 H, Ar*H*), 6.65 (d, J = 9 Hz, 1 H, Ar*H*), 4.67 (t, J = 9 Hz, 1 H, OCH_2), 4.00 (dd, J = 9 Hz, 1 Hz, 1 H, OCH_2), 3.72 (s, 3 H, OMe), 3.60 (m, 1 H, ArC*H*), 2.10−1.58 (m, 4 H, CCH_2CH_2CH); low-resolution CIMS, *m*/*z* (rel intensity) 219 (MH^+, 100). Anal. ($C_{13}H_{14}O_3$) C, H.

5-Methoxy-1,8,9,9a-tetrahydro-2-oxabenzo[*cd*]azulene-6-carbonitrile ((±)-22). Trimethylsilyl cyanide (0.80 g 1.10 mL, 8.0 mmol) dissolved in 10 mL of CH_2Cl_2 was added dropwise under argon to a stirred solution of ketone (±)-**21** (1.35 g, 6.19 mmol) and ZnI_2 (100 mg, 0.31 mmol) in 100 mL of CH_2Cl_2. The mixture was heated at reflux for 6 h and then allowed to cool to room temp. The solvent was evaporated, and the residue was redissolved in 100 mL of toluene. Amberlyst-15 acidic resin (1 g) was added, and the flask was fitted with a Dean−Stark trap and reflux condenser, and the mixture was heated at reflux for 3 h. The resin was removed by filtration through diatomaceous earth, and the filter cake was washed with toluene. The filtrate was treated with a mixture of activated carbon and $MgSO_4$ to decolorize it, then filtered again through diatomaceous earth and evaporated under reduced

pressure to yield a light-yellow oil. Column chromatography (silica gel, EtOAc/hexanes, 1:9) afforded (±)-**22** (0.70 g, 50%) as a white solid: mp 72−74 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (t, J = 2 Hz, 1 H, vinyl C*H*), 6.70 (s, 2 H, Ar*H* × 2), 4.73 (t, J = 3 Hz, 1 H, OCH_2), 4.13 (t, J = 2 Hz, 1 H, OCH_2), 3.83 (s, 3 H, OMe), 3.45 (m, 1 H, ArC*H*), 2.37 (m, 3 H), 2.02 (m, 1 H); low-resolution CIMS, *m*/*z* (rel intensity) 228 (MH^+, 100). Anal. ($C_{14}H_{13}NO_2$) C, H, N.

***C*-(5-Methoxy-1,6,7,8,9,9a-hexahydro-2-oxabenzo[*cd*]azulen-6-yl)methylamine Hydrochloride ((±)-23 HCl).** An aqueous slurry of activated Raney nickel 2800 (Aldrich) was placed into a 500 mL glass hydrogenation flask with 100 mL of absolute EtOH. The magnetic catalyst was further activated by sonication, and when the activated catalyst was held back with a ferromagnet, the inactive nonmetallic nickel could be decanted along with the solvent. The unsaturated nitrile (±)-**22** (1.04 g, 4.56 mmol) was dissolved in 350 mL of absolute EtOH and added to the flask. Anhydrous ammonia gas was bubbled through the solution for 1 min, and the flask was then placed on a Parr apparatus and shaken under 60 psi of H_2 for 2 days. The solution was filtered through diatomaceous earth (Caution! Dry Raney nickel is pyrophoric!), the filter and catalyst were washed with EtOH, and the filtrate was concentrated under reduced pressure to yield 1.07 g of a clear oil. The oil was purified by Kugelrohr distillation at 140−150 °C/0.1 Torr to afford the mixture of diastereomeric free bases as a water white oil. The mixture of free bases was dissolved in MeOH and carefully neutralized to pH 5 with 1 N methanolic HCl, and the MeOH was removed under vacuum to yield 1.23 g (98%) of (±)-**23** as a white solid: mp 225 °C (dec). ^{1}H NMR (300 MHz, MeOD-d_3) δ 6.90 (dd, 1 H, Ar*H*), 6.75 (d, J = 8 Hz, 1 H, Ar*H*), 4.86 (dd, 1 H, ArC*H*), 4.13−4.00 (m, 3 H, ArC*H*), 3.94 (2 × s, 3 H, OMe), 3.87−3.76 (m, 2 H, CH_2N), 2.31−1.65 (m, 6 H, CHCH_2CH_2CH_2CH); low-resolution ESIMS, *m*/*z* (rel intensity) 234 (MH^+, 100). Anal. ($C_{14}H_{20}ClNO_2$) C, H, N.

***C*-(3-Bromo-5-methoxy-1,6,7,8,9,9a-hexahydro-2-oxabenzo-[*cd*]azulen-6-yl)methylamine Hydrochloride ((±)-4·HCl).** Amine hydrochloride (±)-**22** (1.05 g, 3.89 mmol) was dissolved in anhydrous MeOH (100 mL), and a solution of Br_2 (622 mg, 3.89 mmol) in 5 mL of MeOH was added dropwise at 0 °C. Following complete addition of Br_2, the solution was allowed to warm to room temperature. Once the bromine color had discharged (after 30 min), excess Et_2O was added to precipitate the product. After the solid was collected by suction filtration, the diastereomeric mixture of crude products (4:1 by ^{1}H NMR) was dissolved in 10 mL of H_2O, neutralized to pH 11 with 2 N aqueous NaOH, extracted with 3 × 50 mL of CH_2Cl_2, and purified by radial chromatography (silica/gypsum, 1:1 hexanes/THF, under an argon/ammonia atmosphere). Under these optimized conditions, the mixture of diastereomeric free bases eluted together in the forerun, but some pure minor diastereomer could be obtained free of the major isomer in the tail fractions. The pure major diastereomer could not be obtained despite many attempts to purify it by chromatography and crystallization. The hydrochloride salts were prepared by dissolving the free bases in MeOH and neutralizing to pH 4 with 1 N methanolic HCl. Recrystallization of the salts of the mixture and the minor isomer from benzene yielded an ~8:1 mixture of major and minor isomers (±)-*syn*-**4** (1.16 g) and pure minor isomer (±)-*anti*-**4** (0.13 g, 9%) as white solids. Efforts to obtain an X-ray quality crystal of either isomer were not successful. The total yield of both isomers was 85%. (±)-*anti*-**4**: mp 255 °C (dec); ^{1}H NMR (300 MHz, MeOD-d_3) δ 6.88 (s, 1 H, Ar*H*), 4.76 (t, J = 9 Hz, 1 H, OCH_2), 4.02 (t, J = 9 Hz, 1 H, OCH_2), 3.87−3.78 (m, 2 H, ArC*H* × 2), 3.76 (s, 3 H, OMe), 3.17−3.10 (m, 2 H, CH_2N), 2.18−1.39 (m, 6 H, CHCH_2CH_2CH_2CH); irradiation at 6.88 ppm produces a positive ^{1}H NOE at 3.76 ppm and vice versa; COSY and 2D NOESY spectra are provided in the Supporting Information; low-resolution ESIMS, *m*/*z* (rel intensity) 312/314 (MH^+, 51/49). Anal. ($C_{14}H_{19}BrClNO_2$ + 0.3 equiv of benzene) C, H, N. (±)-*syn*-**4** (as hydrobromide): mp 248 °C; low-resolution ESIMS, *m*/*z* (rel intensity) 312/314 (MH^+, 51/49). Anal. ($C_{14}H_{19}BrClNO_2$ + 0.2 equiv of benzene) C, H, N.

Pharmacology. Cellular Assays. Cells stably expressing the rat 5-HT_{2A}, human 5-HT_{2A}, or rat 5-HT_{2C} receptor[19] were maintained in Dulbecco's modified Eagle's medium (DMEM), containing 10% dialyzed fetal bovine serum (Gibco BRL) and supplemented with 2 mM l-glutamine, 50 units/L penicillin, 50 μg/L streptomycin, and 300 mg/L Geneticin for rat or 30 mg/L Zeocin for human. The cells were cultured at 37 °C in a H_2O-saturated air atmosphere with 5% CO_2. For radioligand binding assays, cells were split into 100 mm^2 culture dishes when they reached 90% confluency. Upon reaching 100% confluency, the cells were washed with sterile filtered phosphate-buffered solution and incubated in serum-free Opti-MEM for 5 h. After incubation, the cells were scraped from dishes and harvested by centrifugation (15000*g*, 20 min) and placed immediately in a freezer at −80 °C until the assay was performed. For IP accumulation experiments the cells were seeded into 24-well plates and assays were performed when 70% confluency was achieved. A more detailed description of the methods may be found in Kurrasch-Orbaugh et al.[16]

Radioligand Competition Binding Assays. Competition binding experiments were carried out in a total volume of 500 μL with 0.20 nM [^{125}I]DOI. Previously harvested cells were resuspended and added to each well containing assay buffer (50 mM Tris, 0.5 mM EDTA, 10 mM $MgCl_2$; pH 7.4), radioligand, and test compound (or in the case of the saturation assays, cinanserin, mianserin, or 5-HT). Incubation was carried out at 25 °C for 60 min and terminated by rapid filtration using a prechilled Packard 96-well harvester with GF/B UniFilters that had been preincubated for 30 min in 0.3% polyethylenimine. The filters were rinsed using chilled wash buffer (10 mM Tris, 154 mM NaCl) and left to dry overnight. The following day, Microscint-O was added and radioactivity was determined using a TopCount (Packard) scintillation counter. GraphPad Prism (GraphPad Software, San Diego, CA) was used to analyze the saturation and competition binding curves.

Inositol Phosphate Accumulation Studies in Cells Expressing the 5-HT_{2A} Receptor. Accumulation of inositol phosphates was determined using a modified version of a previously published protocol.[33] Briefly, cells expressing the rat 5-HT_{2A} receptor were labeled for 18−20 h in CRML medium containing 1.0 μCi/mL [^{3}H]-myo-inositol. After the cells were pretreated with 10 μM pargyline/10 mM LiCl for 15 min, the cells were exposed to a test drug for 30 min at 37 °C under an air atmosphere with 5% CO_2. The assay was terminated by aspirating the medium and adding 10 mM formic acid. Following incubation for 16 h at 4 °C, the [3H]inositol phosphates were separated from the cellular debris on Dowex-1 ion-exchange columns and eluted with 1.0 M ammonium formate and 0.10 M formic acid. The vials were counted for tritium using a TriCarb scintillation counter (Packard Instrument Corp.).

Drug Discrimination Studies. As a measure of hallucinogenic activity, (±)-**2** was examined for its effects in rats that were trained to discriminate a training drug from saline. A two-lever drug discrimination paradigm was employed as a screen for potential hallucinogenic activity. Briefly, rats were trained to discriminate saline injection from the effects of ip injection of LSD tartrate (186 nmol/kg) for study of 5-HT_{2A} receptor-mediated (LSD-like) effects. The response of the animal to ip injections of the test drug was quantified by counting the number of presses on the appropriate drug lever. Potencies of test drugs were measured using ED_{50} values for those drugs that substituted completely for the training drug, LSD. Drugs that do not substitute completely for the training drug are scored as producing either "partial substitution" (PS) or "no substitution" (NS). At least 59% of the tested rats must have selected the drug lever for a score of PS to be assigned, and 80% of those tested must have selected the drug lever for full substitution. The precise methodology has been described in detail elsewhere.[34−37] The target compounds that were tested in the drug discrimination assay are presented along with their ED_{50} values and 95% confidence intervals (CI).

Molecular Modeling. Ligand structures were drawn and energy-minimized (Powell method, 0.01 kcal mol^{-1} $Å^{-1}$ gradient termination, MMFF94s force field, MMFF94 charges, 1000 maximum

iterations) using the Sybyl 7.0 modeling program.[38] The in silico activated homology model of the h5-HT_{2A} receptor was prepared as previously described.[14] Virtual dockings of energy-minimized ligands to the h5-HT_{2A} receptor were performed using the program GOLD 2.2[27] and scored using GOLDScore with default settings except for constraints. The GOLDScore fitness algorithm was constrained to orientations containing ligand−protein interactions implicated by site-directed mutagenesis and previous modeling[14] to possibly be essential for binding. Distance constraints of 2−3 Å were set between the side chain carbonyl carbon of $D155^{(3.32)}$ and the amine nitrogen of the ligand,[39,40] from $S159^{(3.36)}$ and $T160^{(3.37)}$ to the ligand 2-position oxygen,[41] and between $S239^{(5.43)}$ and the ligand 5-position oxygen.[42,43] The highest ranked docking output structures were merged with the h5-HT_{2A} receptor model and analyzed with Sybyl.

Merged ligand−receptor structures were energy-minimized as a subset based on the ligand molecule (aggregates set to monomers at >6 Å radius from the ligand, monomers at >12 Å radius ignored, Powell method, 0.1 kcal mol^{-1} $Å^{-1}$ gradient termination, MMFF94s force field, MMFF94 charges, distance dielectric of 4, 1000 maximum iterations). Constraints for subsequent molecular dynamics simulations and minimizations in Sybyl were defined as above for GOLD; however, hydrogen bond constraints were defined as a distance range constraint of 1−2 Å between each polar residue's hydrogen and the appropriate oxygen atom on the ligand. Constrained molecular dynamics simulations were then run on the energy-minimized ligand−receptor structures (constraints, force field, charges, and dielectric as outlined above, aggregates as above plus backbone atoms, NTV ensemble at 300 K, Boltzmann distribution of initial velocities, 5000 steps of 1 fs, and 5 fs snapshots). Structures with the lowest potential energy after the first 1000 fs of equilibration period were then energy-minimized as outlined above with defined constraints.

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Supporting Information Available: X-ray crystallographic coordinates, 2D NMR spectra for stereochemical determination, and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Abstracted in part from the Ph.D. thesis of T. H. McLean, Purdue University, 2006.
(2) Shulgin, A.; Shulgin, A. *PIHKAL: A Chemical Love Story*; Transform Press: Berkeley, CA, 1991.
(3) Chambers, J. J.; Kurrasch-Orbaugh, D. M.; Parker, M. A.; Nichols, D. E. Enantiospecific synthesis and pharmacological evaluation of a series of super-potent, conformationally restricted 5-HT(2A/2C) receptor agonists. *J. Med. Chem.* **2001**, *44*, 1003−1010.
(4) Parker, M. A.; Marona-Lewicka, D.; Lucaites, V. L.; Nelson, D. L.; Nichols, D. E. A novel (benzodifuranyl)aminoalkane with extremely potent activity at the 5-HT2A receptor. *J. Med. Chem.* **1998**, *41*, 5148−5149.
(5) Nichols, D. E.; Barfknecht, C. F.; Long, J. P.; Standridge, R. T.; Howell, H. G.; Partyka, R. A.; Dyer, D. C. Potential psychotomimetics. 2. Rigid analogs of 2,5-dimethoxy-4-methylphenylisopropylamine (DOM, STP). *J. Med. Chem.* **1974**, *17*, 161−166.
(6) Chambers, J. J.; Parrish, J. C.; Jensen, N. H.; Kurrasch-Orbaugh, D. M.; Marona-Lewicka, D.; Nichols, D. E. Synthesis and pharmacological characterization of a series of geometrically constrained 5-HT2A/2C receptor ligands. *J. Med. Chem.* **2003**, *46*, 3526−3535.
(7) Cheng, H. C.; Long, J. P.; Nichols, D. E.; Barfknecht, C. F.; Rusterholz, D. B. Effects of rigid amphetamine analogs on vascular strips: studies of 2-aminotetrahydronaphthalene and 2-aminoindan derivatives. *Arch. Int. Pharmacodyn. Ther.* **1974**, *208*, 264−273.
(8) Monte, A. P.; Marona-Lewicka, D.; Lewis, M. M.; Mailman, R. B.; Wainscott, D. B.; Nelson, D. L.; Nichols, D. E. Substituted naphthofurans as hallucinogenic phenethylamine−ergoline hybrid molecules with unexpected muscarinic antagonist activity. *J. Med. Chem.* **1998**, *41*, 2134−2145.
(9) Monte, A. P.; Marona-Lewicka, D.; Cozzi, N. V.; Nelson, D. L.; Nichols, D. E. Conformationally-restricted tetrahydro-1-benzoxepin analogs of hallucinogenic phenethylamines. *Med. Chem. Res.* **1995**, *5*, 651−663.
(10) Monte, A. P.; Marona-Lewicka, D.; Parker, M. A.; Wainscott, D. B.; Nelson, D. L.; Nichols, D. E. Dihydrobenzofuran analogues of hallucinogens. 3. Models of 4-substituted (2,5-dimethoxyphenyl)-alkylamine derivatives with rigidified methoxy groups. *J. Med. Chem.* **1996**, *39*, 2953−2961.
(11) Makriyannis, A. Knittel, J. Conformational-analysis of amphetamine in solution based on unambiguous assignment of the diastereotopic benzylic protons in the H-1-NMR spectra. *Tetrahedron Lett.* **1981**, *22*, 4631−4634.
(12) Nichols, D. E. Hallucinogens. *Pharmacol Ther.* **2004**, *101*, 131−181.
(13) McLean, T. H.; Chambers, J. J.; Parrish, J. C.; Braden, M. R.; Marona-Lewicka, D.; Kurrasch-Orbaugh, D. M.; Nichols, D. E. *C*-(4,5,6-trimethoxyindan-1-yl)-methanamine: a mescaline analogue designed using a homology model of the 5-HT_{2A} receptor. *J. Med. Chem.*, in press.
(14) Chambers, J. J. Nichols, D. E. A homology-based model of the human 5-HT2A receptor derived from an in silico activated G-protein coupled receptor. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 511−520.
(15) Berg, K. A.; Maayani, S.; Goldfarb, J.; Scaramellini, C.; Leff, P.; Clarke, W. P. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* **1998**, *54*, 94−104.
(16) Kurrasch-Orbaugh, D. M.; Watts, V. J.; Barker, E. L.; Nichols, D. E. Serotonin 5-hydroxytryptamine 2A receptor-coupled phospholipase C and phospholipase A2 signaling pathways have different receptor reserves. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 229−237.
(17) Kilts, J. D.; Connery, H. S.; Arrington, E. G.; Lewis, M. M.; Lawler, C. P.; Oxford, G. S.; O'Malley, K. L.; Todd, R. D.; Blake, B. L.; Nichols, D. E.; Mailman, R. B. Functional selectivity of dopamine receptor agonists. II. Actions of dihydrexidine in D2L receptor-transfected MN9D cells and pituitary lactotrophs. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1179−1189.
(18) Alves, I. D.; Cowell, S. M.; Salamon, Z.; Devanathan, S.; Tollin, G.; Hruby, V. J. Different structural states of the proteolipid membrane are produced by ligand binding to the human delta-opioid receptor as shown by plasmon-waveguide resonance spectroscopy. *Mol. Pharmacol.* **2004**, *65*, 1248−1257.
(19) Kenakin, T. Agonist selective receptor states: the new level of selectivity? *Pharm. News.* **1998**, *5*, 20−25.
(20) Azadi-Ardakani, M. Wallace, T. W. 3,6-Dimethoxybenzocyclobutenone, a reagent for quinone synthesis. *Tetrahedron* **1988**, *44*, 5939−5952.
(21) Coutts, R. T. Malicky, J. L. Synthesis of analogs of the hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). II. Ring-methoxylated 1-amino-and 2-aminoindanes. *Can. J. Chem.* **1974**, *52*, 381−389.
(22) Curran, D. P.; Xu, J. *o*-Bromo-*p*-methoxyphenyl ethers. Protecting/radical translocating (PRT) groups that generate radicals from C−H bonds β to oxygen atoms. *J. Am. Chem. Soc.* **1996**, *118*, 3142−3147.
(23) Green, I. R.; de Koning, C. B.; Hugo, V. I. An investigation into the electronic effects of substituents at the 6-position on the biological activity of isochromanquinones. *S. Afr. J. Chem.* **1999**, *52*, 112−119.
(24) Togo, H.; Kikuchi, O. Double carbon−carbon bond formations via both intramolecular and intermolecular radical reactions with tributyltin hydride to give 2,3-dihydrobenzofuran and 2,3-dihydroindole derivatives. *Tetrahedron Lett.* **1988**, *29*, 4133−4134.
(25) Togo, H. Kikuchi, O. The reactions of allyl *o*-bromoaryl ethers, *N*-allyl *o*-bromo-acetanilide, and related compounds with tributyltin hydride in the presence of activated olefins. *Heterocycles* **1989**, *28*, 373−381.
(26) Parrish, J. C. Nichols, D. E. Serotonin 5-HT_{2A} receptor activation induces 2-arachidonoylglycerol (2-AG) release through a phospholipase C-dependent mechanism. *J. Neurochem.*, in press.
(27) Jones, G.; Willett, P.; Glen, R. C. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J. Mol. Biol.* **1995**, *245*, 43−53.
(28) Ho, B. Y.; Karschin, A.; Branchek, T.; Davidson, N.; Lester, H. A. The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT1A receptor: a site-directed mutation study. *FEBS Lett.* **1992**, *312*, 259−262.

(29) Almaula, N.; Ebersole, B. J.; Ballesteros, J. A.; Weinstein, H.; Sealfon, S. C. Contribution of a helix 5 locus to selectivity of hallucinogenic and nonhallucinogenic ligands for the human 5-hydroxytryptamine2A and 5-hydroxytryptamine2C receptors: direct and indirect effects on ligand affinity mediated by the same locus. *Mol. Pharmacol.* **1996**, *50*, 34−42.
(30) Sealfon, S. C.; Chi, L.; Ebersole, B. J.; Rodic, V.; Zhang, D.; Ballesteros, J. A.; Weinstein, H. Related contribution of specific helix 2 and 7 residues to conformational activation of the serotonin 5-HT2A receptor. *J Biol. Chem.* **1995**, *270*, 16683−16688.
(31) Schiess, P.; Rutschmann, S.; Toan, V. V. Formation of substituted benzocyclobutenes through flash vacuum pyrolysis. *Tetrahedron Lett.* **1982**, *23*, 3669−3672.
(32) Hellwinkel, D.; Kosack, T. Successive enveloping of the benzene nucleus with saturated rings. *Liebigs Ann. Chem.***1985**, 226−238.
(33) Berg, K. A.; Clarke, W. P.; Sailstad, C.; Saltzman, A.; Maayani, S. Signal transduction differences between 5-hydroxytryptamine type 2A and type 2C receptor systems. *Mol. Pharmacol.* **1994**, *46*, 477−484.
(34) Appel, J. B.; Cunningham, K. A. The use of drug discrimination procedures to characterize hallucinogenic drug actions. *Psychopharmacol. Bull.* **1986**, *22*, 959−967.
(35) Oberlender, R.; Nichols, D. E. Drug discrimination studies with MDMA and amphetamine. *Psychopharmacology (Berlin)* **1988**, *95*, 71−76.
(36) Colpaert, F. C.; Niemegeers, C. J.; Janssen, P. A. A drug discrimination analysis of lysergic acid diethylamide (LSD): in vivo agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, a LSD-antagonist. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 206−214.
(37) Monte, A. P.; Waldman, S. R.; Marona-Lewicka, D.; Wainscott, D. B.; Nelson, D. L.; Sanders-Bush, E.; Nichols, D. E. Dihydrobenzofuran analogues of hallucinogens. 4. Mescaline derivatives. *J. Med. Chem.* **1997**, *40*, 2997−3008.
(38) *Sybyl*, version 7.0; Tripos Inc. (1699 South Hanley Road, St. Louis, MO 63144).
(39) Wang, C. D.; Gallaher, T. K.; Shih, J. C. Site-directed mutagenesis of the serotonin 5-hydroxytrypamine2 receptor: identification of amino acids necessary for ligand binding and receptor activation. *Mol. Pharmacol.* **1993**, *43*, 931−940.
(40) Kristiansen, K.; Kroeze, W. K.; Willins, D. L.; Gelber, E. I.; Savage, J. E.; Glennon, R. A.; Roth, B. L. A highly conserved aspartic acid (Asp-155) anchors the terminal amine moiety of tryptamines and is involved in membrane targeting of the 5-HT(2A) serotonin receptor but does not participate in activation via a "salt-bridge disruption" mechanism. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 735−746.
(41) Almaula, N.; Ebersole, B. J.; Zhang, D.; Weinstein, H.; Sealfon, S. C. Mapping the binding site pocket of the serotonin 5-hydroxytryptamine2A receptor. Ser3.36(159) provides a second interaction site for the protonated amine of serotonin but not of lysergic acid diethylamide or bufotenin. *J. Biol. Chem.* **1996**, *271*, 14672−14675.
(42) Johnson, M. P.; Wainscott, D. B.; Lucaites, V. L.; Baez, M.; Nelson, D. L. Mutations of transmembrane IV and V serines indicate that all tryptamines do not bind to the rat 5-HT2A receptor in the same manner. *Brain Res.* **1997**, *49*, 1−6.
(43) Shapiro, D. A.; Kristiansen, K.; Kroeze, W. K.; Roth, B. L. Differential modes of agonist binding to 5-hydroxytryptamine(2A) serotonin receptors revealed by mutation and molecular modeling of conserved residues in transmembrane region 5. *Mol. Pharmacol.* **2000**, *58*, 877−886.

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