1-(2-Aminoethyl)-3-methyl-8,9-dihydropyrano[3,2-e]indole: A Rotationally Restricted Phenolic Analog of the Neurotransmitter Serotonin and Agonist Selective for Serotonin (5-HT_2-Type) Receptors

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A series of rotationally restricted phenolic analogs of the neurotransmitter serotonin has been synthesized with the 5-hydroxyindole portion of serotonin replaced by a dihydropyrano[3,2-e]-indole (1, 3, 4, and 5) and a dihydropyrano[2,3-f]indole (2). The receptor binding profile of these compounds has been studied and compared to the natural substrate serotonin. The dihydropyrano-[3,2-e]indole derivatives (1, 3, 4, and 5) possess lower affinity for 5-HT_1 receptors but equal or greater affinity for 5-HT_2 receptors. Like serotonin, these compounds dose-dependently stimulated phosphatidylinositol turnover in rat brain slices. Moreover, the response to 1-(2-aminoethyl)-3-methyl-8,9-dihydropyrano[3,2-e]indole (5, CP-132,484) and 1-(2-aminoethyl)-8,9-dihydropyrano-[3,2-e]indole (4) is selectively antagonized by 5-HT_2 receptor antagonists establishing these tryptamines as selective 5-HT_2 receptor agonists. The high affinity and potency of 5 for 5-HT_2 receptors suggests that the C5-hydroxy group in serotonin can function as a hydrogen bond acceptor in a 5-HT_2 receptor with a directionality of interaction which is down and away from C6 in serotonin (Figure 5). Furthermore, the potent affinity of these compounds for 5-HT_2 receptors coupled with their poor affinity for 5-HT_1 receptors indicates that the aminoethyl side chain of serotonin adopts significantly different conformations in 5-HT_1 versus 5-HT_2 receptors.

Introduction

The study of the anatomical localization, function, and physiological importance of the family of serotonin receptors has been hampered by the lack of receptor specific tools.[1] While the neurotransmitter serotonin [5-hydroxytryptamine, 5-HT] has been implicated in a wide array of biological functions, from disorders in feeding, sexual, and social behaviors to control of the cardiovascular system,[2] the specific receptors responsible for any of these events have not been identified because of the paucity of receptor subtype specific agonists and antagonists. Until very recently, 8-OH-DPAT [8-hydroxy-2-(*N*,*N*-dipropylamino)tetralin] was the only 5-HT receptor subtype selective agonist known. This 5-HT_{1A} receptor agonist has led to the development of novel serotonergic anxiolytics, i.e. the buspirone and gepirone class of drugs, which are partial 5-HT_{1A} agonists.[3] The discovery of other 5-HT receptor subtype specific agonists should aid in the development of novel treatments for diseases associated with serotonin receptor dysfunction.

In our laboratories we have sought selective agonists for 5-HT receptors via the synthesis of conformationally and rotationally restricted analogs of the natural ligand, serotonin.[4] The premise of our work was that by limiting the molecular freedom of the important aspects of the serotonin molecule and studying the resulting pharmacology of the conformationally or rotationally restricted 5-HT analogs in comparison with the natural substrate serotonin, we could define molecular recognition requirements for specific 5-HT receptor subtypes. This line of research led to the discovery of CP-93,129 [3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one].[4a] This molecule incorporated the C5-hydroxy group of 5-HT[5] into the amide tautomer of a pyridone, thus creating a rotationally restricted phenolic analog of serotonin which was almost entirely selective as an agonist for the 5-HT_{1B} receptor subtype. This high degree of selectivity of CP-93,129 for the 5-HT_{1B} receptor was attributed to the fixed character of the C=O in the amide (pyridone) which required hydrogen bond accepting interactions with the 5-HT_{1B} receptor to occur specifically in a planar directionality defined by the nonrotating amide oxygen lone pairs of electrons. This finding not only gave us a novel tool with which we could use to understand the anatomical distribution, function, and physiological importance of the 5-HT_{1B} receptor, but it also provided a direction for further research involving other rotationally restricted phenolic analogs of 5-HT. Since the directionality of hydrogen bond accepting interactions between CP-93,-

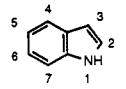
(1) Peroutka, S. J. 5-Hydroxytryptamine Receptor Subtypes. *Annu. Rev. Neurosci.* **1988**, *11*, 45-60.

(2) Glennon, R. A. Central Serotonin Receptors as Targets for Drug Research. *J. Med. Chem.* **1987**, *30*, 1-12.

(3) Robinson, C. P. Buspirone Hydrochloride (Bespar®; Buspar®) - A New Anxiolytic Agent. *Drugs Today* **1987**, *23*, 311-319.

(4) a) Macor, J. E.; Burkhart, C. A.; Heym, J. H.; Ives, J. L.; Lebel, L. A.; Newman, M. E.; Nielsen, J. A.; Ryan, K. Schulz, D. W.; Torgersen, L. K.; Koe, B. K. 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]-pyrid-5-one: A Potent and Selective Serotonin (5-HT_{1B}) Ligand and Rotationally Restricted Phenolic Analog of 5-Methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J. Med. Chem.* **1990**, *33*, 2087-2093. (b) Macor, J. E.; Newman, M. E. Synthesis of a Rotationally Restricted Phenolic Analog of 5-Methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole (RU-24969). *Heterocycles* **1990**, *31*, 805-809. (c) Macor, J. E.; Ryan, K. Synthesis of Some Conformationally Restricted Serotonin Analogs. *Heterocycles* **1990**, *31*, 1497-1504. (d) Macor, J. E.; Newman, M. E. Synthesis of a Dihydropyrano[3,2-e]indole as a Rotationally Restricted Phenolic Analog of the Neurotransmitter Serotonin. *Tetrahedron Lett.* **1991**, *32*, 3345-3348. (e) Macor, J. E.; Ryan, K.; Newman, M. E. The Synthesis of Pyrano-[3,2-e]indoles and Pyrano[2,3-f]indoles as Rotationally Restricted Analogs of the Neurotransmitter Serotonin. *Tetrahedron* **1992**, *48*, 1039-1052.

(5) For simplicity of discussion, the numbering of the serotonin derivatives contained in this manuscript follows the IUPAC numbering of the indole heterocycle:



The dihydropyrano[3,2-e]indoles synthesized in this manuscript are properly named in the Experimental Section of this manuscript.

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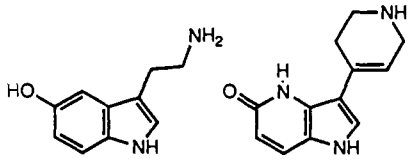
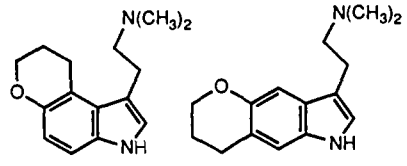
129 and the 5-HT_{1B} receptor occur in the plane of the aromatic ring as defined by the amide, hydrogen bond accepting interactions between other 5-HT receptors might require alternate directionalities, and this assumption led us to the synthesis of dihydropyrano[3,2-*e*]indole (1) and dihydropyrano[2,3-*f*]indole (2).[4d,e] The affinity of the dihydropyrano[2,3-*f*]indole derivative (2) for 5-HT receptors was very poor when compared to the natural substrate serotonin (Table I) indicating that that replacement of the 5-hydroxyindole portion of 5-HT with the dihydropyrano[2,3-*f*]indole heterocycle in 2 was deleterious to recognition by serotonin receptors. This lack of affinity of 2 for serotonin receptors could be a result of either unfavorable steric interactions between the indole with C6 substitution[6] (2) and 5-HT receptors and/or the result of a poorly aligned hydrogen bonding interaction between the oxygen in the dihydropyran ring and 5-HT receptors. We believed that both contribute to the lack of affinity of 2 for serotonin receptors.





However, the diminished affinity demonstrated by the dihydropyrano[3,2-*e*]indole derivative (1) for 5-HT_1 receptors (i.e. 5-HT_{1A} and 5-HT_{1D}, compared to 5-HT) coupled with its relatively high affinity for 5-HT_2-like receptors[7] (i.e. 5-HT_{1C} and 5-HT_2, compared to 5-HT) appeared to be a novel receptor binding profile for such a close analog of serotonin (Table I), especially since indolealkylamines usually are relatively nonselective within the serotonin family of receptors.[8] Therefore, we attempted to optimize this 5-HT_2-type receptor affinity and selectivity via the synthesis of simple analogs of 1. Specifically, we wished to examine the effect of the presence or absence of methyl groups on the indole and aminoethyl nitrogens on the interactions between these compounds and 5-HT_2 receptors. This manuscript presents the results of this study.

Results

Chemistry. The synthesis of the dihydropyrano[3,2-*e*]indole derivatives (1, 3, 4, and 5) is shown in Scheme I. All of these tryptamines start from 8,9-dihydropyrano[3,2-*e*]indole (6), the synthesis of which has been described previously.[4e] The location of the methyl groups in the final products determined the chemical route to each compound. For example, the synthesis of *N*,*N*-dimethyltryptamines was more easily accomplished via the indole-3-glyoxamides, while the synthesis of the primary tryptamines was more easily accomplished via the 3-(2-nitroethenyl)indoles. The formation of 1, proceeding directly from 6 via the indole-3-glyoxamide 7, has been previously described.[4e] While 1 can be directly converted to its indole *N*-methyl analog 3 via direct alkylation of the indole nitrogen, this reaction is problematic since formation of a quaternary nitrogen competes with formation of 3. Only the use of a crown ether and base allowed for the formation of reasonable amounts of 3. A more expeditious approach to 3 involved a high-yielding direct alkylation of the resonance-stabilized anion of the indole nitrogen of 7 using sodium hydride and methyl iodide forming the *N*-methylindole-3-glyoxamide 8. Straightforward reduction of the glyoxamide in 8 using borane,[9] followed by the decomposition of the resulting amine–borane complex afforded 3 in good yield.

The synthesis of the primary tryptamine analogs (4 and 5) proceeded from the indole-3-carboxaldehyde (9), which was readily available from 6 via Vilsmeir–Haack methodology ($POCl_3$/DMF). Heating 9 in nitromethane in the presence of a trace of ammonium acetate (Henry reaction) afforded the nitroolefin derivative 10 in almost quantitative yield as an orange solid, which precipitated directly from the reaction solution analytically pure. Complete reduction of 10 using lithium aluminum hydride afforded the primary tryptamine analog 4. Since direct methylation of the indole nitrogen in 10 failed, alkylation of the aldehyde 9 with sodium hydride and methyl iodide was used to form the *N*-methylindole-3-carboxaldehyde 11. Reaction of 11 with nitromethane as described above, followed by the reduction of 12 using lithium aluminum hydride led to the primary tryptamine 5.

Pharmacology. The dihydropyrano[2,3-*f*]indole analog 2 of serotonin appeared to be uniformly and considerably less potent than serotonin at all 5-HT receptors (Table I). The affinity of the dihydropyrano[3,2-*e*]indole analogs of 5-HT (1, 3, 4, and 5) at 5-HT_1-type (5-HT_{1A} and 5-HT_{1D}) receptors was also low when compared to serotonin (Table I). However, the binding potency of 1, 3, 4, and 5 at 5-HT_2 type (5-HT_2 and 5-HT_{1C}) receptors was similar to that of the natural substrate, serotonin (Table I). While there did not appear to be any trend within the series of compounds at 5-HT_{1C} receptors, the primary tryptamines 4 and 5 appeared to possess greater affinity for the 5-HT_2 receptor than the tertiary tryptamines 1 and 3, especially when the radioligand used in the binding assay was an agonist ([^{125}I]DOI, Table I). The primary tryptamines 4 and 5 were as potent as serotonin in this assay, while the tertiary tryptamines 1 and 3 were slightly less potent binders than serotonin in this assay. These results led us to examine the receptor-mediated effects of these compounds at cortical 5-HT_2 receptors.

In the rat brain cerebral cortical slice preparation, agonist stimulation of 5-HT_2 receptors results in the hydrolysis of phosphatidylinositol (PI) leading to the

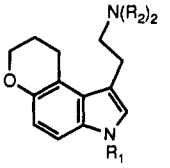
(6) Substitution at C6 of the indole nucleus of tryptamines generally reduces or abolishes their affinity for serotonin receptors. For example, see Glennon, R. A. and Gessner, P. K. Serotonin Receptor Binding Affinities of Tryptamine Analogies. *J. Med. Chem.* 1979, *22*, 428–432.

(7) By present conventions (i.e. using second messenger effects), the 5-HT_{1C} receptor and 5-HT_2 receptor appear to be in the same receptor family since both receptors are positively coupled to phosphatidylinositol hydrolysis. For a further discussion, see Hartig, P. R. Molecular Biology of 5-HT Receptors. *Trends in Pharmacol. Sci.* 1989, *10*, 64–69.

(8) Glennon, R. A.; Chaurasia, C.; Titeler, M. Binding of Indolylalkylamines at 5-HT_2 Serotonin Receptors: Examination of a Hydrophobic Binding Region. *J. Med. Chem.* 1990, *33*, 2777–2784.

(9) The reduction of indole-3-glyoxamides has been an important approach in the synthesis of tryptamine derivatives. For a discussion of this work, see Remers, W. A. Indole Aldehydes and Ketones. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Taylor, E. C., Eds.; John Wiley and Sons: New York, 1979; Vol. 25, Part III, p 405.

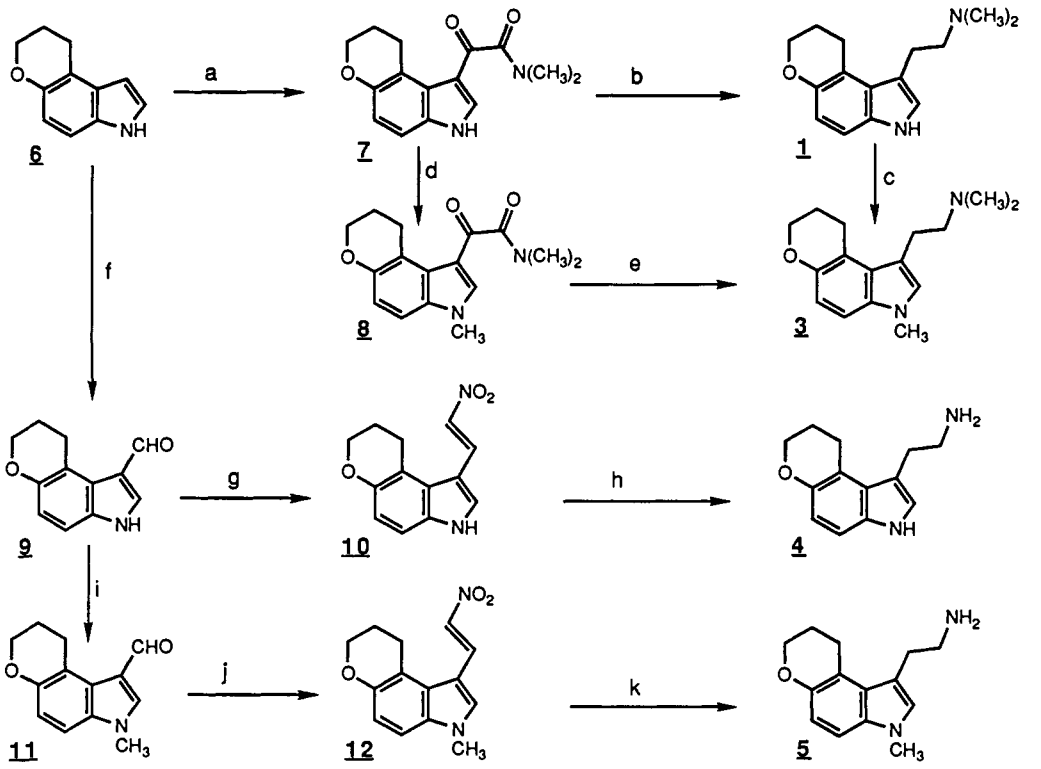
Table I. Binding Data on Pyrano[3,2-*e*]indoles (1–5)



	IC$_{50}$,[a] nM				
	5-HT$_{1A}$	5-HT$_{1D}$	5-HT$_{1C}$	5-HT$_2$(DOI)	5-HT$_2$(ket)
serotinin	5.2 ± 1.5 [15]	3.0 ± 0.3 [3]	81 ± 30 [3]	20 ± 1 [4]	2400 ± 400 [4]
1 (R_1 = H, R_2 = CH_3)	1900 ± 300 [3]	220 ± 20 [3]	150 ± 60 [4]	63 ± 19 [4]	1900 ± 600 [4]
2 (pyrano[2,3-*f*]indole)	1200 ± 300 [6]	1200 ± 200 [3]	2500 ± 300 [4]	920 ± 60 [3]	5900 ± 1500 [4]
3 (R_1 = CH_3, R_2 = CH_3)	5400 ± 100 [3]	9300 ± 1000 [3]	100 ± 15 [4]	81 ± 35 [3]	1000 ± 100 [3]
4 (R_1 = H, R_2 = H)	610 ± 130 [3]	110 ± 30 [3]	41 ± 7 [3]	14 ± 8 [3]	2200 ± 1000 [3]
5 (R_1 = CH_3, R_2 = H)	5300 ± 700 [3]	4200 ± 800 [3]	100 ± 75 [3]	14 ± 2 [3]	800 ± 80 [3]
3-[2-(*N*,*N*-dimethylamino)-ethyl]-5-methoxyindole	11 ± 4 [3]	49 ± 5 [4]	250 ± 80 [3]	130 ± 50 [3]	1800 ± 200 [4]

[a] The number in brackets is the number of experiments. X ± SEM.

Scheme I



a = 1) oxalyl chloride, ether; 2) dimethylamine, ether	e = 1) BH_3 in THF; 2) CsF, Na_2CO_3, EtOH, Δ	i = 1) NaH, THF; 2) CH_3I
b = 1) BH_3 in THF; 2) CsF, Na_2CO_3, EtOH, Δ	f = 1) $POCl_3$ in DMF; 2) NaOH, H_2O, Δ	j = CH_3NO_2, NH_4OAc (cat.), Δ
c = 1) KO*t*Bu, 18-crown-6, THF; 2) CH_3I	g = CH_3NO_2, NH_4OAc (cat.), Δ	k = LAH, THF, Δ
d = 1) NaH, DMF; 2) CH_3I	h = LAH, THF, Δ	

accumulation of inositol phosphates (IP).[10] This system was utilized to determine whether test agents are agonists or antagonists of 5-HT$_2$ receptor coupled PI turnover. In this regard, the response to the endogenous substrate serotonin (100 μM) and to the synthetic 5-HT$_2$ agonist DOI (100 μM) is a 76% and 66% stimulation of IP accumulation, respectively, above basal levels. The 100 μM concentrations of 1, 3, 4, and 5 (dissolved in 3.2% ethanol in 0.033 M HCl) also produced significant elevations in [^{3}H]IP accumulation above basal levels (32%, 42%, 54%, and 155%, respectively, Figure 1), and the magnitude of the response observed for each compound was found to be concentration dependent (Figure 2 and data not shown). There was no significant increase in IP accumulation with the acidified ethanol vehicle (data not shown). The concentration of compounds 1, 3, 4, and 5 producing a half-maximal stimulation of [^{3}H]IP accu-

(10) a) Conn, P. J.; Sanders-Bush, E. Regulation of Serotonin-Stimulated Phosphoinositide Hydrolysis: Relation to the Serotonin 5-HT$_2$ Binding Site. *J. Neurosci.* **1986**, *6*, 3669–3675. (b) Conn, P. J.; Sanders-Bush, E. Serotonin-Stimulated Phosphoinositide Turnover: Mediation by the S$_2$ Binding Site in Rat Cerebral Cortex but Not in Subcortical Regions. *J. Pharmacol. Exp. Ther.* **1985**, *234*, 195–203. (c) Conn, P. J.; Sanders-Bush, E. Selective 5-HT$_2$ Antagonists Inhibit Serotonin Stimulated Phosphatidylinositol Metabolism in Cerebral Cortex. *Neuropharmacology* **1984**, *8*, 993–996. (d) Kendall, D. A.; Naharski, S. R. 5-Hydroxytryptamine-Stimulated Inositol Phospholipid Hydrolysis in Rat Cerebral Cortex Slices: Pharmacological Characterization and Effects of Antidepressants. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 473–479.

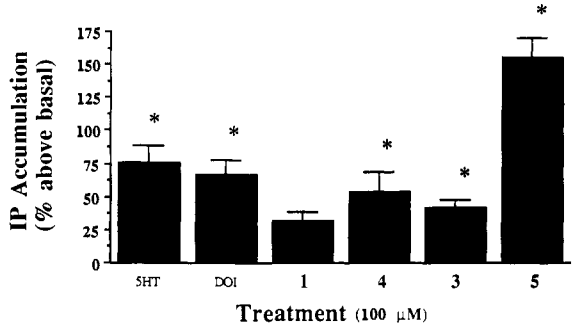


Figure 1. Effect of 5HT, DOI, and compounds **1**, **3**, **4**, and **5** on the accumulation of [^{3}H]inositol phosphates ([^{3}H]IP) in rat brain cerebral cortical slices. The height of each bar represents the mean % increase of [^{3}H]IP accumulation ±SEM of triplicate determinations. Each agent was tested at a final concentration of 100 μM. [*] Indicates significant difference from basal, $p < 0.05$, t-test. The response to compound 1 was significantly ($p < 0.05$) elevated compared to basal in three out of five experiments.

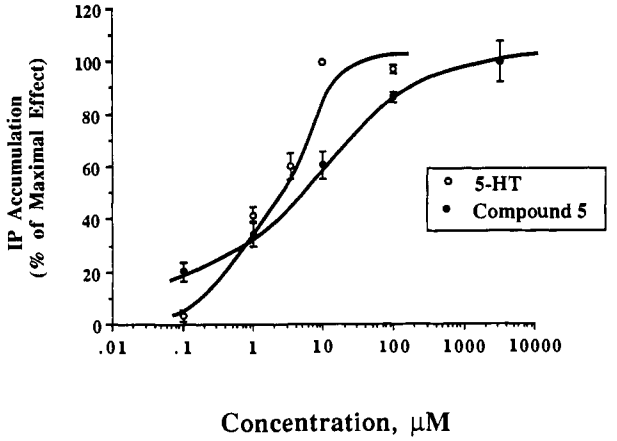


Figure 2. Concentration–effect curves for stimulation of [^{3}H]-IP accumulation by 5HT and compound **5** in rat brain cerebral cortical slices. Each point represents the mean ±SEM of 3–10 determinations each performed in triplicate.

mulation (EC_{50}) was estimated from concentration–response curves similar to those depicted in Figure 2. The maximal response to **5** was 2–3-fold greater than that observed with 5-HT (data not shown). In contrast, the maximal response to **4** was approximately equal to that observed with 5-HT, while the maximal responses of **1** and **3** were less than that of 5-HT (data not shown). In this system, the response to 5-HT typically varies over a range of 50–100% stimulation of IP accumulation above basal levels. The EC_{50} value for 5-HT is 2.62 ± 0.48 μM ($n = 10$). **5** and **4** are approximately equipotent with 5-HT producing half-maximal effects on 5-HT_2 receptor coupled PI turnover at approximately 3–5 μM. **3** and **1** are less potent than these compounds having EC_{50} values between 100–200 μM. Therefore, the rank order of agonist potency at cortical 5-HT_2 receptors was **5** ≃ 5-HT > **4** ≫ **3** > **1**.

To determine whether the PI responses to this new series of 5-HT_2 agonists are mediated by stimulation of a 5-HT_2 receptor subtype, the effects of **5** and **4** on PI turnover were tested in the presence and absence of the muscarinic antagonist atropine (1 μM), the α_1-adrenergic antagonist prazosin (1 μM), and the 5-HT_2-selective antagonists ketanserin (100 nM) and ritanserin (100 nM) (Figure 3). The muscarinic receptor antagonist atropine and the α_1-adrenergic receptor antagonist prazosin were unable to block test-agent stimulation of [^{3}H]IP accumulation at doses that completely inhibit carbachol (muscarinic agonist)- or norepinephrine (α_1-adrenergic agonist)-induced PI turnover.[11] In contrast, both of the 5-HT_2-selective antagonists completely inhibited the response to each of the agents tested (Figure 3). The response to **5** (5 μM) was also blocked by 10 nM ketanserin. The response of 5-HT (100 μM) was blocked by ketanserin with a K_i of 0.76 ± 0.06 nM ($n = 6$) and by ritanserin with a K_i of 0.59 ± 0.47 nM ($n = 4$).

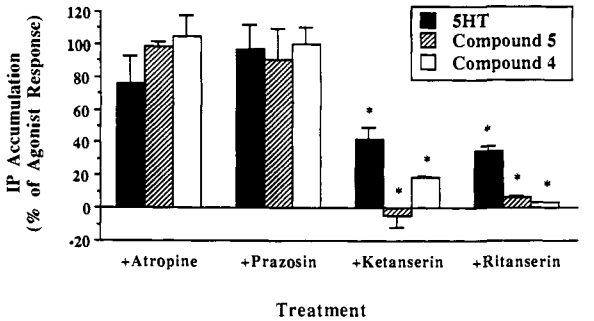


Figure 3. Effect of atropine (1 μM), prazosin (1 μM), ketanserin (100 nM), and ritanserin (100 nM) on the stimulation of [^{3}H]IP accumulation by 5HT (100 μM), compound **5** (5 μM), and compound **4** (3.3 μM) in rat brain cerebral cortical slices. The height of each bar represents the mean ±SEM of triplicate determinations. Data represents % of response induced by corresponding agonists. [*] Indicates significant reduction when compared to agonist alone (normalized to 100%), $p < 0.05$, t-test.

Discussion

While the pyrano[2,3-*f*]indole analog **2** of serotonin did not appear to have appreciable affinity for 5-HT_1 or 5-HT_2 receptors compared to serotonin, the isomeric pyrano-[3,2-*e*]indole analogs (**1**, **3**, **4**, and **5**) appeared to possess affinity for the 5-HT_2-type receptors (i.e. 5-HT_{1C} and 5-HT_2) similar to, if not more potent than, the natural substrate (5-HT) based on their receptor binding profiles (Table I). The affinity of **1**, **3**, **4**, and **5** for the 5-HT_1 receptors which are present in the primate central nervous system (i.e. 5-HT_{1A} and 5-HT_{1D}) was low when compared to serotonin. While **1** and **4** had the highest degree of 5-HT_1 activity among this series of compounds, IC_{50} values for these compounds were 40 times less potent than serotonin itself. These compounds are poor 5-HT_1 ligands, although the marginal selectivity of **1** and **4** for the 5-HT_{1D} receptor versus the 5-HT_{1A} receptor is intriguing since few compounds have appeared in the literature with any selectivity for the 5-HT_{1D} receptor. This poor affinity for 5-HT_1 receptors is significant because the minor modification (i.e. the fused dihydropyran ring) to serotonin incorporated in **1** and **2** had major affects on the affinity of these compounds for 5-HT_1 receptors.

This loss of activity for 5-HT_1 receptors could be explained by either of two hypotheses. It is possible that the directionality of the C5-oxygen in the pyrano[3,2-*e*]-indole derivatives (**1**, **3**, **4**, and **5**) might be less than optimal for hydrogen bond accepting interactions in 5-HT_1 receptors. While this hypothesis might then suggest that the pyrano[2,3-*f*]indole **2**, which possesses the opposite directionality of the C5-oxygen lone pairs of electrons, should have potent 5-HT_1 receptor affinity, the steric effects of C6 substitution in **2** probably inhibit potent binding to 5-HT receptors.[6] An alternative hypothesis

(11) Nomura, S.; Zorn, S. H.; Enna, S. J. Selective Interaction of Tricyclic Antidepressants with a Subclass of Rat Brain Cholinergic Receptors. *Life Sci.* **1987**, *40*, 1751–1760.

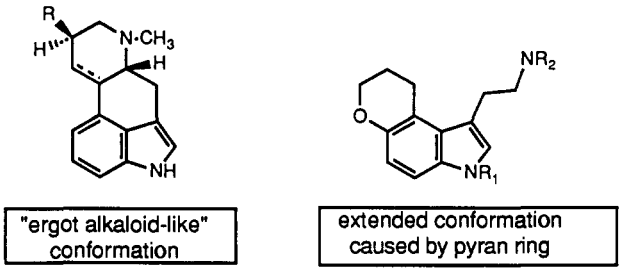


Figure 4.

to explain the low affinity of these compounds for 5-HT$_1$ receptors is that the low affinity of **1**, **3**, **4**, and **5** for 5-HT$_1$ receptors might be a result of steric interference between the dihydropyran ring and the C3-(aminoethyl) side chain which inhibits the tryptamine from adopting an "ergot alkaloid like" conformation which probably approximates the conformation of serotonin in 5-HT$_1$ receptors (Figure 4). Molecular modeling calculations confirm that the aminoethyl side chain in **1** would be severely energetically disfavored from adopting an ergot alkaloid like conformation, and that the lowest energy state conformation of this side chain is fully extended away from the dihydropyran ring. While **2** could achieve an "ergot alkaloid like" conformation, the steric interference at C6 probably destroys all affinity for 5-HT receptors, especially if this is the area where the hydrogen bonding interaction occurs between 5-HT receptors and the C5-hydroxyl group in serotonin. On the basis of binding and PI turnover data, the active conformation of the aminoethyl side chain of serotonin required by a 5-HT$_2$ receptor is easily achieved by **1**, **3**, **4**, and **5**. The synthesis of conformationally restricted analogs of **1** could examine the requirements of the aminoethyl side chain of serotonin in 5-HT receptors, and this is being pursued in our laboratories.

From both binding and functional data, it is apparent that the degree of methylation of the nitrogen atoms in **1**, **3**, **4**, and **5** has a significant effect on the affinity, selectivity, and potency of this series of compounds for the 5-HT$_2$ receptor. It has been known that 5-HT$_1$ affinity can be severely diminished via alkylation of the indole nitrogen.[8] This is likely a result of steric interference in the case of 5-HT$_1$ receptors, and the lack of affinity of 2-methylserotonin for 5-HT$_1$ receptors probably is a result of a similar steric interaction in 5-HT$_1$ receptors. As expected, the products (**3** and **5**, respectively) of indole N1-methylation of **1** and **4** were essentially devoid of affinity for 5-HT$_1$ receptors compared to serotonin. Accordingly, these indole *N*-CH$_3$ tryptamines (**3** and **5**) have the highest degree of 5-HT$_2$ receptor selectivity versus 5-HT$_1$ receptors in this series *when compared to serotonin*. Agonist efficacy of **1**, **3**, **4**, and **5** at cortical 5-HT$_2$ receptors[12] was measured by examining receptor-coupled phosphatidylinositol (PI) turnover (Figure 1).[10] While all four compounds had a 5-HT$_2$ receptor agonist-like effect (i.e. increase in PI turnover as measured by an increase in inositol phosphate (IP) accumulation in the presence of lithium ion), the primary tryptamines (**4** and **5**) were the most efficacious in this response with **5** being 2–3 times more efficacious than the natural substrate (5-HT) (data not shown). IP accumulation induced by **4** and **5** was potently antagonized by the 5-HT$_2$ antagonists ketanserin and ritanserin, but not by the muscarinic antagonist atropine or by the α_1-adrenergic antagonist prazosin, providing further evidence that these agents induce PI turnover by selective stimulation of the 5-HT$_2$ receptor subtype in cerebral cortex.

The results of this work suggest some important molecular recognition elements concerning cortical 5-HT$_2$ receptors. Since **5** is as at least as potent and efficacious as 5-HT as an agonist at cortical 5-HT$_2$ receptors, it can be inferred that **5** and 5-HT interact with cortical 5-HT$_2$ receptors in a similar, if not identical fashion. From this understanding, the hydrogen-bonding function of the C5-oxygen in **5** within a 5-HT$_2$ receptor can be described as that of a hydrogen bond acceptor. This is a significant conclusion, especially in light of the fact that recent modeling studies[13] of this receptor suggest the opposite role for the C5-hydroxyl group in 5-HT (i.e. as a hydrogen bond donor) within a 5-HT$_2$ receptor. The results of our studies are consistent with the C5-hydroxyl group in 5-HT functioning as a *hydrogen bond acceptor* in a 5-HT$_2$ receptor. Accordingly, the receptor (possibly a serine or threonine in the binding domain of the receptor) functions as the hydrogen bond donor in this receptor binding interaction. Because **5** represents a rotationally restricted phenolic analog of 5-HT, the directionality of this hydrogen bonding interaction with a 5-HT$_2$ receptor can also be described. Figure 5 depicts the area where a potent hydrogen bond accepting interaction[14] can occur between **5** (as hydrogen bond acceptor) and a 5-HT$_2$ receptor (as the hydrogen bond donor). This interaction, which is depicted by the yellow contour, is down and away from C6 in the 5-HT molecule or **5** as depicted in Figure 5. This conclusion may represent an important clue for the construction of a pharmacophoric model of the 5-HT$_2$ receptor. Further research in our laboratories will attempt to further develop such a model.

An alternative explanation for the hydrogen bonding interaction between **5** and the 5-HT$_2$ receptor might hypothesize that the receptor (i.e. a serine or threonine in the binding domain of the receptor) can function either as a hydrogen bond donor or receptor, depending on the nature of the ligand presented. This would also be entirely consistent with our results, and at this point in time, we are unable to differentiate between these two hypotheses. In either case, one would expect that the contour in Figure 5 to define the directionality of that hydrogen bonding interaction.

The results of this study also give some novel insight into the active conformation of the aminoethyl side chain

(12) It is believed that the type of 5-HT$_2$ receptor in cortical tissue is primarily the "classical" 5-HT$_2$ or S$_2$ receptor (as opposed to the 5-HT$_{1C}$ receptor) since little mRNA for the 5-HT$_{1C}$ receptor can be found in the cortex. Further discussion of this can be found in (a) Mengod, G.; Nguyen, H.; Le, H.; Waeber, C.; Lübbert, H.; Palacios, J. M. The Distribution and Cellular Localization of the Serotonin 1C Receptor mRNA in the Rodent Brain Examined By In Situ Hybridization Histochemistry. Comparison with Receptor Binding Distribution. *Neuroscience* **1990**, *35*, 577–591. (b) Molineux, S. M.; Jessel, T. M.; Axel, R.; Julius, D. 5-HT$_{1C}$ Receptor is a Prominent Serotonin Receptor in the Central Nervous System. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6793–6797. (c) Claustre, Y.; Rouquier, L.; Scatton, B. Pharmacological Characterization of a Serotonin-Stimulated Phosphoinositide Turnover in Brain Regions of the Immature Rat. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 1051–1056.

(13) Hibert, M. F.; Trumpp-Kallmeyer, S.; Bruinvels, A.; Hoflack, J. Three-Dimensional Models of Neurotransmitter G-Binding Protein-Coupled Receptors. *Mol. Pharmacol.* **1991**, *40*, 8–15.

(14) Contours were created with GRIN and GRID, version 5.15, supplied by Molecular Discovery Limited, Oxford OX2 9LL, England. See Goodford, P. J. A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.* **1985**, *28*, 849–857. The contours represent areas of favorable hydrogen bonding interactions between the 5-HT$_2$ ligands (hydrogen bond acceptor) and a serine-like hydroxyl probe [hydrogen bond donor] at an energy value for bonding of −4.2 kcal/mol.

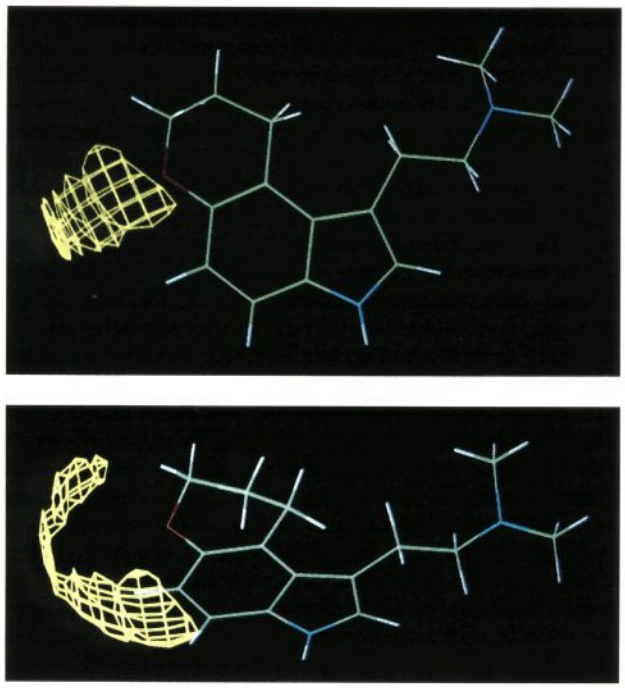


Figure 5. Contours were created with GRIN and GRID, version 5.15, supplied by Molecular Discovery Ltd., Oxford OX2 9LL, England. The yellow contour/grid in the two different views represents the area where a potent hydrogen bond (-4.2 kcal/mol) interaction can occur between compound **5** (as hydrogen bond acceptor) and a cortical 5-HT_2 receptor (as hydrogen bond donor). The directional nature of this hydrogen bonding interaction can be inferred from the equal efficacy and potency of compound **5** when compared with 5-HT at cortical 5-HT_2 receptors.

of serotonin in 5-HT receptors. Since **4** and **5** have comparable or better efficacy than serotonin at 5-HT_2 receptors, it would appear that **4** and **5** can easily adopt the active configuration of the aminoethyl side chain required by a 5-HT_2 receptor. As stated before, this configuration is probably not like that of an ergot alkaloid (Figure 4) due to energy considerations. This would suggest that the conformation of the aminoethyl side chain of serotonin in a 5-HT_2 receptor is extended away from the dihydropyrano[3,2-*e*]indole nucleus as depicted in Figure 4, but further studies are needed to confirm this hypothesis. However, because **4** and **5** have considerably reduced affinity for 5-HT_1 receptors when compared to the natural substrate serotonin, these results suggest that *the active conformation of the aminoethyl side chain of serotonin differs significantly between 5-HT_2 and 5-HT_1 receptors*. While ergot alkaloids are potent ligands at both 5-HT_1 and 5-HT_2 receptors, it is possible ergolines do not have coincident binding orientations within 5-HT_1 and 5-HT_2 receptors.

Further research needs to be done to explore the possibility that the conformational requirements of the aminoethyl side chain in serotonin are significantly different between 5-HT_1 and 5-HT_2 receptor subtypes. Incorporation of the aminoethyl group of **5** into different conformationally rigid systems should provide keener insight into these different conformational requirements between 5-HT receptor subtypes, and targets of this nature are presently being pursued in our group.

Separation of 5-HT_{1C} receptor affinity from 5-HT_2 receptor affinity has not yet been addressed. While compounds **1**, **3**, **4**, and **5** all show approximately the same affinity for the 5-HT_{1C} receptor, there is a defined rank order of agonist activity at cortical 5-HT_2 receptors (i.e. **5** $\simeq$ 5-HT > **4** $\gg$ **3** > **1**). Therefore, within this small series of compounds, **5** could be regarded as the most selective agonist for cortical 5-HT_2 receptors (versus 5-HT_{1A}, 5-HT_{1D}, and 5-HT_{1C} receptors). Since **1** demonstrated the least potency and efficacy as an agonist at cortical 5-HT_2 receptors, the potential for it to be used as a starting point for the design of a selective agonist for the 5-HT_{1C} receptor (versus the 5-HT_2 receptor) needs to be explored. Clearly, the functional activity of compounds **1**, **3**, **4**, and **5** at the 5-HT_{1C} receptor needs to be fully characterized, and this work is ongoing. The degree of methylation on the basic amine in the aminoethyl side chain may provide a clue towards an understanding of the SAR differentiation between cortical 5-HT_2 receptors and 5-HT_{1C} receptors. From this small group of compounds, it would appear that the 5-HT_2 receptor is less tolerant of methylation at the basic amine in the aminoethyl side chain of serotonin than the 5-HT_{1C} receptor, since the primary tryptamines (**5** and **4**) were considerably more potent and efficacious at cortical 5-HT_2 receptors than the tertiary tryptamines (**1** and **3**). Further work in this area should validate this observation.

Conclusion

1-(2-Aminoethyl)-3-methyl-8,9-dihydropyrano[3,2-*e*]indole (**5**) represents an important new tool for the study of 5-HT_2 receptors. It is a direct and simple tryptamine analog of serotonin with extremely low affinity for 5-HT_1 receptors when compared to serotonin. That is, the 5-HT_1 affinity of 5-HT has been removed from **5** via only minor modifications. Compound **5** appears to display full agonist activity at cortical 5-HT_2 receptors with an efficacy that exceeds the natural substrate. Therefore, **5** can be viewed as a serotonin mimic at the 5-HT_2 receptor, and this suggests that (1) the C5-hydroxy group of serotonin can function as a hydrogen bond acceptor in a 5-HT_2 receptor with a directionality of interaction which is down and away from C6 in serotonin as depicted in Figure 5; and (2) the conformational requirements of the aminoethyl side chain in serotonin differ between 5-HT_1 receptors and 5-HT_2 receptors. The construction of analogs of serotonin which incorporate both rotational restriction of the C5-hydroxy group and conformational rigidity of the aminoethyl side chain should provide further understanding of the specific molecular recognition requirements of 5-HT receptors. Our laboratories are eagerly pursuing this line of research.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover open capillary melting point apparatus and are uncorrected. Infrared spectra were obtained from a Perkin-Elmer IR-283B infrared spectrophotometer, and NMR spectra were recorded on either a Bruker AM-300 (300 MHz), Varian XL300 (300 MHz), or Varian XL250 (250 MHz) spectrometer. NMR data are reported in parts per million (δ) and are referenced to the proton or carbon signal from the sample solvent. Low-resolution mass spectra were obtained on a Finnigan 4310 instrument; high-resolution mass spectra (EI and FAB) were obtained on a Kratos Concept IS instrument. Elemental analyses were performed at Central Research Division, Pfizer Inc, Groton, CT. Elemental analyses were within $\pm 0.40\%$ of the calculated values.

Commercial reagents (Aldrich Chemical Co.) were utilized without further purification, including Aldrich anhydrous solvents. Diethyl ether was dried via distillation over sodium hydride. Chromatography refers to column chromatography

performed using 32–63 μm silica gel (approximately 50 g silica gel per gram of material to be chromatographed) and executed under nitrogen pressure (flash chromatography) conditions. Room temperature (rt) refers to 20–25 °C. The synthesis of compounds 1, 2, 6, and 7 have been reported elsewhere.[4e]

1-[2-(Dimethylamino)ethyl]-3-methyl-8,9-dihydropyrano-[3,2-*e*]indole (3). Method A. To a stirred solution of 1 (0.100 g, 0.41 mmol) and 18-crown-6 (0.120 g, 0.45 mmol, 1.1 equiv) in anhydrous tetrahydrofuran (5 mL) at room temperature under nitrogen was added potassium *tert*-butoxide (0.050 g, 0.45 mmol, 1.1 equiv) in one portion. The resulting reaction mixture was stirred at room temperature for 15 min, and then methyl iodide (30.6 μL, 0.49 mmol, 1.2 equiv) was added. The reaction was then stirred at room temperature for 3 h. A saturated solution of sodium hydrogen carbonate (10 mL) was added, and the resulting aqueous mixture was extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was chromatographed using elution with 5% triethylamine in ethyl acetate to afford 3 (0.051 g, 48%) as a clear, colorless oil identical in its physical and spectral properties to the material synthesized in method B.

Method B. To a stirred solution of 8 (0.72 g, 2.51 mmol) in anhydrous THF (7 mL) at 0 °C was added dropwise a solution of borane in THF (1.0 M, 10 mL, 10.0 mmol, 4.0 equiv). The resulting reaction solution was stirred at rt under nitrogen for 20 h. A saturated solution of sodium hydrogen carbonate (15 mL) was added carefully to the reaction solution, and the resulting aqueous mixture was extracted with ether (2 × 30 mL). The organic extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting white foam (1.06 g) was dissolved in absolute ethanol (15 mL), and sodium carbonate (1.06 g) and cesium fluoride (1.06 g) were added to this solution. The resulting mixture was heated at reflux under nitrogen for 20 h. The reaction mixture was then filtered through Celite, and the filtrate was evaporated under reduced pressure. Chromatography of the residue using silica gel (approximately 50 g) and elution with 6% triethylamine in ethyl acetate afforded 3 (0.34 g, 1.32 mmol, 52%) as a clear, colorless oil: IR (neat) 1610, 1580, 1540, 1485, 1440, 1415 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.00 (d, J = 8.8 Hz, 1 H), 6.80 (s, 1 H), 6.74 (d, J = 8.8 Hz, 1 H), 4.18 (t, J = 5.1 Hz, 2 H), 3.66 (s, 3 H), 3.23 (t, J = 6.6 Hz, 2 H), 3.06–3.01 (m, 2 H), 2.61–2.55 (m, 2 H), 2.34 (s, 6 H), 2.13–2.05 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 148.3, 132.3, 127.2, 126.1, 113.3, 112.9, 112.9, 108.1, 65.9, 62.3, 45.6, 32.6, 25.6, 22.8, 22.7; LRMS (m/z, rel inten) 258 (M^+, 46), 200 (72), 184 (12), 58 (100). Anal. ($C_{16}H_{22}N_2O$) C, H, N.

1-(2-Aminoethyl)-8,9-dihydropyrano[3,2-*e*]indole (4). To a stirred mixture of lithium aluminum hydride (0.70 g, 18.4 mmol, 5.3 equiv) in anhydrous THF (15 mL) was added 10 (0.85 g, 3.48 mmol) as a solid portionwise. The resulting pink mixture was stirred at rt under nitrogen for 20 h. Ethyl acetate (15 mL) was then added carefully followed by water (1 mL) and sodium sulfate decahydrate (5 g). The resulting mixture was stirred at rt under nitrogen for 1 h and filtered through Celite, and the filtrate was evaporated under reduced pressure. The residual oil was chromatographed using silica gel (approximately 50 g) and elution with methanol/triethylamine/ethyl acetate (3:2:15) to afford 4 (0.45 g, 2.08, mmol, 60%) as a white foam: ^1H NMR ($CDCl_3$) δ 8.70 (br s, NH), 7.04 (d, J = 8.7 Hz, 1 H), 6.89 (s, 1 H), 6.70 (d, J = 8.8 Hz, 1 H), 4.17 (t, J = 5.0 Hz, 2 H), 3.18 (t, J = 6.5 Hz, 2 H), 2.99 (s, 4 H), 2.10–2.02 (m, 2 H), 1.81 (br s, NH_2); ^{13}C NMR ($CDCl_3$) δ 148.4, 131.7, 125.7, 123.2, 113.8, 113.2, 113.2, 110.2, 65.9, 43.5, 31.2, 22.9, 22.7. This foam was dissolved in methylene chloride/methanol (100:1, 10 mL), and maleic acid (0.241 g, 2.08 mmol) was added. The resulting salt was filtered to afford 4·maleate (0.68 g, 98% salt formation) as a white solid: mp 162.0–164.0 °C with effervescence; IR (KBr) 1620, 1575, 1540, 1520, 1495, 1470, 1460 cm^{-1}; ^1H NMR (CD_3OD) δ 7.05 (s, 1 H), 7.04 (d, J = 8.8 Hz, 1 H), 6.57 (d, J = 8.8 Hz, 1 H), 6.22 (s, 2 H [maleate olefin]), 4.97 (br s, 5 H), 4.11 (t, J = 5.1 Hz, 2 H), 3.20 (s, 4 H), 3.15 (t, J = 6.6 Hz, 2 H), 2.10–2.01 (m, 2 H); ^{13}C NMR (CD_3OD) δ 170.8, 149.6, 136.6, 133.6, 126.4, 125.0, 114.2, 113.4, 111.4, 110.9, 66.8, 42.5, 26.4, 23.9, 23.9; LRMS (m/z, rel inten) 216 (74, M^+), 187 (82), 186 (100), 158 (44), 143 (22), 72 (27). Anal. ($C_{13}H_{16}N_2O \cdot C_4H_4O_4 \cdot {}^1/_4H_2O$) C, H, N.

1-(2-Aminoethyl)-3-methyl-8,9-dihydropyrano[3,2-*e*]indole (5). To a stirred mixture of lithium aluminum hydride (0.82 g, 21.6 mmol, 5.3 equiv) in anhydrous THF (20 mL) was added 12 (1.05 g, 4.07 mmol) as a solid portionwise. The resulting reaction mixture was stirred at rt under nitrogen for 24 h and then heated at reflux for 2 h. Sodium sulfate decahydrate (5 g) was then added carefully, the resulting mixture was stirred at rt under nitrogen for 24 h and filtered through Celite, and the filtrate was evaporated under reduced pressure. The residual oil was chromatographed using silica gel (approximately 50 g) and elution with methylene chloride/methanol/ammonium hydroxide (9:1:0.1) to afford 5 (0.42 g, 1.82 mmol, 45%) as a purple oil: ^1H NMR ($CDCl_3$) δ 7.02 (d, J = 8.8 Hz, 1 H), 6.81 (s, 1 H), 6.75 (d, J = 8.8 Hz, 1 H), 4.18 (t, J = 5.1 Hz, 2 H), 3.68 (s, 3 H), 3.19 (t, J = 6.6 Hz, 2 H), 2.99–2.98 (m, 4 H), 2.13–2.04 (m, 2 H), 1.97 (br s, NH_2); ^{13}C NMR ($CDCl_3$) δ 148.3, 132.5, 127.7, 126.0, 113.3, 112.9, 112.2, 108.1, 65.9, 43.7, 32.7, 31.0, 22.9, 22.7. This oil was dissolved in ethyl acetate (20 mL), maleic acid (0.211 g, 1.82 mmol) was added as a solid, and the resulting mixture was stirred at rt under nitrogen for 24 h. The resulting solid was filtered to yield 5·maleate (0.63 g, 100% salt formation) as a white solid: mp 200 °C with effervescence; IR (KBr) 1620, 1565, 1555, 1550, 1490, 1475, 1455, 1420 cm^{-1}; ^1H NMR (CD_3OD) δ 7.03 (d, J = 8.8 Hz, 1 H), 6.96 (s, 1 H), 6.63 (d, J = 8.8 Hz, 1 H), 6.21 (s, 2 H [maleate olefin]), 4.92 (br s, 4 H), 4.12 (t, J = 5.1 Hz, 2 H), 3.68 (s, 3 H), 3.18 (s, 4 H), 3.14 (t, J = 6.8 Hz, 2 H), 2.09–2.00 (m, 2 H); ^{13}C NMR (CD_3OD) δ 170.9, 149.8, 136.7, 134.2, 129.3, 126.9, 114.2, 113.8, 110.0, 109.4, 66.8, 42.4, 32.8, 26.2, 23.8; LRMS (m/z, rel inten) 230 (32, M^+), 200 (100), 185 (11), 172 (10). Anal. ($C_{14}H_{18}N_2O \cdot C_4H_4O_4$) C, H, N.

***N,N*-Dimethyl-3-methyl-8,9-dihydropyrano[3,2-*e*]indole-1-glyoxamide (8).** To a stirred solution of 7[4e] (0.85 g, 3.12 mmol) in anhydrous DMF (10 mL) was added sodium hydride (60% in oil, 0.138 g, 3.45 mmol, 1.1 equiv), and the slowly effervescing mixture was stirred at rt under nitrogen for 30 min. Methyl iodide (0.21 mL, 3.37 mmol, 1.1 equiv) was then added dropwise to the reaction mixture, and the resulting mixture was stirred at rt under nitrogen for 12 h. The resulting reaction mixture was evaporated under reduced pressure, and the residue was placed in a saturated solution of sodium hydrogen carbonate (20 mL). This aqueous mixture was extracted with ethyl acetate (3 × 25 mL), and the organic extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure to afford an oil. Crystallization of this oil using ether afforded 8 (0.78 g, 2.72 mmol, 87%) as a white crystalline solid: mp 143.5–145.5 °C; IR (KBr) 1635, 1530, 1490, 1440, 1425 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 (s, 1H), 7.02 (d, J = 8.8 Hz, 1 H), 6.82 (d, J = 8.8 Hz, 1 H), 4.17 (t, J = 5.1 Hz, 2 H), 3.72 (s, 3 H), 3.42 (t, J = 6.6 Hz, 2 H), 3.05 (s, 3 H), 3.00 (s, 3 H), 2.05–1.97 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 185.2, 168.9, 151.5, 141.1, 133.1, 125.7, 116.2, 115.4, 114.2, 108.6, 66.2, 37.6, 34.2, 33.8, 25.5, 22.5; LRMS (m/z, rel inten) 286 (20, M^+), 214 (100), 186 (10), 158 (10). Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

8,9-Dihydropyrano[3,2-*e*]indole-1-carboxaldehyde (9). To a stirred solution of phosphorus oxychloride (1.2 mL, 12.9 mL, 1.1 equiv) in anhydrous DMF (4 mL) was added dropwise a solution of 6[4e] (2.00 g, 11.55 mmol) in anhydrous DMF (3 mL). The resulting mixture was heated at 40 °C under nitrogen for 1 h. The reaction solution was then poured onto ice (25 g), and an aqueous solution of sodium hydroxide (10% by weight, 20 mL) was added to this mixture. The resulting mixture was heated at reflux under nitrogen for 2 h. The resulting solution was cooled, the pH was adjusted to neutrality using 10% HCl, and this aqueous mixture was extracted with ethyl acetate (3 × 75 mL). The organic extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure, and the residual oil was passed through silica gel filter (approx 100 g) followed by a solution of ethyl acetate/hexanes (1:1, 2 L). The latter 1.5 L was evaporated under reduced pressure to afford 9 (2.02 g, 87%) as an off-white solid: mp 116.0–118.0 °C; IR (KBr) 1660, 1635, 1620, 1585, 1515, 1500, 1465, 1455, 1450, 1420, 1405 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.04 (s, 1 H), 9.73 (br s, NH), 7.87 (d, J = 2.8 Hz, 1 H), 7.16 (d, J = 8.8 Hz, 1 H), 6.81 (d, J = 8.8 Hz, 1 H), 4.20 (t, J = 5.1 Hz, 2 H), 3.31 (t, J = 6.6 Hz, 2 H), 2.13–2.05 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 185.3, 151.1, 136.2, 132.0, 124.7, 120.8, 115.1, 114.8, 111.0, 66.1, 25.1, 22.3; LRMS (m/z, rel inten) 201 (100,

M^+), 186 (12), 173 (31), 158 (12), 145 (75), 130 (17), 117 (25). Anal. ($C_{12}H_{11}NO_2$) C, H, N.

1-(2-Nitroethenyl)-8,9-dihydropyrano[3,2-*e*]indole (10). A solution of **9** (0.503 g, 2.50 mmol) and ammonium acetate (0.150 g, 1.94 mmol, 0.8 equiv) in nitromethane (8 mL) was heated at reflux under nitrogen for 2 h. The resulting reaction mixture was cooled, and the precipitated solid was filtered to afford **10** (0.582 g, 2.38 mmol, 95%) as a bright orange solid: mp 270 °C dec violently; IR (KBr) 1605, 1520, 1505, 1480, 1460, 1425 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.18 (br s, NH), 8.50 (d, J = 13.1 Hz, 1 H), 8.36 (s, 1 H), 8.04 (d, J = 13.0 Hz, 1 H), 7.21 (d, J = 8.7 Hz, 1 H), 6.68 (d, J = 8.7 Hz, 1 H), 4.12 (t, J = 5.0 Hz, 2 H), 3.10 (t, J = 6.5 Hz, 2 H), 2.08–1.99 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 150.3, 135.8, 131.5, 131.0, 130.8, 125.4, 113.8, 113.3, 111.7, 108.3, 65.3, 22.7, 21.8. Anal. ($C_{13}H_{12}N_2O_3$) C, H, N.

3-Methyl-8,9-dihydropyrano[3,2-*e*]indole-1-carboxaldehyde (11). To a stirred solution of **9** (1.48 g, 7.35 mmol) in anhydrous THF (20 mL) was added sodium hydride (60% in oil, 0.32 g, 8.00 mmol, 1.1 equiv), and the resulting effervescing mixture was stirred at rt under nitrogen for 15 min. Methyl iodide (0.46 mL, 7.39 mmol, 1.0 equiv) was then added dropwise, and the resulting reaction solution was stirred at rt under nitrogen for 1 h. A saturated solution of sodium hydrogen carbonate (15 mL) was added to the reaction mixture, and this aqueous mixture was extracted with ethyl acetate (3 × 25 mL). The organic extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure, and the residual oil was passed through a silica gel filter (100 g) followed first by methylene chloride/hexanes (1 L, 1:1) and then by 3% methanol in methylene chloride (1 L). The latter filtrate was evaporated under reduced pressure to afford **11** (1.31 g, 6.09 mmol, 83%) as an off-white solid: mp 147.5–148.5 °C; IR (KBr) 1640, 1610, 1585, 1520, 1490, 1475, 1455, 1430, 1420 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.96 (s, 1 H), 7.71 (s, 1 H), 7.07 (d, J = 8.8 Hz, 1 H), 6.84 (d, J = 8.8 Hz, 1 H), 4.20 (t, J = 5.1 Hz, 2 H), 3.79 (s, 3 H), 3.28 (t, J = 6.6 Hz, 2 H), 2.13–2.05 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 184.1, 151.3, 138.8, 132.9, 125.5, 119.4, 115.1, 114.8, 108.9, 66.2, 33.8, 25.1, 22.3; LRMS (m/z, rel inten) 215 (100, M^+), 187 (15), 186 (13), 159 (54), 158 (16), 144 (10), 131 (16), 130 (16). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

3-Methyl-1-(2-nitroethenyl)-8,9-dihydropyrano[3,2-*e*]indole (12). A mixture of **11** (1.12 g, 5.20 mmol) and ammonium acetate (0.30 g, 3.9 mmol, 0.75 equiv) in nitromethane (15 mL) was heated at reflux under nitrogen for 3 h. The reaction mixture was evaporated under reduced pressure, and the residual solid was dissolved in methylene chloride (20 mL). This solution was passed through a silica gel filter (approx 50 g) followed by methylene chloride (500 mL). This filtrate was evaporated under reduced pressure to afford an orange solid (1.3 g). Recrystallization of this solid in methylene chloride/hexanes (1:3) afforded **12** (1.25 g, 4.84 mmol, 93%) as a bright orange solid: mp 215 °C dec with effervescence; IR (KBr) 1610, 1605, 1590, 1525, 1490, 1465, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.56 (d, J = 13.2 Hz, 1 H), 7.54 (s, 1 H), 7.49 (d, J = 13.2 Hz, 1 H), 7.10 (d, J = 8.9 Hz, 1 H), 6.83 (d, J = 8.9 Hz, 1 H), 4.21 (t, J = 5.1 Hz, 2 H), 3.81 (s, 3 H), 3.18 (t, J = 6.6 Hz, 2 H), 2.17–2.09 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 151.1, 134.8, 132.5, 131.0, 130.8, 126.3, 114.7, 114.0, 109.4, 108.0, 66.0, 33.8, 23.4, 22.1; LRMS (m/z, rel inten) 258 (100, M^+), 241 (21), 211 (35), 184 (40), 168 (23), 154 (25), 139 (31). Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

Pharmacology. (2-[^{3}H](N))*myo*-Inositol 12.3 Ci/mmole), [^{3}H]-8-OH-DPAT, [^{3}H]serotonin, [^{3}H]mesulergine, [^{3}H]ketanserin, and [^{125}I]DOI were purchased from New England Nuclear, Boston, MA. Ketanserin tartrate and ritanserin were purchased from Research Biochemicals Inc. (Natick, MA). 5-HT, creatine sulfate, atropine sulfate and all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Binding Experiments. Receptor binding was conducted by using methods previously reported in the literature: 5-HT_{1A} using rat cortex and [^{3}H]-8-OH-DPAT;[15] 5-HT_{1C} using pig choroid plexus and [^{3}H]mesulergine;[16] 5-HT_{1D} using bovine caudate and [^{3}H]serotonin;[17] 5-HT_2 using rat anterior cortex and [^{3}H]-ketanserin;[18] and 5-HT_2 using rat anterior cortex and [^{125}I]DOI.[19] The concentration of radioligand used in competition studies was approximately equal to the K_D of the binding system.

Measurement of Inositol Phosphate Accumulation in Rat Brain Slices. [^{3}H]Inositol phosphate ([^{3}H]IP) accumulation in rat brain slices was examined using a modification[20] of the method originally described by Brown and co-workers.[21] In brief, rat brain cerebral cortical slices (0.30 × 0.30 mm) were prepared using a McIlwain tissue chopper and then incubated for 60 min at 37 °C in a modified Krebs–Ringer buffer (NaCl, 118 mM; KCl, 5 mM; $CaCl_2$, 1.3 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; and dextrose, 11.1 mM) oxygenated with 95% O_2/5% CO_2. During this time the buffer was changed at 15-min intervals, with 10 mM LiCl added before the final preincubation period. The slices were then preincubated for an additional 30 min at 37 °C with [^{3}H]*myo*-inositol (1 μCi/tube) in the presence of LiCl and 10 μM pargylline. After the prelabeling period, slices were exposed to the various test agents for 45 min under the same experimental conditions. Brain slices were exposed to antagonists 5–10 min prior to agonist exposure. The reaction was terminated by the addition of a chloroform/methanol (1:2 v/v) solution, and the water-soluble [^{3}H]IP's isolated by a batch technique employing a Dowex AG 1-X8 anion-exchange resin.[20]

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