

C-(4,5,6-Trimethoxyindan-1-yl)methanamine: A Mescaline Analogue Designed Using a Homology Model of the 5-HT_{2A} Receptor

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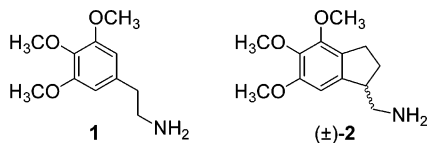
Received March 10, 2006

A conformationally restricted analogue of mescaline, C-(4,5,6-trimethoxyindan-1-yl)-methanamine, was designed using a 5-HT_{2A} receptor homology model. The compound possessed 3-fold higher affinity and potency than and efficacy equal to that of mescaline at the 5-HT_{2A} receptor. The new analogue substituted fully for LSD in drug discrimination studies and was 5-fold more potent than mescaline. Resolution of this analogue into its enantiomers corroborated the docking experiments, showing the *R*-(+) isomer to have higher affinity and potency and to have efficacy similar to that of mescaline at the 5-HT_{2A} receptor.

Introduction

The mechanism of action of mescaline (**1**), the hallucinogenic component of psychoactive cactus *Lophophora williamsii* and of most other drugs that possess hallucinogenic properties, is believed to involve the activation of brain serotonin-2A (5-HT_{2A}) receptors.¹ Recent SAR investigations in our laboratory into the chemical and topological properties of potent hallucinogenic phenethylamines have led to the design and synthesis of a number of methoxylated phenethylamine analogues where the methoxy groups have been constrained into furan or dihydrofuran rings.^{2–6} Although this strategy has proven successful when applied to 2,5-dimethoxy-substituted compounds, it failed in the design of mescaline analogues.⁷ We therefore hypothesized that when mescaline binds to the 5-HT_{2A} receptor, the distal 3,5-methoxy groups must be conformationally mobile or adopt out-of-plane orientations.

Recently we developed a homology model of the 5-HT_{2A} receptor based on an *in silico* activated form of bovine rhodopsin.⁸ A variety of agonist ligands, including mescaline, LSD, and psilocin, were docked using an unbiased docking routine to give reasonable bound orientations of the ligands within the receptor. When mescaline was docked into this model, we observed that the 3- and 5-methoxy groups adopted out-of-plane conformations (Figure 1A) allowing hydrogen bonding with serine residues, yet maintaining the aromatic edge-face interaction with F6.52⁽³⁴⁰⁾, shown by mutagenesis studies to be essential for agonist activity.⁹ Also, the side chain was in an approximately gauche conformation, whereas in the 2,5-dimethoxy compound series the side chain was in a more nearly anti orientation. Single-point energy calculations performed on mescaline in the docked conformation (isolated from the protein) indicate that this conformation is higher in energy than the *in vacuo* global conformational energy minimized form of mescaline in which the side chain is extended into an anti conformation.



Inspection of the putative bound conformation of **1** immediately suggested the synthesis of conformationally restricted

analogue **2**, which maintains the embedded mescaline structure in a low-energy conformation. Subsequent synthesis and testing of (±)-**2** revealed that it possessed mescaline-like properties *in vitro* and *in vivo* and had greater potency than **1** in several assays.

Results and Discussion

Computational Modeling. From docking studies of the enantiomers of **2**, we predicted that the *R* enantiomer would be more potent than mescaline because of a predominance of highly similar and plausible binding orientations obtained from unbiased genetic algorithm searches. The docked conformer of (*R*)-**2** (Figure 1B) most resembled that obtained for **1**, indicating that the amine-bearing side chain adopts a gauche orientation upon binding to the receptor, apparently to optimize hydrogen bonding of all three aromatic methoxy groups with the hydroxyl groups of S3.36⁽¹⁵⁹⁾, T3.37⁽¹⁶⁰⁾, and S5.43⁽²³⁹⁾, residues thought to be potentially important for agonist binding to the 5-HT_{2A} receptor.^{6,9–11} In all docked conformations obtained for (*R*)-**2**, the protonated amine of the ligand forms the essential ionic interaction with the carboxylate of D3.32⁽¹⁵⁵⁾.¹²

By contrast, unbiased docking of (*S*)-**2** into the homology model gave numerous orientations, none of which included the salt bridge between the protonated amine of the ligand and the carboxylate of D3.32⁽¹⁵⁵⁾. As this interaction is essential for agonist activity, the docking routine was repeated with a 2–3 Å distance constraint between the amine nitrogen and carboxyl carbon atoms, almost exclusively returning orientations with the ligand tilted up to place the amine in the same position in space as seen in the *R* isomer, at the expense of the hydrogen bonds between the 6-methoxy and the hydroxy of S3.36⁽¹⁵⁹⁾ and between the 4-methoxy and the hydroxy of S5.43⁽²³⁹⁾. To test this prediction, **2** was resolved into its enantiomers and each isomer was assayed for 5-HT_{2A} binding affinity and potency at stimulating phosphoinositide turnover.

Synthesis. Racemic (±)-**2** was readily synthesized beginning with indanone **3** (Scheme 1). Ketone **3** was functionalized utilizing reaction with trimethylsilyl cyanide and directly dehydrated to indene-1-carbonitrile **4** by treatment with Amberlyst-15 acidic resin. Subsequent two-step catalytic hydrogenation of **4**, first at low pressure over 5% Pd/C in ethanol to reduce the olefin, followed by nitrile reduction over activated Raney nickel in ethanol, yielded the final racemic compound (±)-**2**.

Fractional crystallization of various diastereomeric salts of (±)-**2** failed to resolve the enantiomers, as did attempted

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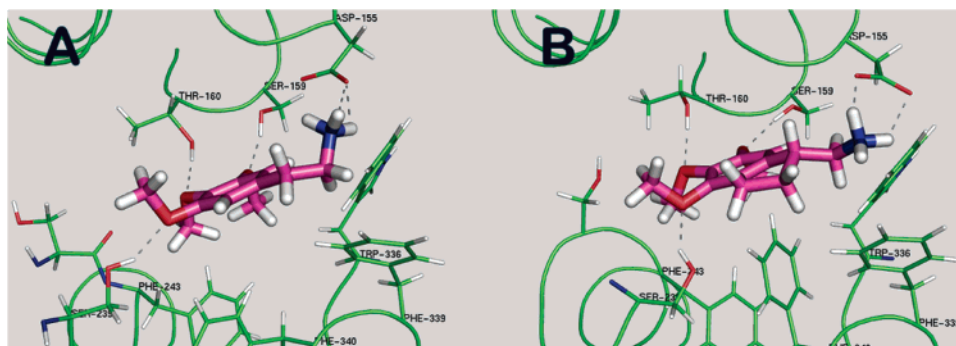
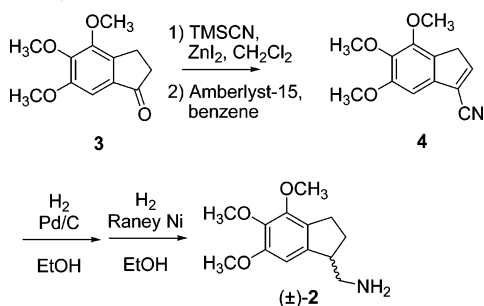
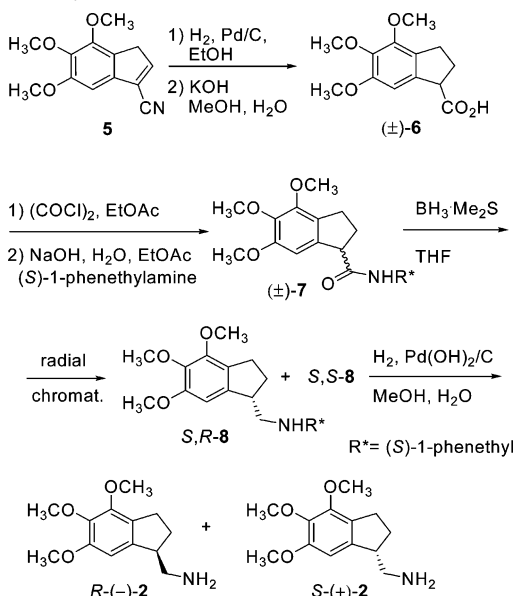


Figure 1. (A) Top view of mescaline (**1**) virtually docked into the 5-HT_{2A} receptor homology model with key residues known to be important for binding. (B) Similar view of the docked *R*-enantiomer of **2**. The view is from the extracellular face of the receptor, with ASP155 in TM3, PHE243 in TM5, and PHE339 and PHE340 in TM6.

Scheme 1. Synthesis of (±)-**2**



Scheme 2. Synthesis of the Enantiomers of **2**



enantioselective acylation of the amine using *Candida antarctica* B lipase (Novozyme 435).¹³ After several unsuccessful attempts to induce chirality by stereoselective reduction of various prochiral intermediates, we attempted the separation of diastereomeric covalent derivatives of acid (±)-**6** as shown in Scheme 2.¹⁴ Unsaturated nitrile **4** was catalytically hydrogenated at low pressure over Pd/C in ethanol, followed by alkaline hydrolysis of the intermediate saturated nitrile to afford acid (±)-**6**. The mixture of diastereomeric amides (±)-**7**, derived from coupling of (±)-**6** with (*S*)-(+)-1-phenethylamine, was reduced using borane, and the resulting secondary amines (*S,R*)- and (*S,S*)-**8** were separated by radial chromatography on a silica rotor. Catalytic *N*-debenzylation of the free base of each diastereomer over Pd(OH)₂ on carbon in wet methanol afforded the enantiomers of **2** in optically pure form.

Table 1. Results of Radioligand Competition Binding Studies at Cloned Receptors

compd	K _i (SEM), nM	
	r5-HT _{2A} (±)-[¹²⁵ I]DOI	r5-HT _{2C} (±)-[¹²⁵ I]DOI
(±)- 2	130(20)	60(10)
(<i>R</i>)-(+)- 2	69(6)	nd ^a
(<i>S</i>)-(–)- 2	1120(180)	nd ^a
mescaline (1)	360(70)	380(60)
psilocin ^b	25(5)	nd ^a
LSD ^b	3.5(0.6)	5.5(0.3)

^a nd = not determined. ^b Data from Chambers et al.⁶

Table 2. Results of the IP₃ Accumulation Studies at Cloned Rat 5-HT_{2A} Receptors

compd	EC ₅₀ at 5-HT _{2A} (SEM), nM	max 5-HT stimulation (SEM), %
(±)- 2	6100(740)	83(4)
(<i>R</i>)-(+)- 2	3200(400)	88(1)
(<i>S</i>)-(–)- 2	> 50000	59(4)
mescaline (1)	11300(1600)	86(10)
psilocin ^a	2300(290)	50(2)
LSD ^a	9.8(3.7)	20(3)

^a Data from Chambers et al.⁶

Determination of the absolute configuration was accomplished by X-ray crystallography of a single crystal of diastereomeric intermediate (*S,S*)-(–)-**7**. This compound was obtained in diastereopure form by the resolution of acid (±)-**6** by fractional crystallization of its quinine salt from ethanol and condensation of each enantiomer of **6** with (*S*)-1-phenethylamine. HPLC analysis showed the diastereomeric excess of **7** to be greater than 99%. X-ray crystallography of one diastereomer established the configuration at the indan-1-position to be *S*, and by inference, the other diastereomer must have the *R* configuration.

Reduction of each diastereomer of **7** by borane–dimethyl sulfide in THF afforded secondary amines (*S,R*)- and (*S,S*)-**8** without racemization. Once it was discovered that (±)-**8** could be resolved by radial chromatography, the tedious fractional crystallization step was omitted and (±)-**8** was synthesized in racemic form and then resolved as shown in Scheme 2.

Pharmacological investigation of conformationally restricted analogue (±)-**2** and its enantiomers was conducted, and the results are compared directly to those obtained for mescaline (Tables 1 and 2).

The competition radioligand binding experiments confirmed the model-derived qualitative prediction that **2** would possess reasonable affinity for the 5-HT_{2A} receptor. When compared to **1**, racemic **2** possessed nearly 3-fold higher affinity for the 5-HT_{2A} receptor, was 2 times more potent at stimulating IP₃ accumulation, and had intrinsic activity comparable to that of mescaline in this pathway.

Pharmacological results also confirmed the prediction that (*R*)-**2** would be a better ligand at the 5-HT_{2A} receptor than (*S*)-**2**, displaying a 16-fold higher affinity and greater than 16-fold higher potency than its distomer.

In vivo drug discrimination tests indicate that **2** substitutes fully for LSD in an animal model of hallucinogenesis.^{15,16} In particular, (\pm)-**2** had an ED₅₀ (95% confidence interval) of 6.6 (3.8–11.5) μ mol/kg, whereas mescaline had an ED₅₀ of 34 (21–54) μ mol/kg. For comparison purposes, the potent hallucinogen LSD had an ED₅₀ of 0.037 (0.023–0.058) μ mol/kg in this assay.

Conclusion

Development of a human 5-HT_{2A} receptor homology model has provided an additional tool for the design of new chemical probes for this receptor. Although **2** is a relatively simple structure, it was designed on the basis of clues provided by the model, and the side chain orientation was counterintuitive on the basis of our previous SAR studies. The new ligand was shown to possess higher affinity and potency than mescaline. Furthermore, the model correctly predicted that (*R*)-**2** would be the more potent enantiomer, based on unbiased docking results and their correlation with key ligand–receptor interactions previously identified by mutagenesis. Although this computer-based model appears useful in explaining much of the empirical data concerning the SAR of 5-HT_{2A} receptor agonists, there is little doubt that it does not accurately represent the *exact* structure of the “activated” binding site. Nevertheless, it can serve as a work in progress, subject to study and refinement through an iterative procedure involving molecular modeling, conformationally restricted analogue synthesis, and mutagenesis studies. With this approach, the model will be incrementally improved to expand our knowledge of the agonist binding site. Until directly measured and detailed structural information for this receptor becomes available, this method appears promising.

Experimental Section

General. All reagents were commercially available and were used without further purification unless otherwise indicated. Indanone **3** was obtained in excellent yield by intramolecular cyclization of 3-(2,3,4-trimethoxyphenyl)propionic acid by a modification of the method of Koo.¹⁷ 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. ¹H 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 CDCl₃, 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 results are within $\pm 0.4\%$ 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.

4,5,6-Trimethoxy-3H-indene-1-carbonitrile, 4. To a solution of 4,5,6-trimethoxyindanone¹⁸ **3** (1.0 g, 4.5 mmol) in CH₂Cl₂ (60 mL) under argon were added ZnI₂ (40 mg) and trimethylsilyl cyanide (0.66 mL, 4.95 mmol). The mixture was heated at reflux for 5 h, and then the solvent was evaporated. The resulting oil was

redissolved in 40 mL of benzene, 0.5 g of Amberlyst-15 cation exchange resin was added, and the flask was fitted with a Dean–Stark trap. The solution was heated at reflux for 3 h until no more water was collected. After filtration through diatomaceous earth to remove the resin, the benzene was evaporated, and the resulting residue was chromatographed on silica (EtOAc/hexanes, 1:1) to give **4** (0.71 g, 68%) as a yellow oil that solidified on standing: mp 95–97 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.16 (t, *J* = 2 Hz, 1 H, ArCCH), 6.78 (s, 1 H, ArH), 3.91 (s, 3 H, OCH₃), 3.84 (s, 3 H, OCH₃), 3.79 (s, 3 H, OCH₃), 3.50 (d, *J* = 2 Hz, 2 H, ArCH₂); low-resolution EIMS *m/z* (rel intensity) 231 (M⁺, 100). Anal. (C₁₃H₁₃NO₃) C, H, N.

(\pm)-(2,3-Dihydro-4,5,6-trimethoxy-1H-inden-1-yl)amino-methane Hydrochloride, (\pm)-2**.** Unsaturated nitrile (\pm)-**4** (1.40 g, 6.05 mmol) dissolved in absolute EtOH (250 mL) was placed in a 500 mL glass Parr hydrogenation flask containing 250 mg of 5% Pd/C and shaken for 20 min under 20 psi of H₂. The solution was then filtered through diatomaceous earth to remove the catalyst and placed back into the hydrogenation flask along with 1 g of fresh activated Raney nickel 2800. Ammonia gas was bubbled through the solution for 1 min, and the flask was then shaken under 45 psi of H₂ 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 (\pm)-**2** hydrochloride (1.58 g, 96%) as a white solid. An analytical sample was recrystallized from 95% EtOH (4.8 g, 87%): mp 245 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.82 (m, 1 H, ArCH₂CH₂, *J* = 3.3 Hz), 2.34 (m, 1 H, ArCH₂CH₂, *J* = 3.2 Hz), 2.85 (m, 1 H, ArCH₂, *J* = 3.1 Hz), 2.97 (dt, 2 H, CH₂N, *J* = 5.0, 3.0 Hz), 3.20 (m, 1 H, ArCH₂), 3.36 (m, 1 H, ArCH, *J* = 6.3 Hz), 3.72 (s, 3 H, ArOCH₃), 3.80 (s, 3 H, ArOCH₃), 3.84 (s, 3 H, ArOCH₃), 6.65 (s, 1 H, ArH); MS (CI) *m/z* 238 (M + H), 221 (M + H – NH₃). Anal. (C₁₃H₂₀ClNO₂) C, H, N.

4,5,6-Trimethoxyindan-1-carboxylic Acid, (\pm)-6**.** Indenecarbonitrile (\pm)-**4** (14.0 g, 60.5 mmol) was dissolved in absolute EtOH (300 mL) and added to a glass hydrogenation bottle containing 1 g of 10% Pd/C. The mixture was shaken under 20 psi of H₂ for 1 h at room temperature to reduce the olefin selectively. The catalyst was removed by filtration through diatomaceous earth, and the EtOH was removed by rotary evaporation to afford the intermediate indan-1-carbonitrile as an oil. This residue was redissolved in 100 mL of MeOH in a flask with magnetic stirring, and 100 mL of 3 N aqueous KOH was added. The mixture was held at reflux under argon for 12 h. The basic solution was washed once with 50 mL of Et₂O, then acidified carefully to pH 1 with concentrated HCl and then extracted with 3 \times 100 mL of Et₂O. The ether extracts were washed with brine, then dried over anhydrous Na₂SO₄ and evaporated to give the acid as an oil that solidified upon standing (14.3 g, 94%): mp 80–83 °C; ¹H NMR (300 MHz, CDCl₃) δ 6.73 (s, 1 H, ArH), 4.00 (dd, *J* = 6, 3 Hz, 1 H, ArCH), 3.87 (s, 3 H, OCH₃), 3.82 (s, 6 H, 2 \times OCH₃), 3.05 (m, 1 H, ArCH₂), 2.87 (m, 1 H, ArCH₂), 2.46–2.28 (m, 2 H, ArCH₂CH₂); low-resolution ESIMS *m/z* (rel intensity) 253 (M + H, 100). Anal. (C₁₃H₁₆O₅) C, H.

Resolution of the Enantiomers of 4,5,6-Trimethoxyindan-1-carboxylic Acid, (\pm)-6**.** The racemic acid (11.6 g, 46.0 mmol) was dissolved in 100 mL of THF and added to a solution of quinine (14.9 g, 46.0 mmol) in 100 mL of THF. The precipitate that formed was collected by suction filtration and dried to give the 1:1 mixture of diastereomeric salts: $[\alpha]^{20}_D$ –113.1° (*c* 1.00, MeOH). The solid was recrystallized five times from absolute EtOH to constant specific rotation: $[\alpha]^{20}_D$ –108.9° (*c* 1.00, MeOH). The mother liquors from all the EtOH recrystallizations were combined and evaporated to near dryness. Addition of 200 mL of Et₂O led to formation of a precipitate, which was collected by suction filtration and dried. The optical rotation for this fraction of the salt derived from the mother liquor was measured: $[\alpha]^{20}_D$ –116.8° (*c* 1.00, MeOH). The salt obtained from the EtOH recrystallization and the precipitate derived from the mother liquor fraction had melting points of 256–260 °C, with decomposition. The free carboxylic acid from each fraction was obtained by acidifying an aqueous

solution of the corresponding quinine salt to pH 1 with concentrated HCl, extracting with CH_2Cl_2 , and removing solvent. The rotation of the acid derived from recrystallized salt (4.7 g) was $[\alpha]_D^{20} +4.97^\circ$ (*c* 1.00, benzene), and that of the acid derived from the mother liquor (6.8 g) was $[\alpha]_D^{20} -3.18^\circ$ (*c* 1.00, benzene). The enantiomeric purity of the acids was determined in the following step by HPLC analysis of the corresponding α -methylbenzylamides 7.

(R)-(-)-4,5,6-Trimethoxy-N-((S)-1-phenethyl)-2,3-dihydroindene-1-carboxamide, (R,S)-7. Carboxylic acid (R)-(+)-6 (2.0 g, 7.9 mmol) was dissolved in EtOAc (10 mL) with a drop of DMF and stirred under argon. Oxalyl chloride (1.4 g, 11.1 mmol) was added dropwise, and the solution was stirred vigorously for 15 min. Approximately half of the solvent volume was removed by rotary evaporation to azeotrope excess oxalyl chloride. Another 10 mL of EtOAc was added to dissolve the intermediate acid chloride. (S)- α -Methylbenzylamine (1.35 g, 11.1 mmol) was dissolved in another 10 mL of EtOAc and added to the flask containing the acid chloride, followed immediately by 20 mL of 2 N aqueous NaOH. The flask was shaken vigorously on a Vortex mixer for 1 min, then poured into a separatory funnel. The solvent layers were allowed to separate. The organic fraction was washed with 1 N aqueous HCl to remove excess amine and then dried (Na_2SO_4), filtered, and evaporated to give the crude amide. Column chromatography (silica; hexanes/EtOAc, 3:2) provided the amide (R,S)-7 (2.0 g, 71%) as a white crystalline solid. A 1 mM solution in acetonitrile was analyzed for diastereomeric purity by HPLC using an analytical reversed-phase C8 silica column (3.0 mm \times 150 mm) with a 25 μL injection loop, UV (254 nm), and ESIMS detection. Gradient elution over 25 min (35:65 to 50:50 acetonitrile/ H_2O containing 0.1% trifluoroacetic acid) with a flow rate of 1 mL/min provided adequate resolution to determine any contamination by the other diastereomer. By comparison with a sample of the 1:1 mixture of diastereomeric amides, the absence of any shoulder peaks indicated that the sample was >99% diastereomerically pure: HPLC $t_R = 9.73$ min; mp 116–117 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.28–7.16 (m, 5 H, ArH), 6.45 (s, 1 H, ArH), 5.70 (d, *J* = 8 Hz, 1 H, NH), 5.12 (m, *J* = 7 Hz, 1H, PhCHNH), 3.85 (s, 3 H, OCH₃), 3.78 (s, 3 H, OCH₃), 3.67 (s, 3 H, OCH₃), 3.82–3.76 (t, 1 H, ArCH), 3.00–2.90 (m, 1 H, ArCH₂), 2.83–2.75 (m, 1 H, ArCH₂), 2.45–2.34 (m, 1 H, ArCHCH₂), 2.25–2.15 (m, 1 H, ArCHCH₂), 1.39 (d, 3 H, *J* = 7 Hz, CH₃); low-resolution ESIMS *m/z* (rel intensity) 355 (M^+ , 100); $[\alpha]_D^{20} -35.73^\circ$ (*c* 1.00, CHCl_3). Anal. ($\text{C}_{21}\text{H}_{25}\text{NO}_4$) C, H, N.

(S)-(-)-4,5,6-Trimethoxy-N-((S)-1-phenethyl)-2,3-dihydroindene-1-carboxamide, (S,S)-7. Carboxylic acid (S)-(-)-6 (2.44 g, 9.52 mmol) was dissolved with stirring under argon in EtOAc (15 mL) with a drop of DMF. Oxalyl chloride (1.69 g, 13.3 mmol) was added dropwise, and the solution was stirred vigorously for 15 min. Approximately half of the solvent volume was removed by rotary evaporation to azeotrope excess oxalyl chloride. Another 15 mL of EtOAc was added to dissolve the intermediate acid chloride. (S)- α -Methylbenzylamine (1.62 g, 13.3 mmol) was dissolved in another 15 mL of EtOAc and added to the flask containing the acid chloride, followed immediately by 40 mL of 2 N aqueous NaOH. The flask was shaken vigorously on a Vortex mixer for 1 min and then poured into a separatory funnel. The solvent layers were allowed to separate. The organic fraction was washed with 1 N aqueous HCl to remove excess amine and was then dried (Na_2SO_4) and evaporated to afford the crude amide. Column chromatography (silica; hexanes/EtOAc, 3:2) provided the amide (S,S)-7 (2.84 g, 84%) as a white crystalline solid. Diastereomeric purity determination using the same RP-HPLC method described for the R,S amide above showed an ~80:20 mixture of amide diastereomers. A single recrystallization from absolute EtOH was sufficient to bring the diastereomeric purity to >99% by HPLC analysis, yielding 2.27 g (67%) of pure (S,S)-7.

A crystal of sufficient quality for X-ray structural determination was produced by vapor diffusion of Et_2O into a concentrated solution of the amide in EtOAc. The absolute configuration at the stereocenter adjacent to the amide carbonyl carbon was found to be *S*, and by inference, the other amide must therefore be the *R,S*

isomer. HPLC $t_R = 9.85$ min; mp 114–115 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.27–7.17 (m, 5 H, ArH), 6.42 (s, 1 H, ArH), 5.75 (d, *J* = 8 Hz, 1 H, NH), 5.12 (m, *J* = 7 Hz, 1H, PhCHNH), 3.81 (s, 3 H, OCH₃), 3.76 (s, 3 H, OCH₃), 3.66 (s, 3 H, OCH₃), 3.80–3.73 (t, 1 H, ArCH), 2.99–2.85 (m, 1 H, ArCH₂), 2.82–2.75 (m, 1 H, ArCH₂), 2.38–2.18 (m, 2 H, ArCHCH₂), 1.41 (d, 3 H, *J* = 7 Hz, CH₃); low-resolution ESIMS *m/z* (rel intensity) 356 ($\text{M} + \text{H}$, 100); $[\alpha]_D^{20} -8.96^\circ$ (*c* 1.00, CHCl_3). Anal. ($\text{C}_{21}\text{H}_{25}\text{NO}_4$) C, H, N.

(S)-(-)-N-(((R)-4,5,6-Trimethoxyindan-1-yl)methyl)-1-phenethylamine, (S,R)-8. Amide (R,S)-7 (0.5 g, 1.4 mmol) was dissolved in dry THF with magnetic stirring under argon at room temperature. A 10 M $\text{BH}_3\text{-SMe}_2$ complex solution (1 mL, 10 mmol) was added in one portion, and the mixture was stirred for 2 h. Upon complete consumption of starting material, indicated by TLC, 15 mL of 1 N methanolic HCl was carefully added to quench excess borane reagent. Concentrated HCl (3 mL) was added, and the solution was brought to reflux for 20 min to hydrolyze the intermediate boramine complex. The solvent was evaporated to near dryness, 10 mL of MeOH was added, and the solvent was again evaporated to remove residual trimethylborate by azeotropic distillation. The residue was redissolved in 1 N aqueous HCl and extracted with Et_2O to remove nonpolar contaminants, and the solution was basified to pH 11 with 6 N aqueous NaOH. The free amine was extracted into 3 \times 20 mL of CH_2Cl_2 . The combined extract was dried over anhydrous Na_2SO_4 , filtered, and then evaporated to give (R,S)-8 as an oil (470 mg, 98%). An analytical sample was prepared by radial chromatography on a 4 mm silica/gypsum rotor, eluting with EtOAc/hexanes, 20:30. The hydrochloride proved too hygroscopic for elemental analysis. The subsequent N-debenzylation step proceeded much more smoothly with the free amine, but care had to be taken to exclude air for the long-term storage of the free base: mp 220–221 $^\circ\text{C}$ (hydrochloride); ^1H NMR (hydrochloride, 300 MHz, CD_3OD) δ 7.44–7.32 (m, 5 H, ArH), 6.51 (s, 1 H, ArH), 4.39 (q, 1 H, CHNH, *J* = 7 Hz), 3.72 (s, 3 H, OCH₃), 3.69 (s, 3 H, OCH₃), 3.65 (s, 3 H, OCH₃), 3.41 (m, 1 H, ArCHCH₂), 3.26–3.23 (m, 2 H, CH₂NH), 2.73 (m, 2 H, ArCH₂), 2.57 (dd, 6 Hz, 1 H, ArCHCH₂, *J* = 2 Hz), 2.28 (m, 1 H, ArCHCH₂), 1.66 (d, 3 H, CH₃, *J* = 7 Hz); low-resolution ESIMS *m/z* (rel intensity) 342 ($\text{M} + \text{H}$, 100); $[\alpha]_D^{20} -58.09^\circ$ (*c* 1.00, MeOH, HCl salt). Anal. ($\text{C}_{21}\text{H}_{27}\text{NO}_3$) C, H, N.

(S)-(-)-N-(((S)-4,5,6-Trimethoxyindan-1-yl)methyl)-1-phenethylamine, (S,S)-8. The same procedure was followed as for the reduction of (S,S)-7 (0.5 g, 1.4 mmol). (S,S)-8 was obtained as an oil (475 mg, 99%). An analytical sample was prepared by radial chromatography on a 4 mm silica/gypsum rotor, eluting with EtOAc/hexanes, 20:30: mp 225–227 $^\circ\text{C}$ (hydrochloride); ^1H NMR as hydrochloride (300 MHz, CD_3OD) δ 7.52–7.38 (m, 5 H, ArH), 6.46 (s, 1 H, ArH), 4.41 (q, 1 H, CHNH, *J* = 7 Hz), 3.74 (s, 3 H, OCH₃), 3.69 (s, 3 H, OCH₃), 3.67 (s, 3 H, OCH₃), 3.32 (m, 1 H, ArCHCH₂), 3.14–3.09 (dd, 4 Hz, 1 H, CH₂NH, *J* = 9 Hz), 2.97–2.68 (m, 2 H, CH₂NH, ArCH₂), 2.26 (m, 1 H, ArCHCH₂), 1.86 (m, 1 H, ArCHCH₂), 1.66 (d, 3 H, CH₃, *J* = 7 Hz); low-resolution ESIMS *m/z* (rel intensity) 342 ($\text{M} + \text{H}$, 100); $[\alpha]_D^{20} -15.77^\circ$ (*c* 1.00, MeOH, as HCl salt). Anal. ($\text{C}_{21}\text{H}_{27}\text{NO}_3$) C, H, N.

Alternative Procedure for Obtaining (S,R)-8 and (S,S)-8 by Chromatographic Separation. The diastereomeric mixture of amides (\pm)-7, obtained by condensation of the racemic carboxylic acid (\pm)-6 with (S)-(+)-1-phenethylamine, as described for the enantiomers above, was dissolved in THF with stirring under argon, and 10 equiv of 1 M $\text{BH}_3\text{-THF}$ complex was added in one portion at room temperature. Once consumption of starting material was complete, as indicated by TLC, excess 1 N methanolic HCl was carefully added to quench excess borane, and the solution was brought to reflux for 30 min to hydrolyze the boramine complex. The solvent was evaporated under reduced pressure, and the residue was dissolved in aqueous 1 M NaOH and extracted with CH_2Cl_2 . The organic extracts were combined and evaporated to afford a quantitative yield of the diastereomeric mixture as an oil. This material was separated 500 mg at a time by radial chromatography (3:2 hexanes/EtOAc; oven-dried 4 mm silica gel rotor). The S,S

diastereomer was the first to elute, followed by the *S,R*. In the case of incomplete resolution, the fractions containing a mixture of diastereomers were combined, evaporated, and rechromatographed. Both diastereomers were analytically identical to the material prepared by the previous method.

(R)-(-)-(4,5,6-Trimethoxyindan-1-yl)methanamine Hydrochloride, (R)-(-)-2·HCl. (*R,S*)-**8** free base (100 mg, 0.29 mmol) was dissolved in 7 mL of MeOH with a drop of H₂O and placed into a 25 mL glass Ace hydrogenation flask containing a suspension of 100 mg of wet 20% Pd(OH)₂/C in 5 mL of MeOH and a magnetic stirbar. The flask was pressurized to 40 psi of H₂ and stirred for 6 h. Once all starting material had been consumed, as indicated by TLC, the reaction was filtered through diatomaceous earth to remove the catalyst (Caution! Pd(OH)₂ and MeOH spontaneously ignite in air) and the solvent was evaporated to give a clear oil. The hydrochloride salt was formed by dissolving the free amine in MeOH and neutralizing to pH 4 with 1 N methanolic HCl, then evaporating the solvent. The hydrochloride salt was obtained in solid form by dissolving the residue in a minimum amount of *i*-PrOH, followed by addition of Et₂O and collection of the resulting solid by suction filtration. The (*R*)-**2**·HCl obtained (77 mg, 96%), pure by TLC and NMR, was recrystallized from absolute EtOH for elemental analysis to yield a white crystalline solid (69 mg, 86%): mp 170–171 °C; ¹H NMR (300 MHz, CD₃-OD) δ 6.64 (s, 1 H, ArH), 3.78 (s, 3 H, OCH₃), 3.76 (s, 3 H, OCH₃), 3.70 (s, 3 H, OCH₃), 3.36–3.25 (m, 2 H, CH₂NH₃), 2.94–2.72 (m, 3 H, ArCH, ArCH₂), 2.34–2.22 (m, 1 H, ArCH₂CH₂), 1.86–1.75 (m, 1 H, ArCH₂CH₂); low-resolution ESIMS *m/z* (rel intensity) 238 (M + H, 30), 221 (M + H) – NH₃, 100; [α]_D²⁰ –14.40° (c 1.00, MeOH, as HCl salt). Anal. (C₁₃H₂₀CINO₃) C, H, N.

(S)-(+)-(4,5,6-Trimethoxyindan-1-yl)methanamine Hydrochloride, (S)-(+)-2·HCl. Following a procedure similar to that used for (*R,S*)-**8** above, (*S,S*)-**8** free base (150 mg, 0.44 mmol) was dissolved in 10 mL of MeOH with a drop of H₂O and placed into a 25 mL glass Ace hydrogenation flask containing a suspension of 150 mg of wet 20% Pd(OH)₂/C in 5 mL of MeOH and a magnetic stir bar. The flask was pressurized to 40 psi of H₂ and stirred for 6 h. Once all starting material had been consumed, as indicated by TLC, the mixture was filtered through diatomaceous earth to remove the catalyst and the solvent was evaporated to give a clear oil. The hydrochloride salt was formed by dissolving the free amine in MeOH and neutralizing to pH 4 with 1 N methanolic HCl, then evaporating the solvent. The hydrochloride salt was recrystallized from *i*-PrOH/Et₂O. After filtration and drying under vacuum, 114 mg (95%) of (*S*)-(+)-**2**·HCl was obtained as a white powder, sufficiently pure for elemental analysis: mp 173–175 °C; ¹H NMR (300 MHz, CD₃OD) δ 6.64 (s, 1 H, ArH), 3.78 (s, 3 H, OCH₃), 3.76 (s, 3 H, OCH₃), 3.70 (s, 3 H, OCH₃), 3.36–3.25 (m, 2 H, CH₂NH₃), 2.94–2.72 (m, 3 H, ArCH, ArCH₂), 2.34–2.22 (m, 1 H, ArCH₂CH₂), 1.86–1.75 (m, 1 H, ArCH₂CH₂); low-resolution ESIMS *m/z* (rel intensity) 238 (M + H, 30), 221 (M + H) – NH₃, 100; [α]_D²⁰ +14.10° (c 1.00, MeOH, as HCl salt). Anal. (C₁₃H₂₀CINO₃) C, H, N.

Pharmacology. Cells stably transfected to express the rat 5-HT_{2A} or rat 5-HT_{2C} receptor¹⁹ were maintained in Dulbecco's modified Eagle medium, 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. The cells were cultured at 37 °C in a H₂O-saturated atmosphere of 95% air and 5% CO₂. For radioligand binding assays, cells were split into 100 mm² 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 and pelleted by centrifugation (2000g, 10 min), resuspended in assay bugger, and pelleted again (15000g, 20 min). The supernatant was aspirated, and the pellet was placed immediately in a freezer at –80 °C until the assay was performed. For IP₃ accumulation experiments the cells were seeded into 48-well plates and assays were performed when 70% confluency was achieved.

Radioligand Competition Binding Assays. Competition binding experiments were carried out in a total volume of 500 μL with 0.20 nM [¹²⁵I]DOI. Previously harvested cells were resuspended in assay buffer and added to each well containing assay buffer (50 mM Tris, 0.5 mM EDTA, 10 mM MgCl₂; pH 7.4), radioligand, and test compound. 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 Uni-filters 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 Triphosphate 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.²⁰ Briefly, cells expressing the rat 5-HT_{2A} receptor were labeled for 18–20 h in CRML medium containing 1.0 μCi/mL [³H]-myoinositol. 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 atmosphere of 5% CO₂. The assay was terminated by aspirating the medium and adding 10 mM formic acid. After 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 Beckman LS6500 scintillation counter.

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 the test drug was quantified by counting the number of presses on the appropriate drug lever. Potencies of test drugs were measured using ED₅₀ 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 “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.^{15,16,21,22} The target compounds that were tested in the drug discrimination assay are presented along with their ED₅₀ values and 95% confidence intervals.

Molecular Modeling and Docking. 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.²³ The *in silico* activated homology model of the h5-HT_{2A} receptor was prepared as previously described.⁸ Virtual dockings of energy-minimized ligands to the h5-HT_{2A} receptor were performed using the program GOLD 2.2²⁴ 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 modeling⁸ to 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.^{11,25} 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 of >6 Å radius from the ligand, monomers of >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 dynam-

ics 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 lowest potential energy after the first 1000 fs equilibration period were then energy-minimized as outlined above with defined constraints.

Acknowledgment. The authors are indebted to Stewart Frescas for synthesis of the indanone starting material, for funding by NIH Grant DA02189 from NIDA, a grant from the Heffter Research Institute, a Purdue Presidential Distinguished Fellowship to T.H.M., and a Purdue Research Foundation Fellowship to J.J.C. The work was conducted in a facility constructed with support from Research Facilities Improvement Program Grant C06-14499 from the National Center for Research Resources of the National Institutes of Health.

Supporting Information Available: X-ray crystallographic coordinates for determination of absolute configuration; elemental analysis results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- Nichols, D. E. Hallucinogens. *Pharmacol. Ther.* **2004**, *101*, 131–181.
- Chambers, J. J.; Kurrasch-Orbaugh, D. M.; Nichols, D. E. Translocation of the 5-alkoxy substituent of 2,5-dialkoxyarylalkylamines to the 6-position: effects on 5-HT(2A/2C) receptor affinity. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1997–1999.
- Chambers, J. J.; Kurrasch-Orbaugh, D. M.; Parker, M. A.; Nichols, D. E. Enantiospecific synthesis and pharmacological evaluation of a series of superpotent, conformationally restricted 5-HT(2A/2C) receptor agonists. *J. Med. Chem.* **2001**, *44*, 1003–1010.
- 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.
- 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-HT_{2A} receptor. *J. Med. Chem.* **1998**, *41*, 5148–5149.
- 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-HT_{2A/2C} receptor ligands. *J. Med. Chem.* **2003**, *46*, 3526–3535.
- 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.
- Chambers, J. J.; Nichols, D. E. A homology-based model of the human 5-HT_{2A} receptor derived from an in silico activated G-protein coupled receptor. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 511–520.
- Roth, B. L.; Willins, D. L.; Kristiansen, K.; Kroeze, W. K. 5-Hydroxytryptamine₂-family receptors (5-hydroxytryptamine_{2A}, 5-hydroxytryptamine_{2B}, 5-hydroxytryptamine_{2C}): where structure meets function. *Pharmacol. Ther.* **1998**, *79*, 231–257.
- Ballesteros, J. A.; Weinstein, H. Integrated methods for the construction of three-dimensional models and computational probing of structure–function relations in G protein-coupled receptor. *Methods Neurosci.* **1995**, *25*, 366–428.
- Wang, C. D.; Gallaher, T. K.; Shih, J. C. Site-directed mutagenesis of the serotonin 5-hydroxytryptamine₂ receptor: identification of amino acids necessary for ligand binding and receptor activation. *Mol. Pharmacol.* **1993**, *43*, 931–940.
- Almaula, N.; Ebersole, B. J.; Zhang, D.; Weinstein, H.; Sealfon, S. C. Mapping the binding site pocket of the serotonin 5-hydroxytryptamine_{2A} 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.
- Yang, B.; Izumi, T.; Zhang, S. S. Stereoselectivity of amidation catalyzed by lipase. *Gaodeng Xuexiao Huaxue Xuebao* **2001**, *22*, 1332–1337.
- McLean, T. H. Conformationally-Restricted Analogs of Hallucinogenic Phenethylamines as Functionally-selective 5-HT_{2A} Agonists. Ph.D. Thesis, Purdue University, West Lafayette, IN, 2006.
- Appel, J. B.; Cunningham, K. A. The use of drug discrimination procedures to characterize hallucinogenic drug actions. *Psychopharmacol. Bull.* **1986**, *22*, 959–967.
- Glennon, R. A. Do classical hallucinogens act as 5-HT₂ agonists or antagonists? *Neuropsychopharmacology* **1990**, *3*, 509–517.
- Koo, J. Studies in polyphosphoric acid cyclizations. *J. Am. Chem. Soc.* **1953**, *75*, 1891–1895.
- Safir, S. R. Sedative Indanones. U.S. Patent 3,454,565, October 5, 1966 (American Cyanamid, Co).
- Julius, D.; Huang, K. N.; Livelli, T. J.; Axel, R.; Jessell, T. M. The 5HT₂ receptor defines a family of structurally distinct but functionally conserved serotonin receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 928–932.
- 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.
- 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.
- Oberlender, R.; Nichols, D. E. Drug discrimination studies with MDMA and amphetamine. *Psychopharmacology (Berlin)* **1988**, *95*, 71–76.
- Sybyl 7.0; Tripos Inc. (1699 South Hanley Road, St. Louis, MO 63144).
- 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.
- 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.

JM060272Y