Articles

Dihydrobenzofuran Analogues of Hallucinogens. 4.[1] Mescaline Derivatives[2]

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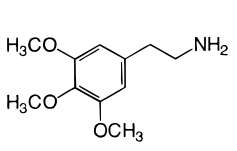
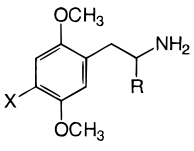
Dihydrobenzofuran and tetrahydrobenzodifuran functionalities were employed as conformationally restricted bioisosteres of the aromatic methoxy groups in the prototypical hallucinogen, mescaline (**1**). Thus, 4-(2-aminoethyl)-6,7-dimethoxy-2,3-dihydrobenzofuran hydrochloride (**8**) and 1-(8-methoxy-2,3,5,6-tetrahydrobenzo[1,2-*b*:5,4-*b*′]difuran-4-yl)-2-aminoethane hydrochloride (**9**) were prepared and evaluated along with **1** for activity in the two-lever drug discrimination (DD) paradigm in rats trained to discriminate saline from LSD tartrate (0.08 mg/kg). Also, **1**, **8**, and **9** were assayed for their ability to displace [^{3}H]ketanserin from rat cortical homogenate 5-HT$_{2A}$ receptors and [^{3}H]8-OH-DPAT from rat hippocampal homogenate 5-HT$_{1A}$ receptors. In addition, these compounds were evaluated for their ability to compete for agonist and antagonist binding to cells expressing cloned human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors. Finally, agonist efficacy was assessed by measurement of phosphoinositide hydrolysis in NIH 3T3 cells expressing the rat 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors. Although **1** fully substituted for LSD in the DD assays (ED$_{50}$ = 33.5 μmol/kg), neither **8** nor **9** substituted for LSD, with just 50% of the rats administered **8** selecting the drug lever, and only 29% of the rats administered **9** selecting the drug lever. All of the test compounds had micromolar affinity for the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in rat brain homogenate. Curiously, the rank order of affinities of the compounds at 5-HT$_{2A}$ sites was opposite their order of potency in the behavioral assay. An evaluation for ability to stimulate phosphoinositide turnover as a measure of functional efficacy revealed that all the compounds were of approximately equal efficacy to serotonin in 5-HT$_{2C}$ receptors. At 5-HT$_{2A}$ receptors, however, **8** and **9** were significantly less efficacious, eliciting only 61 and 45%, respectively, of the maximal response. These results are consistent with the proposed mechanism of action for phenethylamine hallucinogens, that such compounds must be full agonists at the 5-HT$_{2A}$ receptor subtype. In contrast to the 2,5-dimethoxy-substituted phenethylamines, where rigidification of the methoxy groups had no deleterious effect on activity, the loss of activity in the 3,4,5-trioxygenated mescaline analogues may suggest that the 3 and 5 methoxy groups must remain conformationally mobile to enable receptor activation.

Introduction

Peyote (*Lophophora williamsii*), a member of the *Cactaceae* family, represents one of the earliest known hallucinogenic plants, the use of which was exclusively limited to the New World.[3] The main constituent of peyote, mescaline (3,4,5-trimethoxy-β-phenethylamine, **1**), was first identified by Heffter in 1896 and was recognized as the active hallucinatory principle on the basis of rudimentary pharmacological testing and self experiments.[4] Following the first chemical synthesis of mescaline in 1919,[5] this alkaloid served as the prototypical structure in more than 75 years of structure−activity relationship (SAR) studies linking molecular structure to hallucinogen-like, or psychedelic,[6] activity.[7−12] In addition, the psychic−behavioral effects produced by mescaline were frequently used as a benchmark against which the effects of other hallucinogens were compared, and the potencies of novel compounds prepared in SAR investigations were for many years expressed in terms of "mescaline units".[13,14]



1, Mescaline



2

The successive SAR studies of **1**-like hallucinogens have ultimately led to agents of general structure **2** (R = H, CH$_3$; X = alkyl, halo, alkoxy, alkylthio, etc.) which currently are among some of the most potent hallucinogens known.[8,12,15,16] As such, compounds **2** have

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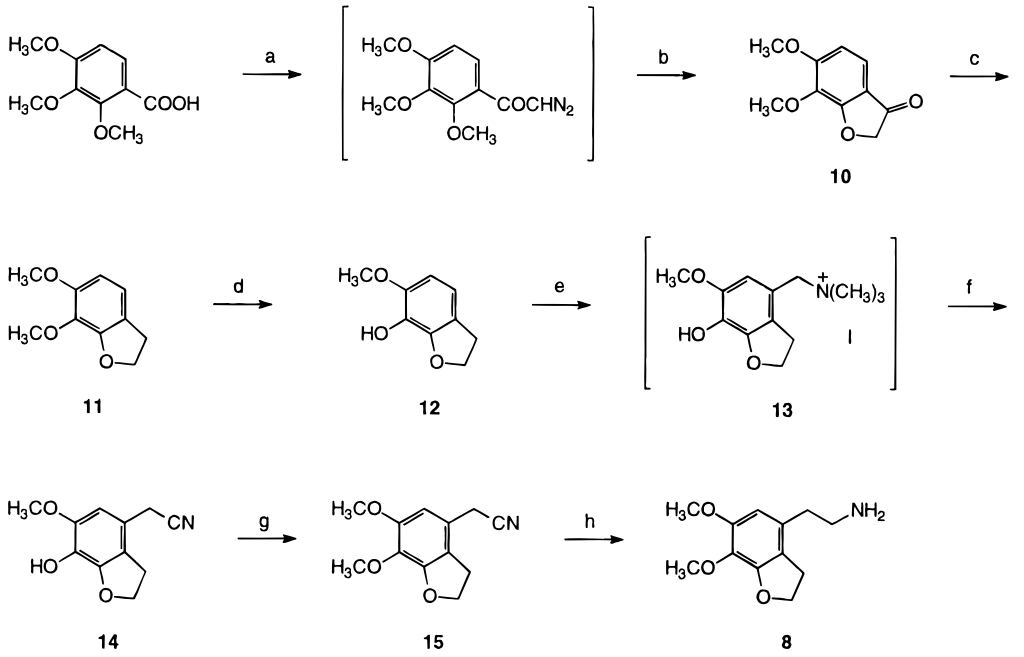
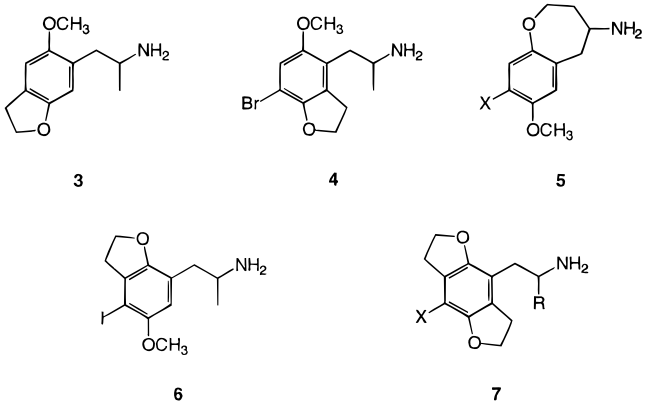
Scheme 1[a]

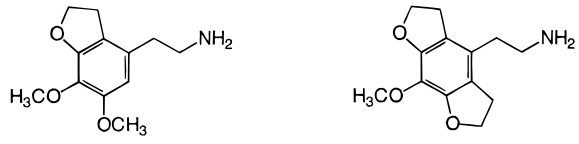


[a] Reagents: (a) $(COCl)_2$, PhH; CH_2N_2, Et_2O; (b) AcOH; (c) Pd/C, H_2, EtOH; (d) BCl_3, CH_2Cl_2; (e) H_2CO, $(CH_3)_2NH$ (aqueous), heat; CH_3I, iPrOH; (f) KCN, H_2O, heat; (g) CH_3I, K_2CO_3, Me_2CO, reflux; (h) Pd/C, H_2, EtOH, HCl.

typically been shown to have low nanomolar affinities for serotonin 5-HT_{2A} and 5-HT_{2C} receptors,[17−25] the primary sites believed to be involved in the mediation of the unique behavioral effects of hallucinogenic drugs.[26−29] Given the high potency and site-selectivity of **2** derivatives, they have served as convenient prototypes for further SAR studies using rigid analogs to probe the topography of the serotonin 5-HT_2 agonist binding sites.[30,31] Recently, compounds **3**,[32] **4**,[33,34] **5**,[35] and **6** and **7**[36] have all been synthesized and evaluated pharmacologically in our laboratory for hallucinogen-like behavioral activity and serotonin receptor affinities, to study the possible "active" conformations of the aromatic methoxy groups of **2** at the serotonin agonist binding site. We have reported that the dihydrobenzofuran structure serves as an effective, conformationally-restricted bioisostere of the aromatic methoxy group, and that the orientation of alkoxy groups exemplified by compound **7** most likely represents the active conformation of these groups during serotonin receptor activation.[36]



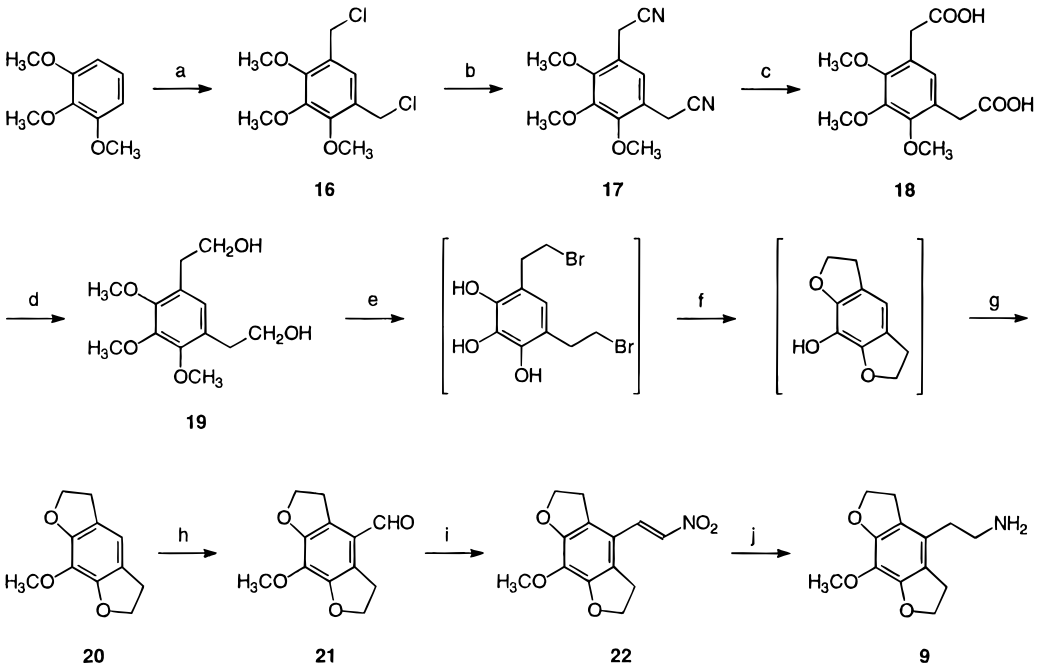
As an extension of our previous efforts with dihydrobenzofuranyl analogs of hallucinogenic phenethylamines, we sought to apply a similar design rationale and our established synthetic methodology to the mescaline prototype **1**, a structure containing three aromatic methoxy groups. Because **1** has been shown to have action at both serotonin and dopamine (DA) receptors,[37,38] and because hallucinogenic activity has generally been correlated with the overall hydrophobicity of the drug molecule,[39−41] it was hypothesized that **8** and **9** could ultimately have potential as probes of 5-HT, DA, or other central neurotransmitter receptors. As molecules with increased hydrophobicity relative to **1**, they might also be useful in evaluating the role of this physicochemical property in the production of hallucinogenic effects. Thus, we now report on the synthesis and preliminary pharmacological evaluation of rigid analogs of mescaline in which one (**8**) or two (**9**) of the three aromatic methoxy groups of **1** are tethered into rotationally constrained dihydrofuran rings.



Chemistry

The dihydrobenzofuranyl mescaline analog **8** was synthesized successfully as shown in Scheme 1. Construction of the furan ring was accomplished using the method of Jung and Abrecht.[42] Thus, 2,3,4-trimethoxybenzoic acid was converted to its corresponding diazomethylketone which was cyclized to the benzofuranone **10** upon treatment with acetic acid. Catalytic hydrogenation of the ketone then afforded the parent dihydrobenzofuran **11** in excellent yield. Initial unsuccessful attempts to elaborate the side chain of **11** involved the standard sequential procedure of electrophilic formylation, condensation of the aldehyde with nitromethane, and subsequent reduction of the nitrostyrene with

Scheme 2[a]



[a] Reagents: (a) H_2CO, HCl (g), concd HCl, dioxane; (b) NaCN, DMSO; (c) concd HCl, heat; (d) BH_3, THF; H_2O; (e) BBr_3 (excess), CH_2Cl_2; CH_3OH; (f) K_2CO_3, Me_2CO, reflux; (g) CH_3I, K_2CO_3, Me_2CO, reflux; (h) Cl_2CHOCH_3, $SnCl_4$, CH_2Cl_2; H_2O, ice; (i) CH_3NO_2, NH_4OAc; (j) $LiAlH_4$, THF; H_2O.

$LiAlH_4$ as described previously.[43] Because the 5-position of **11** was more susceptible to electrophilic attack, this position was first blocked by aromatic bromination which occurred in a facile and near-quantitative manner. (The bromine atom would then be removed during the subsequent LAH reduction step). Attempts to formylate the 5-bromo compound at the 4-position, however, gave complex mixtures of products that were extremely difficult to purify, and this method was abandoned. A possible explanation for the difficulties with this formylation may lie in the fact that some demethylation of the 7-methoxy group may have occurred in the presence of the Lewis acid catalyst. The steric crowding of this group causes it to lie out of plane, thus decreasing the overlap between the 7-methoxy lone pair electrons and the aromatic π system. Consequently, the 7-methoxy group is more basic than a planar aromatic ether and would be expected to be more labile to cleavage by Lewis acids such as the tin(IV) chloride used in this formylation step.

Fortunately, an alternative method for constructing phenethylamines existed in the literature that was well suited for mescaline-like compounds.[44,45] This methodology effects direct aromatic substitution at the 4-position as shown in Scheme 1. Thus, the 7-methoxy group of **11** was regioselectively demethylated using boron trichloride in dichloromethane.[46] The ring was functionalized at the 4-position of **12** under mild Mannich conditions, and the resulting crude Mannich base was quaternized to the methiodide salt **13** by addition of iodomethane to the reaction mixture.[47] Displacement of trimethylamine from **13** with cyanide ion produced the phenylacetonitrile **14** in good yield. This phenol was then remethylated with iodomethane to give **15**, and catalytic hydrogenation of the nitrile in the presence of HCl afforded the desired target compound **8** in good overall yield.

The tetrahydrobenzodifuran mescaline analog **9** was synthesized successfully as shown in Scheme 2. Chloromethylation of 1,2,3-trimethoxybenzene according to the method of Dallacker *et al.*[48] proceeded in fair yield to give the bis-chloromethyl compound **16**. Both chlorine atoms of **16** were displaced with cyanide using KCN in DMSO to afford the bis-nitrile **17**.[49] After acidic hydrolysis to the corresponding bis-acetic acid **18**, reduction using the selective borane−THF method of Yoon *et al.*[50] gave diol **19** in reasonable yield considering the difficulty of workup for this water-soluble intermediate.

The two dihydrofuran rings were formed simultaneously employing a methodology developed recently in our laboratory.[34,36] Thus, essentially conducting three steps in one pot, **19** was first tri-demethylated and dibrominated with excess BBr_3, quenching with methanol to avoid aqueous workup of the highly water soluble and unstable pyrogallol intermediate. Removal of methanol under vacuum and immediate reflux with potassium carbonate in acetone led to ring closure to give the cyclized, phenolic intermediate. Once the complete disappearance of the dibromo compound was noted by TLC, iodomethane was added to the reaction mixture to O-methylate the remaining phenolic oxygen, giving **20** in excellent overall yield from **19**. Final elaboration of the sidechain then proceeded according to standard methods.[43] Friedel−Crafts formylation of **20** using tin(IV) chloride catalyst[51,52] gave aldehyde **21** in modest yield. The low yield was likely due to the partial cleavage of the out-of-plane 8-methoxy group, as discussed above for the synthesis of the monofuran derivative **8**. Nitrostyrene **22** was formed by condensation of **21** with nitromethane using ammonium acetate as the catalyst, and the final target compound **9** was obtained in good yield by LAH reduction of **22**.

Table 1. Results of the Drug Discrimination Studies in LSD-Trained Rats and Radioligand Competition Studies at [^{3}H]Ketanserin-Labeled Rat 5-HT$_{2A}$ and [^{3}H]8-OH-DPAT-Labeled Rat 5-HT$_{1A}$ Receptors

	DD studies			K_i (nanomolar)	
drug	ED$_{50}$ (μmol/kg)	95% CI	n^a	5-HT$_{2A}$ sites	5-HT$_{1A}$ sites
1	33.5	(20.9−53.5)	8−12	5500 ± 600	6900 ± 800
8	50% @ 57.8	NS[b]	8−12	2500 ± 300	6200 ± 400
9	29% @ 55.2	NS[b]	8−12	NT[c]	NT[c]
23	50% @ 16.2	NS[b]	6−15	2100 ± 200	6900 ± 800

[a] Number of animals tested at each dose. [b] NS = no substitution. [c] Not tested in this preparation.

Pharmacology

Compounds **1**, **8**, and **9** were initially evaluated in the two-lever drug discrimination assay in a group of rats trained to discriminate the effects of ip injections of saline from those of LSD tartrate (0.08 mg/kg), according to methods described previously (Table 1).[53] For the compounds not fully substituting for LSD in this assay, the percentage of rats selecting the drug lever at the given dosage levels is reported in the table. Additionally, **1**, **8**, and **9** were tested for their ability to compete for radioligand binding to 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor sites (Table 1).[53] Briefly, the ability of test compounds to displace 0.75 nM [^{3}H]8-OH-DPAT from rat hippcampal homogenate and 0.75 nM [^{3}H]ketanserin from rat frontal cortex homogenate was measured. In addition, the compounds were evaluated in cells expressing cloned human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors that were labeled either with [^{3}H]antagonists or radiolabeled agonists (Table 2). Finally, as a measure of efficacy, compounds were assessed for their ability to stimulate phosphoinositide hydrolysis in cell lines expressing the rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Figures 1 and 2).

Results and Discussion

The results of the drug discrimination (DD) studies in LSD-trained rats and the results of the radioligand competition experiments at [^{3}H]ketanserin-labeled rat 5-HT$_{2A}$ and [^{3}H]8-OH-DPAT-labeled rat 5-HT$_{1A}$ receptors are presented in Table 1. In the behavioral assays, mescaline **1** was the most potent of the test compounds, substituting for LSD with an ED$_{50}$ of 33.5 μmol/kg. Neither of the new rigid analogs fully substituted for LSD. For the dihydrobenzofuran **8**, only 50% of the rats selected the drug lever when administered 57.8 μmol/kg of test drug. When a similar dosage of the tetrahydrobenzodifuran **9** was administered, only 29% of the rats selected the drug lever.

It was initially envisioned that if **8** and **9** retained the necessary pharmacophoric elements, they might have somewhat increased *in vivo* hallucinogen-like activity compared to **1** due to the slightly greater molecular hydrophobicities anticipated. Barfknecht *et al.*[39] previously correlated hallucinogenic activity with octanol−water partition coefficients for a series of substituted phenylalkylamines and found that the optimum hydrophobicity was centered at log P = 3.15. Since mescaline has a log P of only 1.18, it was felt that increased hydrophobicities of **8** and **9** (however, the increase resulting from the added methylene groups is

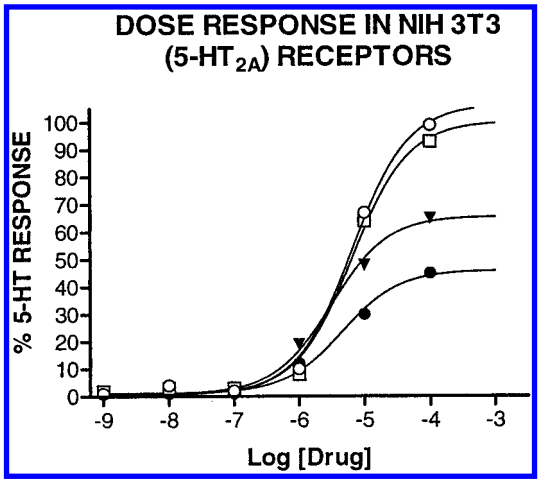


Figure 1. Effects of mescaline **1** (□), **8** (▼), **9** (●), and **23** (○) on phosphoinositide hydrolysis in NIH 3T3 fibroblasts expressing the rat 5-HT$_{2A}$ receptor. The curves are representative of experiments carried out on the same day. The mean E_{max} values (3−5 determinations) for **1**, **23**, **8**, and **9** were 92, 96, 61, and 45%, respectively, of the maximum 5-HT response. The mean EC$_{50}$ values ± SEM were 4 ± 1, 4 ± 2, 3 ± 1, and 2 ± 1 μM, respectively.

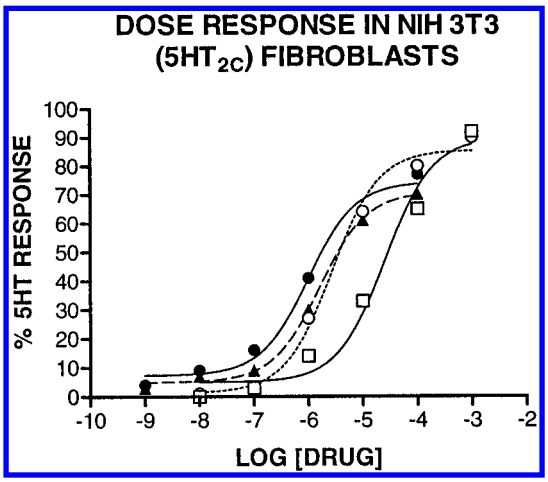


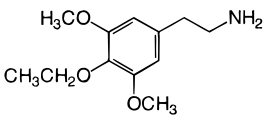
Figure 2. Effects of mescaline **1** (□), **8** (▼), **9** (●), and **23** (○) on phosphoinositide hydrolysis in NIH 3T3 fibroblasts expressing the rat 5-HT$_{2C}$ receptor. The curves are representative of experiments carried out on the same day. The mean E_{max} values (three determinations) of **1**, **23**, **8**, and **9** were 97, 95, 82, and 70% respectively, of the maximum 5-HT response. The mean EC$_{50}$ values ± SEM were 24 ± 1, 2 ± 1, 2 ± 1, and 1 ± 0.2 μM, respectively.

Table 2. Results of the Radioligand Competition Studies of **8** and **9** at Cloned Human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ Receptors (K_i values ± SEM in nanomolar)

	agonist radioligands			antagonist radioligands		
	[^{125}I]DOI		[^{3}H]serotonin	[^{3}H]ketanserin	[^{3}H]rauwolscine	[^{3}H]mesulergine
compound	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_{2B}$	5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_{2C}$
1	551.0 ± 35.3	302.8 ± 2.9	795.1 ± 65.5	NT	NT	NT
8	368.4 ± 17.9	118.3 ± 16.0	523.0 ± 53.0	6405 ± 834	311 ± 38	1105 ± 57
9	335.3 ± 35.7	61.5 ± 5.3	301.5 ± 70.0	4443 ± 319	205 ± 7.6	654 ± 52
23	215.6 ± 25.0	177.2 ± 18.0	555.1 ± 41.0	NT	NT	NT

somewhat offset by the more effective hydrogen-bonding of the constrained heterocyclic oxygens) would enhance their *in vivo* activity. Nevertheless, in the behavioral assays, the rank order of potency for this series decreased with increasing molecular hydrophobicity. In view of the somewhat greater brain concentrations of **8** and **9** that should be achieved, relative to **1**, the ability to activate some essential biological target appeared to have been lost by compounds **8** and **9**. These results are even more surprising when it is recalled that similar transformations leading to molecules such as **7** afforded extremely potent compounds.[36] Clearly, there seem to be some fundamental differences between the molecular mechanisms of action of the 3,4,5- and 2,4,5-substituted phenethylamine hallucinogens.

At this point, it was decided to assay a related compound, 3,5-dimethoxy-4-ethoxyphenethylamine (escaline; **23**), reported to be significantly more potent than mescaline in humans.[10] To our surprise, **23** also failed to produce generalization in LSD-trained rats, but did produce LSD-appropriate responding in 50% of the rats at a dose that was approximately one-half the ED_{50} of mescaline.



23, Escaline

In the *in vitro* assays, none of the test compounds had significant affinity for 5-HT_{1A} receptors, results that are consistent with what is known of the general SAR of hallucinogenic phenylalkylamines. Curiously, however, the rank order of affinities of the test compounds for 5-HT_{2A} receptors was opposite the potencies obtained in the behavioral assays. Thus, while mescaline **1** had the lowest affinity for 5-HT_{2A} sites, the rigid analog **8** had more than twice the potency at these sites. These results are unusual because 5-HT_{2A} receptor affinity of similar phenethylamines typically parallels the potency in behavioral assays for hallucinogenic activity. Indeed, the 5-HT_2 receptor subtype is commonly accepted as the primary site of action through which the unique behavioral effects of this class of drugs are mediated.[26−29] Although mescaline represents one of the least potent hallucinogens, one would still expect its *in vitro* and *in vivo* pharmacological profiles to be in agreement with the known phenylalkylamine SAR. Furthermore, the related 3,5-dimethoxy-4-ethoxyphenethylamine (**23**) had a K_i of 2.1 μM at these 5-HT_{2A} sites, virtually identical to **8**, yet is reported to be 6−8 times more potent than mescaline **1** as an hallucinogen in humans.[10]

The data from the binding studies in cloned human receptors are no more revealing (Table 2). At [^{3}H]-antagonist-labeled 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors, increased rigidification in going from **8** to **9** led to *increased* affinity. Hallucinogens are generally considered to act as agonists, so it was even more surprising when data from the competition studies employing the agonist ligands [^{125}I]DOI and [^{3}H]serotonin were obtained also showing increased affinity as the molecules become more rigid. When one considers that the 4-ethoxy homologue **23** is considerably more potent than **1** as an hallucinogen,[10] a comparison of its affinities for the three 5-HT_2 receptor subtypes with those of **1**, **8**, and **9** revealed no obvious basis for the decreased *in vivo* potencies of **8** and **9**.

This prompted us to examine the relative efficacies of the compounds in activating phosphoinositide turnover in cells expressing the 5-HT_{2A} receptor, anticipating that perhaps compounds **8** and **9** might be partial agonists or antagonists. These compounds were compared to mescaline **1** and **23** (Figure 1). All four phenethylamine derivatives stimulated [^{3}H]-IP_3 hydrolysis in a transfected cell line expressing the 5-HT_{2A} receptor. Consistent with the drug discrimination data, compounds **8** and **9** were partial agonists, eliciting a maximum response less than that obtained with serotonin. Also in agreement with the drug discrimination data, compound **9** was a weaker agonist than was **8**. Curiously, however, in these studies, escaline was a full agonist, in contrast to its low efficacy in the drug discrimination paradigm, with an E_{max} virtually identical to that of mescaline. This latter result contrasts with the partial substitution for **23** in the drug discrimination assay.

Similar phosphoinositide turnover experiments were then carried out in cells expressing the rat 5-HT_{2C} receptor (Figure 2) to determine whether agonist properties at this receptor might provide more insight into the behavioral effects of the four drugs. The profile was very similar to that found for the 5-HT_{2A} receptor. While mescaline was a full agonist at the 5-HT_{2C} receptor, compounds **8** and **9** had somewhat reduced efficacy. Escaline **23**, once again, appeared to be equally efficacious to mescaline **1**. At this receptor, however, **1** had a much larger EC_{50} than did the other three compounds. We believe this latter result has bearing on the drug discrimination results, especially for **23**, as discussed later.

As one final assay to compare these compounds and attempt to identify additional features in their *in vitro* pharmacology that might serve to explain the behavioral results, we evaluated the ability of all four compounds to inhibit the accumulation of [^{3}H]DA or [^{3}H]5-HT in rat whole brain synaptosomes. The IC_{50} values of all compounds were >10 μM, with no obvious trends to parallel the observed behavioral potencies (unpublished results).

We believe that the loss of LSD-like activity in the drug discrimination assay for compounds **8** and **9** may lie in their reduced 5-HT_{2A} agonist efficacies, compared to **1**. This point is particularly emphasized by the lowered degree of substitution of **9** relative to **8**, and the parallel reduction of agonist efficacy. These results support the current hypothesis that hallucinogens are agonists at 5-HT_{2A} receptors.

Somewhat more problematic, however, is the interpretation of the behavioral data for compound **23**. To our knowledge, examples of false negatives among hallucinogen analogues in this assay have been unknown up to the present time. The affinity of the 4-ethoxy homologue **23** for the 5-HT_{2A} receptor, as well as its ability to stimulate phosphoinositide turnover in the cloned receptor, are consistent with its activity as a mescaline-like agent in man. Although the radioligand competition experiments indicate only about a two-fold increase of affinity over **1**, the enhanced hydrophobicity

of **23** could be part of the explanation for its 6−8 fold higher clinical potency.

Escaline **23** also has full efficacy at the 5-HT_{2C} receptor and furthermore has significantly greater potency than mescaline at that site, at least in the cloned rat receptor. That result suggests that 5-HT_{2C} agonism should be a relatively more prominent feature of the pharmacology of **23** compared with **1**. No studies have been carried out to compare the relative strengths of the interoceptive cues produced in rats by 5-HT_{2A} and 5-HT_{2C} receptor agonists. It seems possible, therefore, that the interoceptive cue induced by the increased intensity of the 5-HT_{2C} agonist action of **23** may be masking its 5-HT_{2A} stimulus properties. It might be noted in this context that the EC_{50} values for compounds **8** and **9** (Figure 2) are also significantly lower than for **1**. Thus, while we believe that lowered 5-HT_{2A} efficacy of **8** and **9** is sufficient to explain their behavioral effects, stimulus masking by agonism at the 5-HT_{2C} receptor could also be invoked as a partial explanation. Of course, both mechanisms may be important.

It is possible that other pharmacological factors might be involved in the different behavioral effects of these compounds. For example, it has been reported previously that the behavioral effects of **1** are mediated through both 5-HT and dopamine (DA) receptors.[37] Although we have not examined dopamine receptor affinities for any of these molecules, their ability to inhibit accumulation of dopamine into rat whole brain synaptosomes did not differ significantly (unpublished study). It is still possible that other unidentified variables that we did not consider may be important. The efficacy data at the 5-HT_{2A} receptor, however, seem adequate to explain the decreased LSD-like effects of compounds **8** and **9**.

The decreased efficacy of compounds **8** and **9** at the 5-HT_{2A} receptor relative to **1** or **23** is most interesting. When one incorporates the 2- and 5-methoxy groups of 2,4,5-substituted hallucinogenic amphetamines into dihydrofuran rings, activity is completely retained.[36] In the present instance, however, the same type of increased rigidification leads to decreased agonist efficacy, while at the same time increasing affinity. If the previously proposed receptor model is correct,[36] involving hydrogen bonding sites for the methoxy oxygens, the present results suggest that the 3- and 5-methoxy groups in mescaline-related compounds may not lie in conformations that place them in the plane of the aromatic ring. Implicit in this observation is the possibility that these methoxy groups may reorient themselves upon receptor binding, to achieve a complementary fit and induce an activated conformational state of the receptor. This would necessarily involve out-of-plane conformations for the methoxy groups. Therefore, future molecular modeling studies involving comparisons of 3,4,5- and 2,4,5-trisubstituted phenethylamines should take this possibility into account.

Further pharmacological evaluation of **1**, **8**, **9**, and **23** appears warranted to elucidate fully the mechanism of action of these mescaline analogues, and to understand how the mechanism(s) of action for mescaline might differ from the action of hallucinogenic 2,5-dimethoxyphenylalkylamines. These results also suggest the possibility that 5-HT_{2C} agonists may produce a more robust interoceptive cue than do 5-HT_{2A} agonists, at least in rats.

In summary, we have extended our previously described rigid analog approach, using tetrahydrobenzodifuran functionalities as rotationally restricted bioisosteres of the aromatic methoxy groups of mescaline. Although the rigid analogs synthesized here had increased overall hydrophobicity and enhanced affinity for serotonin 5-HT_2 receptors, they had decreased efficacy and were not as potent as **1** in a behavioral assay for hallucinogen-like activity. These results may point to different (i.e. out-of-plane) methoxy group conformations for 3,4,5-trimethoxyphenylalkylamines related to mescaline as compared with the 2,5-dimethoxyphenylalkylamines. The novel rigid analogs **8** and **9** may serve as useful ligands for probing possible alternate mechanisms of action for mescaline and similar structures, and will be valuable tools in future pharmacological investigations of the SAR of hallucinogenic agents.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ^{1}H-NMR spectra were recorded using either a 500 MHz Varian VXR-500S or 300 MHz Bruker ARX-300 NMR spectrometer. Chemical shifts are reported in δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference (0.03% v/v). Abbreviations used in NMR analyses are as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, q = quartet, p = pentet, m = multiplet, b = broad, Ar = aromatic. Chemical ionization mass spectra (CIMS) using methane as the carrier gas were obtained with a Finnigan 4000 spectrometer. IR measurements were taken with a Perkin-Elmer 1600 Series FTIR spectrophotometer. Elemental analyses were performed by the Purdue University Microanalysis Laboratory and were within ±0.4% of the calculated values unless otherwise noted. Thin-layer chromatography (TLC) was typically performed using Baker-flex silica gel IB2-F, plastic-backed plates with fluorescent indicator (2.5 × 7.5 cm, J.T. Baker), eluting with CH_2Cl_2, and visualizing with UV light at 254 nm and/or I_2 vapor unless otherwise noted. Plates used for radial centrifugal chromatography ("Chromatotron", Harrison Research, Palo Alto, CA) were prepared from Silica Gel 60 PF2-54 containing gypsum. Most reactions were carried out under an inert atmosphere of dry nitrogen.

6,7-Dimethoxybenzofuran-3-one (10). Oxalyl chloride (7.2 mL, 81.0 mmol) in 27 mL of dry benzene was added dropwise to a solution of 12.05 g (56.8 mmol) of 2,3,4-trimethoxybenzoic acid (Aldrich, Milwaukee, WI) in 150 mL of benzene, with two drops of DMF added as a catalyst. The mixture was stirred for 3.5 h, and the volatiles were removed under reduced pressure to give a quantitative yield of crude benzoyl chloride as an oil that spontaneously crystallized to a white solid.

Following literature methods,[55,56] an ethereal solution of diazomethane was cautiously prepared from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) in a well-ventilated fume hood. A solution of 17 g of KOH in 23 mL of water, 66 mL of carbitol (2-(2-ethoxyethoxy)ethanol), and 20 mL of ether was stirred in a Diazald apparatus (Aldrich, Milwaukee, WI) and warmed to 60 °C over an oil bath. Diazald (48.42 g, 226.1 mmol) in 150 mL of anhydrous ether was added slowly to the warm solution, and the clear, yellow distillate that formed was collected and kept cold in an ice−salt bath. The crude benzoyl chloride prepared above was dissolved in 50 mL of anhydrous ether and added dropwise to the cold diazomethane solution. After 10 min, the ice−salt bath was removed, and the mixture was stirred for an additional 2 h. The solvent and excess diazomethane were removed by stirring under reduced pressure (water aspirator), to afford the intermediate diazomethylketone as a viscous yellow-orange oil. To the oil was added

80 mL of glacial acetic acid, giving an immediate, strong evolution of gas and heat. The mixture was allowed to stir in a room temperature water bath for 1.5 h. Solvent removal under reduced pressure gave a transparent red-brown oil that crystallized spontaneously. Recrystallization from methyl ethyl ketone gave 8.30 g (77% from 2,3,4-trimethoxybenzoic acid) of pure product **10** as fluffy, white needles: mp 122−124 °C; ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3, CH_3O), 4.0 (s, 3, CH_3O), 4.7 (s, 2, CH_2), 6.7 (d, 1, Ar*H*), 7.4 (d, 1, Ar*H*); CIMS *m*/*z* 195 (M + 1). Anal. ($C_{10}H_{10}O_4$) C, H.

6,7-Dimethoxy-2,3-dihydrobenzofuran (11). The benzofuranone **10** (7.42 g, 38.2 mmol) was added to a suspension of 2.5 g of 10% Pd−C in 300 mL of absolute ethanol in a Parr flask, and the mixture was shaken under 70 psi of H_2 for 80 h. The reaction mixture was filtered through Celite, and the solvent was removed on the rotary evaporator to give a pale yellow oil. Kugelrohr distillation gave 5.95 g (86%) of essentially pure **11** as a colorless oil. An analytical sample was further purified by radial centrifugal chromatography (1 mm silica plate, CH_2Cl_2): bp 70 °C at 0.02 mm Hg; ^{1}H NMR ($CDCl_3$) δ 3.2 (t, 2, ArCH_2CH$_2$O), 3.8 (s, 3, CH_3O), 3.9 (s, 3, CH_3O), 4.6 (t, 2, ArCH$_2$CH_2O), 6.4 (d, 1, Ar*H*), 6.8 (d, 1, Ar*H*); CIMS *m*/*z* 181 (M + 1). Anal. ($C_{10}H_{12}O_3$) C, H.

7-Hydroxy-6-methoxy-2,3-dihydrobenzofuran (12). The dimethoxy benzofuran **11** (5.72 g, 30.4 mmol) was dissolved in 84 mL of methylene chloride and stirred over a solid CO_2−acetone bath. Boron trichloride (33 mmol as a 1 N CH_2Cl_2 solution) was introduced to the mixture. After 0.5 h of stirring, the cooling bath was allowed to melt and warm slowly to room temperature. Stirring was continued for 4.5 h, and the reaction was cooled on ice and quenched by the dropwise addition of 138 mL of water. The organic phase separated, and the aqueous phase was extracted with methylene chloride (5 × 20 mL). The combined organic extracts were washed with brine (2 × 50 mL) and concentrated under reduced pressure to give a yellow oil. Water (400 mL) was poured over the oil, and 15 mL of 5 N NaOH was added. The basic solution was stirred under nitrogen for 2 h and was then acidified with concentrated HCl. The aqueous mixture was extracted with 5 × 50 mL of CH_2Cl_2, and the extracts were combined. The organic phases were washed with brine, dried ($MgSO_4$), and evaporated to yield a red-orange oil. Kugelrohr distillation gave a colorless oil that spontaneously crystallized to a white solid upon cooling. Recrystallization of this solid from ethyl acetate−hexane afforded 2.98 g (59%) of pure **12** as dense, colorless, needles: mp 71−72 °C; ^{1}H NMR ($CDCl_3$) δ 3.2 (t, 2, ArCH_2CH$_2$O), 3.9 (s, 3, CH_3O), 4.6 (t, 2, ArCH$_2$CH_2O), 5.3 (s, 1, ArOH), 6.4 (d, 1, Ar*H*), 6.7 (d, 1, Ar*H*); CIMS *m*/*z* 167 (M + 1). Anal. ($C_9H_{10}O_3$) C, H.

4-(Cyanomethyl)-7-hydroxy-6-methoxy-2,3-dihydrobenzofuran (14). To a stirred solution of **12** (574 mg, 3.45 mmol) in 5 mL of methanol was added dimethylamine (0.47 mL of a 40% aqueous solution, 3.7 mmol), followed by formaldehyde (0.28 mL of a 37% aqueous solution, 3.7 mmol). The mixture was stirred at room temperature for 30 min and then outfitted with a condenser and brought to gentle reflux over a steam bath. After 4.5 h, TLC analysis indicated the presence of unreacted starting material, so more dimethylamine (0.10 mL of 40% solution, 0.8 mmol) and formaldehyde (0.06 mL of 37% solution, 0.8 mmol) were added to drive the reaction to completion. After three more hours of reflux, the reaction mixture was cooled, and all volatiles were removed under reduced pressure, leaving the crude benzylamine as a white solid. The benzylamine was dried under high vacuum and was taken up, with warming, in 10 mL of 2-propanol. Methyl iodide (0.4 mL, 6.4 mmol) was added, and the mixture was stirred at room temperature. After 2 min, a flocculent white solid began to form. Stirring was continued for 5 h, and the precipitate was collected by vacuum filtration and dried under high vacuum to give 1.11 g (88%) of the quaternary methiodide **13** as a fine white powder that was sufficiently pure to carry on to the next step. Attempts to purify further the methiodide salt by recrystallization resulted in rapid decomposition: mp 159−162 °C (begins to decompose at 97 °C and does not melt cleanly); CIMS *m*/*z* 224 [(M + 1) − CH_3I], 179 [(M + 1) − $N(CH_3)_3^+I^-$].

The crude methiodide salt **13** (5.74 g, 15.7 mmol) was immediately taken up in 63 mL of water containing 7.41 g (113.8 mmol) of potassium cyanide, and the mixture was heated to reflux. All solids dissolved on heating, but as the reaction proceeded a small amount of precipitate became visible. After 5 h, the reaction was cooled and cautiously acidified with 6 N HCl (warning! HCN produced), giving a substantial quantity of solid. The acidified mixture was stirred for 2 h in the fume hood, and the solid material was carefully collected by suction filtration. Extensive air drying on the filter paper afforded 2.54 g (79%) of the title compound **14** as an off-white solid: mp 100−104 °C; ^{1}H NMR ($CDCl_3$) δ 3.2 (t, 2, ArCH_2CH$_2$O) 3.6 (s, 2, ArCH_2CN), 3.9 (s, 3, CH_3O), 4.7 (t, 2, ArCH$_2$CH_2O), 5.3 (s, 1, ArO*H*), 6.4 (s, 1, Ar*H*); CIMS *m*/*z* 206 (M + 1), 1.79. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

4-(Cyanomethyl)-6,7-dimethoxy-2,3-dihydrobenzofuran (15). A mixture of 4-(cyanomethyl)-7-hydroxy-6-methoxy-2,3-dihydrobenzofuran (**14**, 400 mg, 1.95 mmol), powdered anhydrous potassium carbonate (539 mg, 3.90 mmol), and methyl iodide (0.24 mL, 3.9 mmol) in 4.6 mL of acetone was heated at reflux and stirred under N_2 for 20 h. The reaction mixture was cooled, filtered through Celite, and evaporated to give an oily yellow solid. This was taken up in ethyl acetate, washed with 10% sodium bicarbonate (2 × 25 mL) and brine (25 mL), and dried ($MgSO_4$). Filtration, followed by solvent removal under reduced pressure afforded a dark yellow oil that crystallized spontaneously. The oil was purified on the Chromatotron (4 mm silica plate, CH_2Cl_2) to give 345 mg (81%) of **15** as a yellow solid: mp 87−89 °C; ^{1}H NMR ($CDCl_3$) δ 3.2 (t, 2, ArCH_2CH$_2$O), 3.6 (s, 2, ArCH_2CN), 3.8 (s, 3, CH_3O), 3.9 (s, 3, CH_3O), 4.7 (t, 2, ArCH$_2$CH_2O), 6.4 (s, 1, Ar*H*); CIMS *m*/*z* 220 (M + 1), 193. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

4-(2-Aminoethyl)-6,7-dimethoxy-2,3-dihydrobenzofuran Hydrochloride (8). In a Parr hydrogenation flask, 1.85 g (8.44 mmol) of 6,7-dimethoxy-4-(cyanomethyl)-2,3-dihydrobenzofuran **15** and 1.0 g of 10% Pd−C were combined in 125 mL of ethanol containing 2 mL of concentrated HCl. The mixture was shaken under 60 psi of hydrogen for 24 h, during which time the theoretical amount of hydrogen was taken up. Filtration through Celite to remove the catalyst, followed by evaporation of solvent *in vacuo* gave the crude product as a white solid. Recrystallization from ethanol−ethyl acetate gave 1.93 g (88%) of pure **8**·HCl as glistening white crystals: mp 206−208 °C; ^{1}H NMR (HCl salt in DMSO) δ 2.8 (t, 2, NH_3-CH$_2$CH_2Ar), 3.0 (t, 2, NH_3CH_2CH$_2$Ar), 3.1 (t, 2, ArCH_2CH$_2$O), 3.69 (s, 3, CH_3O), 3.74 (s, 3, CH_3O), 4.6 (t, 2, ArCH$_2$CH_2O), 6.4 (s, 1, Ar*H*), 8.1 (bs, 3, NH_3); CIMS *m*/*z* 224 (M + 1). Anal. ($C_{12}H_{17}NO_3$·HCl) C, H, N.

1,5-Bis(chloromethyl)-2,3,4-trimethoxybenzene (16).[48] A mixture of 49.0 g (0.29 mol) of 1,2,3-trimethoxybenzene (Aldrich, Milwaukee, WI), 200 mL of 1,4-dioxane, and 130 mL of concentrated HCl was stirred vigorously, and hydrogen chloride gas was continuously bubbled through the clear solution. Formaldehyde (90 mL of a 37% aqueous solution) was added in three 30 mL portions at 30 min intervals. The formalin addition caused the reaction to become exothermic with the formation of a white precipitate. The mixture was stirred for 3 h, and the HCl gas flow was stopped. After stirring for another 1 h, the mixture (now returned to room temperature) was cooled further on an ice bath, and 150 mL of concentrated HCl was added. The mixture was extracted with 5 × 100 mL of ether, and the extracts were combined and washed with 2 × 100 mL of 10% K_2CO_3, 2 × 100 mL of water, and brine. The organic phase was dried over $MgSO_4$ and filtered through Celite, and the solvent was removed under reduced pressure to afford 65 g (85%) of crude product as an orange oil. The oil was purified by Kugelrohr distillation (bp 120 °C at 0.4 mmHg) to give 31.87 g (41%) of pure **16** as a colorless oil that crystallized slowly on standing: mp 43 °C (lit.[48] mp 43 °C); ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3, ArOC*H*3), 3.99 (s, 6, ArOC*H*3), 4.58 (s, 4, ArC*H*2Cl), 7.12 (s, 1, Ar*H*), CIMS *m*/*z* 265 (M + 1), 229, 195.

1,5-Bis(cyanomethyl)-2,3,4-trimethoxybenzene (17). Following the method of Friedman and Schecter,[49] 20 g (0.075 mol) of the bis-chloro compound **16** was added portionwise to a stirred mixture of 8.5 g (0.17 mol) of sodium cyanide in 65

mL of dry DMSO warmed over an 80 °C oil bath. After 20 min, the reaction appeared complete (TLC) and was poured onto 400 mL of ice. The dark mixture was extracted with 4 × 100 mL of ether, and the extracts were combined and washed with 5 × 100 mL of H_2O and 2 × 100 mL of brine. After drying ($MgSO_4$) and filtration through Celite, the solvent was removed on the rotary evaporator, and the oil was stirred under high vacuum to give 16.2 g (87%) of **17** as a clear, brown oil that was sufficiently pure to carry on to the next step. An analytical sample was prepared by Kugelrohr distillation to give a colorless oil: bp 150 °C at 0.15 mm Hg; ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 4, ArC*H*2CN), 3.85 (s, 3, ArOC*H*3), 3.95 (s, 6, ArOC*H*3), 7.02 (s, 1, Ar*H*); CIMS *m*/*z* 247 (M + 1), 220. Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

1,5-Bis(carboxymethyl)-2,3,4-Trimethoxybenzene (18). The bis-nitrile **17** (11 g, 0.045 mol) was hydrolyzed by reflux for 4 h with 50 mL of concentrated HCl. Some acid precipitated upon cooling to room temperature, and the mixture was thoroughly cooled in an ice−water bath to effect the precipitation of more solid. The water-soluble precipitate was collected on a Büchner funnel and rinsed carefully with ice-cold water. Air-drying the solid overnight, and then under high vacuum, gave 9.38 g (74%) of **18** as a white solid: mp 155−157 °C; ^{1}H NMR (DMSO-d_6) δ 3.45 (s, 4, ArC*H*$_2$COOH), 3.76 (s, 6, ArOC*H*$_3$), 3.79 (s, 3, ArOC*H*$_3$), 6.78 (s, 1, Ar*H*), 12.23 (s, 2, COOH); CIMS *m*/*z* 285 (M + 1), 267, 239, 225. Anal. ($C_{13}H_{16}O_7$) C, H.

1,5-Bis(2-hydroxyethyl)-2,3,4-trimethoxybenzene (19). The diacid **18** (7.7 g, 0.027 mol) was suspended in 250 mL of dry THF and stirred vigorously while cooling on an ice bath. To the mixture was cautiously added 73 mL of BH_3/THF (0.073 mol as a 1 N solution) *via* syringe. The mixture was stirred for 24 h while the ice bath was allowed to melt and warm to room temperature. After carefully quenching the reaction with 40 mL of H_2O, the THF was removed on the rotary evaporator. The aqueous residue was saturated with K_2CO_3 and extracted with 5 × 50 mL of 10% THF in diethyl ether. The extracts were combined and washed with brine, dried ($MgSO_4$), and filtered through Celite. Removal of solvent under reduced pressure gave 5.7 g (82%) of a thick yellow oil that was purified by Kugelrohr distillation (115−135 °C at 0.1 mmHg) to give a waxy solid. The solid was recrystallized from ethyl acetate−petroleum ether to afford 3.7 g (53%) of pure diol **19** as a white solid: mp 52 °C; ^{1}H NMR ($CDCl_3$) δ 2.38 (bs, 2, O*H*), 2.80 (t, 4, ArCH$_2$C*H*$_2$OH, *J* = 6.4 Hz), 3.76 (t, 4, ArC*H*$_2$CH$_2$OH, *J* = 6.4 Hz), 3.83 (s, 6, ArOC*H*$_3$), 3.88 (s, 3, ArOC*H*$_3$), 3.74 (s, 1, Ar*H*); CIMS *m*/*z* 257 (M + 1), 239, 225. Anal. ($C_{13}H_{20}O_5$) C, H.

8-Methoxy-2,3,5,6-tetrahydrobenzo[1,2-*b*:5,4-*b*′]difuran (20). A solution of 6.6 g (0.026 mol) of the diol **19** in 150 mL of CH_2Cl_2 was cooled to −78 °C by stirring over a solid CO_2−acetone bath. Boron tribromide (17 mL, 0.18 mol) was added to the mixture *via* syringe, and the solution was stirred for 17 h while allowing the bath to warm to room temperature and evaporate. The reaction was quenched by cooling again to −78 °C and adding 100 mL of methanol. After warming to room temperature, the volatiles were removed on the rotary evaporator, and another 100 mL of methanol was added and evaporated. The intermediate dibromide (**20**) was further dried by stirring under high vacuum to give an unstable brown oil that was taken directly to the next step.

Under an atmosphere of N_2, acetone (250 mL) and K_2CO_3 (35 g, 0.26 mol) were added to the oil, and the mixture was heated at reflux over an oil bath for 5 h. Iodomethane (1.62 mL, 0.026 mol) was then added to the solution, and reflux was continued for another 6 h. The reaction was cooled to room temperature and filtered through Celite, and the acetone was removed on the rotary evaporator. The dark residue was partitioned between ether and water, and the layers were separated. The ether phase was washed with 3 × 50 mL of 5 N NaOH, 3 × 50 mL of 3 N HCl, and brine, dried over $MgSO_4$, and filtered through Celite. Solvent removal under reduced pressure gave a yellow oil that spontaneously crystallized after standing at room temperature. The crude product was purified on the Chromatotron (two 4 mm silica plates, CH_2Cl_2) to give a solid that was recrystallized from ethyl acetate−hexane to afford 2.8 g (57% from diol **19**) of pure **20** as a waxy, white solid: mp 63−64 °C; ^{1}H NMR ($CDCl_3$) δ 3.12 (td, 4, ArOCH$_2$-C*H*$_2$, *J* = 8.7 Hz, 0.9 Hz), 3.96 (s, 3, ArOC*H*$_3$), 4.59 (t, 4, ArOC*H*$_2$CH$_2$, *J* = 8.7 Hz), 6.67 (s, 1, Ar*H*); CIMS *m*/*z* 193 (M + 1). Anal. ($C_{11}H_{12}O_3$) C, H.

4-Formyl-8-methoxy-2,3,5,6-tetrahydrobenzo[1,2-*b*:5,4-*b*′]difuran (21). A solution of 2.8 g (0.015 mol) of **20** was dissolved in 50 mL of dry CH_2Cl_2 and cooled over an ice bath. Tin(IV) chloride (3.4 mL, 0.029 mol) was added to the solution *via* syringe, and the mixture was stirred for 5 min. Two, 1.3 mL portions (0.029 mol) of α,α-dichloromethyl methyl ether were added at 30 min intervals, the ice bath was removed, and the mixture was allowed to stir at room temperature for 4 h. The reaction was quenched by the addition of 50 mL of ice cold 3 N HCl and diluted with 100 mL of ether, and the layers were separated. The organic phase was washed with 5 × 50 mL of 6 N HCl, 2 × 50 mL of 5% $NaHCO_3$, and brine, dried ($MgSO_4$), and filtered through Celite and a thin pad of silica gel. Removal of solvent under reduced pressure gave a yellow oil that crystallized spontaneously. The solid was recrystallized from ethyl acetate−hexane to give 1.3 g (40%) of **21** as pale yellow needles: mp 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 3.44 (t, 4, ArOCH$_2$C*H*$_2$, *J* = 8.7 Hz), 4.10 (s, 3, ArOC*H*$_3$), 4.68 (t, 4, ArOC*H*$_2$CH$_2$, *J* = 8.7 Hz), 10.08 (s, 1, ArC*H*O); CIMS *m*/*z* 221 (M + 1). Anal. ($C_{12}H_{12}O_4$) C, H.

8-Methoxy-4-(2-nitroethenyl)-2,3,5,6-tetrahydrobenzo-[1,2-*b*:5,4-*b*′]difuran (22). The aldehyde **21** (0.75 g, 3.41 mmol) was stirred under nitrogen with 10 mL of nitromethane and 0.26 g (3.41 mmol) of ammonium acetate over a 100 °C oil bath. After 5 h, the volatiles were removed on the rotary evaporator. The residue was taken up in 10% CH_2Cl_2 in ether, and the organic phase was washed with 2 × 50 mL of water, 4 × 50 mL of 3 N HCl, and 50 mL of brine. After drying ($MgSO_4$), filtration through Celite, and removal of solvent under reduced pressure, the crude yellow-orange product was purified on the Chromatotron (4 mm silica plate, CH_2Cl_2). The pure solid was crystallized from methanol to give 0.49 g (55%) of **22** as bright yellow needles: mp 157−158 °C; ^{1}H NMR ($CDCl_3$) δ 3.29 (t, 4, ArOCH$_2$C*H*$_2$, *J* = 8.7 Hz), 4.03 (s, 3, ArOC*H*$_3$), 4.70 (t, 4, ArOC*H*$_2$CH$_2$, *J* = 8.7 Hz), 7.35 (d, 1, ArCH=C*H*, *J* = 13.5 Hz), 7.96 (d, 1, ArC*H*=CH, *J* = 13.9 Hz); CIMS *m*/*z* 264 (M + 1). Anal. ($C_{13}H_{13}NO_5$) C, H, N.

1-(8-Methoxy-2,3,5,6-tetrahydrobenzo[1,2-*b*:5,4-*b*′]difuran-4-yl)-2-aminoethane Hydrochloride (9). A solution of the nitroolefin **22** (0.53 g, 2.01 mmol) in 50 mL of dry THF was added dropwise to a stirred suspension of 0.25 g (6.04 mmol) of $LiAlH_4$ in 50 mL of dry THF. The mixture was heated at reflux over an oil bath for 3 h and quenched by first cooling in an ice bath and then cautiously adding 5 mL of 5 N KOH with vigorous stirring. The mixture was filtered through Celite, washing the filter cake with ether, and the volatiles were removed on the rotary evaporator. The aqueous residue was taken up in 75 mL of 3 N HCl and washed with 3 × 50 mL of ether. The aqueous phase was cooled over an ice bath and made strongly basic with the addition of 5 N KOH. The free amine was then extracted into 5 × 40 mL of CH_2Cl_2, and the extracts were combined and washed with 2 × 100 mL of brine. After drying ($MgSO_4$), filtration through Celite, and removal of the solvent *in vacuo*, 0.35 g (72%) of the free amine **9** was obtained as a yellow oil. The hydrochloride salt was formed by the addition of 1 N HCl in anhydrous ethanol, and recrystallization from ethanol−ethyl acetate gave 222 mg (40%) of **9**·HCl as a white, crystalline solid: mp 282−283 °C; ^{1}H NMR (free base in $CDCl_3$) δ 1.35 (bs, 2, N*H*$_2$), 2.58 (t, 2, ArCH$_2$C*H*$_2$, *J* = 7.3 Hz), 2.88 (t, 2, ArC*H*$_2$CH$_2$, *J* = 7.3 Hz), 3.10 (t, 4, ArOCH$_2$C*H*$_2$, *J* = 8.7 Hz), 3.93 (s, 3, ArOC*H*$_3$), 4.61 (t, 4, ArOC*H*$_2$CH$_2$, *J* = 8.7 Hz); CIMS *m*/*z* 236 (M + 1), 219; Anal. ($C_{13}H_{17}NO_3$·HCl) C, H, N.

Pharmacology Methods. Drug Discrimination Studies. The procedures for the drug discrimination assays were essentially as described in previous reports.[33,36] Twenty male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200−220 g at the beginning of the drug discrimination study were used as subjects trained to discriminate LSD tartrate from saline. None of the rats had previously received drugs or behavioral training. Water was freely available in

the individual home cages and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions to maintain approximately 80% of free-feeding weight. Lights were on from 0700 to 1900. The laboratory and animal facility temperature was 22−24 °C, and the relative humidity was 40−50%. Experiments were performed between 0830 and 1700 each day, Monday−Friday.

Six standard operant chambers (Model E10-10RF, Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a food hopper (combination dipper pellet trough, Model E14-06, module size 1/2), all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, interfaced through a Med Associates interface to a 486-based microcomputer, controlled reinforcement and data acquisition with a locally-written program.

A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. The drug discrimination procedure details have been described elsewhere.[57,58] After habituation to the experimental conditions (one week after isolation in the individual home-cages and at the beginning of the food deprivation), the rat's initial shaping was started. During the first 2−3 sessions, rats were trained only to associate a characteristic noise (click) after lever pressing with a delivered food pellet (without drug injections). Initially, rats were shaped to lever press on an FR1 schedule so that one food pellet was dispensed for each press. Half of the rats were trained on drug-L (left), saline-R (right) and the other half on drug-R, saline-L to avoid positional preference. Training sessions lasted 15 min and were conducted at the same time each day. Levers were cleaned between animals with 10% ethanol solution to avoid olfactory cues. Only one appropriate lever was present during the first 10 sessions of initial learning (after beginning to administer saline or training drug, i.p. 30 min before sessions). Afterwards, both levers were present during all following phases of training, but reinforcements were delivered only after responses on the appropriate lever. Presses on the incorrect lever had no programmed consequences. As responding rates stabilized (during the next 15 sessions), the schedule of reinforcement was gradually increased to a FR50. Once at the FR50, training continued until an accuracy of at least 85% (number of correct presses × 100/number of total presses) was attained for eight of ten consecutive sessions (approximately 40−60 sessions). Once criterion performance was attained, test sessions were interspersed between training sessions, either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. Test drugs were administered ip 30 min prior to the sessions; test sessions were run under conditions of extinction, with rats removed from the operant chamber when fifty presses were emitted on one lever. If fifty presses on one lever were not completed within 5 min the session was ended and scored as a disruption. Treatments were randomized at the beginning of the study.

The training drug was (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg, 186 nmol/kg, NIDA). All drugs were dissolved in 0.9% saline and were injected intraperitoneally in a volume of 1 ml/kg, 30 min before the sessions.

Data from the drug discrimination study were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (%SDL) for each dose of test compound was determined. The degree of substitution was determined by the maximum %SDL for all doses of the test drug. "No substitution" is defined as 59% SDL or less, and "partial" substitution is 60−79% SDL. If the drug was one which completely substituted for the training drug (at least one dose resulted in a %SDL = 80% or higher) the method of Litchfield and Wilcoxon[59] was used to determine the ED_{50} (log−probit analysis as the dose producing 50% drug-lever responding) and 95% confidence interval (95% CI). This method also allowed for tests of parallelism between dose−response curves of the drug and the training drug. If 50% or more of the animals tested were disrupted at a dose where the nondisrupted rats gave 80% SDL, no ED_{50} was calculated.

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

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

The procedure of Johnson et al.[60] was employed. Briefly, the frontal cortex or hippocampal brain regions from 20−40 rats were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in 4 or 8 volumes of 0.32 M sucrose for frontal cortex or hippocampus, respectively. The homogenates were centrifuged at 36000*g* for 10 min, and the resulting pellets were resuspended in the same volume of sucrose. Separate aliquots of tissue suspension were then frozen at −70 °C until assay.

For each separate experiment, a tissue aliquot was thawed slowly and diluted 1:25 with 50 mM Tris HCl (pH = 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 36500*g* for 20 min with an intermittent wash. The resulting pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na_2EDTA, 0.1% Na ascorbate, and 10 mM pargyline HCl (pH 7.4). In experiments with [^{3}H]ketanserin, either 10 mM $MgCl_2$ or 5.7 mM $CaCl_2$ were included, respectively. A second preincubation for 10 min at 37 °C was conducted, and the tissues were then cooled in an ice bath.

All experiments were performed with triplicate determinations using the appropriate buffer to which 200−400 μg of protein was added, giving a final volume of 1 mL. The tubes were allowed to equilibrate for 15 minutes at 37 °C before filtering through Whatman GF/C filters using a cell harvester (Brandel, Gaithersburg, MD) followed by two 5 mL washes using ice-cold Tris buffer. Specific binding was defined as that displaceable with 10 μM cinanserin in the [^{3}H]ketanserin binding study and with 10 μM 5-HT in the [^{3}H]8-OH-DPAT binding study. Filters were air-dried, placed into scintillation vials with 10 mL of Ecolite scintillation cocktail, allowed to sit overnight before counting at an efficiency of 37% for tritium, and directly counted in a γ counter for [^{125}I]-ligand at an efficiency of 79.4%.

Radioligand Competition Experiments Using Cloned Human Receptors. All chemicals were obtained from the sources previously described.[61] [^{3}H]-5-HT was purchased from DuPont-NEN (Wilmington, DE) or Amersham Corporation (Arlington Heights, IL) at 22.8−26.7 or 81−91 Ci/mmol, respectively. [^{125}I]DOI (2200 Ci/mmole), [^{3}H]rauwolscine (70−90 Ci/mmol), and [^{3}H]ketanserin (60−78.7 Ci/mmol) were purchased from Dupont-NEN (Wilmington, DE).

Membrane Preparation from Transformed Cell Lines. Membranes were prepared essentially as previously described[61] using AV12 cell lines (Syrian hamster fibroblast, ATCC no. CRL 9595) stably transformed with the human 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors. In brief, cells expressing the receptor of interest were grown in suspension and harvested by centrifugation. The cell pellets were then resuspended in a minimal volume of a hypotonic buffer, 50 mM Tris-HCl, pH 7.4, and frozen at −70 °C until needed. On the day of the assay, the membrane suspension was thawed and diluted to 35 mL per 0.5×10^9 cells with 50 mM Tris-HCl, pH 7.4. The combination of hypotonic buffer and vortexing was sufficient to lyse the cells for the membrane preparation. After vortexing, the preparation was centrifuged at 39000*g* for 10 min at 4 °C, and the resulting membrane pellet was resus-

pended and incubated at 37 °C for 10 minutes and then centrifuged at 39000*g* for 10 min at 4 °C. This pellet was resuspended and centrifuged one more time, and the final membrane pellet was resuspended (using a Tissumizer, setting 65 for 15 s) in Tris-HCl, pH 7.4, for cells expressing the human 5-HT_{2B} receptor, in Tris-HCl, pH 7.4, containing $MgCl_2$ and EDTA for [^{125}I]DOI binding to 5-HT_{2A} or 5-HT_{2C} receptors, or in Tris-HCl, pH 7.6, for [^{3}H]ketanserin and [^{3}H]mesulergine binding to 5-HT_{2A} and 5-HT_{2C} receptors, respectively.

5-HT_{2B} [^{3}H]-5-HT Binding Studies. Human 5-HT_{2B} receptor binding assays using [^{3}H]-5-HT were performed as previously described.[61] The assay was automated using a Biomek 1000 (Beckman Instruments, Fullerton, CA). [^{3}H]-5-HT in Tris-HCl containing $CaCl_2$, pargyline, and L-ascorbic acid, adjusted to pH 7.4, was added to drug dilutions, spanning six log units, in water. Then 200 μL of membrane resuspension (approximately 100−150 μg protein) was added with mixing and incubated for 15 min at 37 °C. The total incubation volume was 800 μL, and all incubations were performed in triplicate. The final concentration of $CaCl_2$, pargyline, Tris, and L-ascorbic acid was 3 mM, 10 μM, 50 mM, and 0.1%, respectively. The assay was terminated by vacuum filtration through Whatman GF/B filters which had been presoaked with 0.5% polyethylenimine (w/v) and precooled with 4 mL ice-cold wash buffer (50 mM Tris-HCl, pH 7.4), using a Brandel cell harvester (Model MB-48R; Brandel, Gaithersburg, MD). The filters were then washed rapidly four times with 1 mL of ice-cold wash buffer. The amount of [^{3}H]-5-HT trapped on the filters was determined by liquid scintillation spectrometry (Ready Protein, LS 6000IC, Beckman Instruments, Fullerton, CA). The final [^{3}H]-5-HT concentration for competition studies was approximately 2 nM (range = 1.7−2.5 nM). The actual free radioligand concentration was determined by sampling the supernatant of identical tubes where bound ligand was removed by centrifugation. Nonspecific binding was defined with 10 μM 5-HT or 10 μM 1-naphthylpiperazine (1-NP). The amount of protein was determined by the method of Bradford,[62] with bovine serum albumin as the standard.

5-$HT_{2A,2C}$ [^{125}I]DOI Binding Studies. Human 5-HT_{2A} or 5-HT_{2C} binding studies were performed essentially as described for [^{3}H]-5-HT binding to the 5-HT_{2B} receptor with the following exceptions. The assay buffer contained, in final concentration, 10 μM pargyline, 9.75 mM $MgCl_2$, 0.5 mM (ethylenedinitrilo)-tetraacetic acid, disodium salt (EDTA), 0.1% sodium ascorbate, and 50 mM Tris-HCl, pH 7.4. Incubations were performed at 37 °C for 30 min with approximately 40 and 30 μg protein for the 5-HT_{2A} and 5-HT_{2C} receptors, respectively, and then filtered and washed as described above. The amount of [^{125}I]-DOI trapped on the filters was determined using a γ counter. Nonspecific binding was determined with 10 μM mianserin for 5-HT_{2C} and 1 μM ketanserin for 5-HT_{2A} receptors. The final concentration of [^{125}I]DOI was approximately 0.07−0.15 nM.

[^{3}H]Ketanserin Binding to the Human 5-HT_{2A} Receptor. Membranes were prepared as described above, and the assay conditions were essentially as previously described.[63] Assays consisted of 0.8 mL total volume containing 50 mM Tris-HCl, 100 nM prazosin (to block potential binding of [^{3}H]-ketanserin to α_1-adrenergic receptors), 0.4−0.5 nM [^{3}H]-ketanserin, and varying concentrations of the competing compound of interest (final pH 7.6). Mianserin, 3 μM, was used to define the level of nonspecific binding. Tubes were incubated at 37 °C for 15 min and then rapidly filtered and washed as described above. The amount of [^{3}H]ketanserin trapped on the filters was determined by liquid scintillation spectrometry.

[^{3}H]Rauwolscine Binding to the Human 5-HT_{2B} Receptor. This assay is based on a previously described procedure.[64] Membrane preparation and the filtration binding assay were essentially as described above. Conditions specific to this assay were as follows (all concentrations given as final concentrations): 2 nM [^{3}H]rauwolscine, 500 nM efaroxan (to mask rauwolscine binding to α_2-adrenergic receptors), and 50 mM Tris-HCl, pH 7.4. Tubes were incubated at 37 °C for 20 min and then rapidly filtered as described above. Nonspecific binding was defined in the presence of 10 μM 1-naphthylpiperazine.

[^{3}H]Mesulergine Binding to the Human 5-HT_{2C} Receptor. This assay was adapted from that described by Pazos *et al.*[65] Membranes were prepared as described above. Final concentrations for the 0.8 mL-assays were 0.74−0.82 nM [^{3}H]-mesulergine, varying concentration of competing compound, and 50 nM Tris-HCl, final pH 7.6. Nonspecific binding was determined using 3 mM mianserin. Assay tubes were incubated for 30 min at 37 °C, after which the samples were filtered, washed, and radioactivity determined as for the [^{3}H]-ketanserin binding assay described above.

Statistical Analysis for Radioligand Binding. Nonlinear regression analysis for the competition curves was performed as previously described[61] to determine IC_{50} values. These were converted to K_i values by the method of Cheng and Prusoff.[66]

Measurement of Phosphoinositide Turnover in NIH 3T3 Cells. 5-HT_{2A} and 5-HT_{2C} receptor cDNAs were the gift of David Julius (UCSF, San Francisco, CA). Clonal cell lines were generated in NIH 3T3 fibroblasts (American Type Culture Collection, Rockville, MD) by calcium phosphate precipitation and screening by radioligand binding. Cell lines expressing 5-HT_{2A} receptors (∼5000 fmol/mg protein) and 5-HT_{2C} receptors (∼3000 fmol/mg protein) are referred to as 3T3-2A and 3T3-2C, respectively. [^{3}H]*Myo*-Inositol (20−25 Ci/mmol) was purchased from Dupont/NEN Corporation (Boston, MA). Penicillin, streptomycin, and Dulbecco's modified eagle medium (DMEM) were obtained from GIBCO/BRL (Grand Island, NY). Bovine serum was purchased from Hyclone (Logan, UT) and tissue culture plates, from Falcon (Lincoln Park, NJ).

Fibroblasts were grown in DMEM supplemented with penicillin (5 U/ml), streptomycin (5 mg/ml), and calf serum (9%). For the PI hydrolysis assay, cells were plated into 24-well culture plates and allowed to adhere for 30 h in DMEM supplemented with 9% calf serum. Cells were subsequently washed once with 0.5 mL serum-free DMEM and then incubated for 18 h in 0.5 mL serum-free, inositol-free DMEM supplemented with 5 mCi/mL [^{3}H]*myo*-inositol. Labeled cells were subsequently used for determination of [^{3}H]inositol phosphate (IP) formation as described originally by Berridge *et al.*[67] and modified by Grotoweil and Sanders-Bush.[68] Dose−response curves were fit using Prism, a commercially available computer graphics/analysis program (GraphPad, San Diego, CA).

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References

(1) For parts 1−3 in this series, see refs 32, 33, and 36, respectively.
(2) Abstracted in part from the Ph.D. thesis of A. Monte, Purdue University, 1995.
(3) Kapadia, G. J.; Fayez, M. B. E. Peyote Constituents: Chemistry, Biogenesis, and Biological Effects. *J. Pharm. Sci.* **1970**, *59*, 1699−1727.
(4) Heffter, A. Ueber Cacteenalkaloide. *Chem. Ber.* **1896**, *29*, 216−227.
(5) Späth, E. Uber Die Anhalonium-Alkaloidie. 1. Anhalin and Mezcalin. *Monatsh. Chem.* **1919**, *40*, 129−154.
(6) Jaffe, J. H. Drug Addiction and Drug Abuse. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 553−557.
(7) Braun, U.; Braun, G.; Jacob, P.; Nichols, D. E.; Shulgin, A. T. Mescaline Analogs: Substitutions at the 4-Position. In *NIDA Research Monograph 22, Quantitative Structure-Activity Relationships of Analgesic, Narcotic Antagonists, and Hallucinogens*, Barnett, G., Trsic, M., Willette, R., Eds.; U.S. Government Printing Office: Washington, D.C., 1978; pp 27−32.
(8) Nichols, D. E.; Glennon, R. A. Medicinal Chemistry and Structure-Activity Relationships of Hallucinogens. In *Hallucinogens: Neurochemical, Behavioral, and Clinical Perspectives*; Jacobs, B. L., Ed.; Raven Press: New York, 1984; pp 95−142.
(9) Nichols, D. E. Studies of the Relationship Between Molecular Structure and Hallucinogenic Activity. *Pharmacol. Biochem. Behav.* **1986**, *24*, 335−340.

(10) Shulgin, A. T.; Shulgin, A. Pihkal: A Chemical love Story; Transform Press: Berkeley, 1991; 978 pp.
(11) Nichols, D. E.; Oberlender, R.; McKenna, D. J. Stereochemical Aspects of Hallucinogenesis. In *Biochemistry and Physiology of Substance Abuse*; Watson, R., Ed.; CRC Press: Boca Raton, 1991; pp 1−39.
(12) Nichols, D. E. Medicinal Chemistry and Structure-Activity Relationships. In *Amphetamine and Its Analogs*; Cho, A. K., Segal, D. S., Eds.; Academic Press, Inc.: San Diego, 1994; pp 3−41.
(13) Shulgin, A. T.; Sargent, T.; Naranjo, C. Structure-Activity Relationships of One-Ring Psychotomimetics. *Nature* **1969**, *221*, 537−541.
(14) Shulgin, A. T.; Dyer, D. C. Psychotomimetic Phenylisopropylamines. 5. 4-Alkyl-2,5-dimethoxyphenylisopropylamines. *J. Med. Chem.* **1975**, *18*, 1201−1204.
(15) Glennon, R. A. Hallucinogenic Agents as Discriminative Stimuli: A Correlation with Serotonin Receptor Affinities. *Psychopharmacology* **1980**, *68*, 155−158.
(16) Glennon, R. A.; Young, R.; Benington, F.; Morin, R. D. Behavioral and Serotonin Receptor Properties of 4-Substituted Derivatives of the Hallucinogen 1-(2,5-Dimethoxyphenyl)-2-aminopropane. *J. Med. Chem.* **1982**, *25*, 1163−1168.
(17) Rasmussen, K.; Glennon, R. A.; Aghajanian, G. K. Phenethylamine Hallucinogens in the Locus Coeruleus: Potency of Action Correlates with Rank Order of 5-HT_2 Binding Affinity. *Eur. J. Pharmacol.* **1986**, *132*, 79−82.
(18) Heller, W. A.; Baraban, J. M. Potent Agonist Activity of DOB at 5-HT_2 Receptors in Guinea Pig Trachea. *Eur. J. Pharmacol.* **1987**, *138*, 115−117.
(19) Lyon, R. A.; Davis, K. H.; Titeler, M. [^{3}H]DOB (4-Bromo-2,5-Dimethoxyphenylisopropylamine) Labels a Guanyl Nucleotide-Sensitive State of Cortical 5-HT_2 Receptors. *Mol. Pharmacol.* **1987**, *31*, 194−199.
(20) Lyon, R. A.; Titeler, M.; Seggel, M. R.; Glennon, R. A. Indolealkylamine Analogs Share 5-HT_2 Binding Characteristics with Phenylalkylamine Hallucinogens. *Eur. J. Pharmacol.* **1988**, *145*, 291−297.
(21) Sanders-Bush, E.; Burris, K. D.; Knoth, K. Lysergic Acid Diethylamide and 2,5-Dimethoxy-4-methylamphetamine are Partial Agonists at Serotonin Receptors Linked to Phosphoinositide Hydrolysis. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 924−928.
(22) Titeler, M.; Lyon, R. A.; Glennon, R. A. Radioligand Binding Evidence Implicates the Brain 5-HT_2 Receptor as a Site of Action for LSD and Phenylisopropylamine Hallucinogens. *Psychopharmacology* **1988**, *94*, 213−216.
(23) Pierce, P. A.; Peroutka, S. J. Hallucinogenic Drug Interactions with Neurotransmitter Receptor Binding Sites in Human Cortex. *Psychopharmacology* **1989**, *97*, 118−122.
(24) Seggel, M. R.; Yousif, M. Y.; Lyon, R. A.; Titeler, M.; Roth, B. L.; Suba, E. A.; Glennon, R. A. A Structure-Affinity Study of the Binding of 4-Substituted Analogs of 1-(2,5-Dimethoxyphenyl)-2-aminopropane at 5-HT_2 Serotonin Receptors. *J. Med. Chem.* **1990**, *33*, 1032−1036.
(25) Glennon, R. A.; Raghupathi, R.; Bartyzel, P.; Teitler, M.; Leonhardt, S. Binding of Phenylalkylamine Derivatives at 5-HT_{1C} and 5-HT_2 Serotonin Receptors: Evidence for a Lack of Selectivity. *J. Med. Chem.* **1992**, *35*, 734−740.
(26) Branchek, T.; Adham, N.; Macchi, M.; Kao, H.-T.; Hartig, P. R. [^{3}H]DOB (4-Bromo-2,5-Dimethoxyphenylisopropylamine) and [^{3}H]Ketanserin Label Two Affinity States of the Cloned Human 5-Hydroxytryptamine$_2$ Receptor. *Mol. Pharmacol.* **1990**, *38*, 604−609.
(27) Titeler, M.; Leonhardt, S.; Weisberg, E. L.; Hoffman, B. J. 4-[^{125}I]-Iodo-(2,5-dimethoxy)phenylisopropylamine and [^{3}H]Ketanserin Labelling of 5-Hydroxytryptamine$_2$ (5-HT_2) Receptors in Mammalian Cells Transfected with a Rat 5-HT_2 cDNA: Evidence for Multiple States and Not Multiple 5-HT_2 Receptor Subtypes. *Mol. Pharmacol.* **1990**, *38*, 594−598.
(28) Pierce, P. A.; Kim, J. Y.; Peroutka, S. J. Molecular Structural Basis of Ligand Selectivity for 5-HT_2 Versus 5-HT_{1C} Cortical Receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1992**, *346*, 4−11.
(29) Sanders-Bush, E. Neurochemical Evidence That Hallucinogenic Drugs Are 5-HT_{1C} Receptor Agonists: What Next? In *NIDA Research Monograph 146, Hallucinogens: An Update*; Lin, G. C., Glennon, R. A., Eds.; NIH Publication No. 94-3872, NIDA: Rockville, 1994; pp 203−213.
(30) Nichols, D. E.; Barfknecht, C. F.; Long, J. P.; Stanbridge, R. T.; Howell, H. G.; Partyka, R. A.; Dyer, D. C. Potential Psychotomimetics. 2. Rigid Analogs of 2,5-Dimethoxy-4-methylphenylisopropylamine (DOM, STP). *J. Med. Chem.* **1974**, *17*, 161−166.
(31) Nichols, D. E.; Weintraub, H. J. R.; Pfister, W. R.; Yim, G. K. W. The Use of Rigid Analogues to Probe Hallucinogen Receptors. In *NIDA Research Monograph 22, Quantitative Structure-Activity Relationships of Analgesics, Narcotic Antagonists, and Hallucinogens*; Barnett, G., Trsic, M., Willette, R., Eds.; U.S. Government Printing Office: Washington, D.C., 1978; pp 70−83.
(32) Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Riggs, R. M. Synthesis and Evaluation of 2,3-Dihydrobenzofuran Analogues of the Hallucinogen 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane: Drug Discrimination Studies in Rats. *J. Med. Chem.* **1986**, *29*, 302−304.
(33) Nichols, D. E.; Snyder, S. E.; Oberlender, R.; Johnson, M. P.; Huang, X. 2,3-Dihydrobenzofuran Analogues of Hallucinogenic Phenethylamines. *J. Med. Chem.* **1991**, *34*, 276−281.
(34) Waldman, S. R.; Monte, A. P.; Bracey, A.; Nichols, D. E. One-Pot Claisen Rearrangement/*O*-Methylation/Alkene Isomerization in the Synthesis of *Ortho*-Methoxylated Phenylisopropylamines. *Tetrahedron Lett.* **1996**, *37*, 7889−7892.
(35) Monte, A. P.; Marona-Lewicka, D.; Cozzi, N. V.; Nelson, D. L.; Nichols, D. E. Conformationally Restricted Tetrahydro-1-benzoxepin Analogs of Hallucinogenic Phenethylamines. *Med. Chem. Res.* **1995**, *5*, 651−663.
(36) Monte, A. P.; Marona-Lewicka, D.; Wainscott, D. B.; Nelson, D. L.; Nichols, D. E. Dihydrobenzofuran Analogues of Hallucinogens. 3. Models of 4-Substituted 2,5-Dimethoxyphenylalkylamine Derivatives with Rigidified Methoxy Groups. *J. Med. Chem.* **1996**, *39*, 2953−2961.
(37) Trulson, M. E.; Crisp, T.; Henderson, L. J. Mescaline Elicits Behavioral Effects in Cats by an Action at Both Serotonin and Dopamine Receptors. *Eur. J. Pharmacol.* **1983**, *96*, 151−154, and references therein.
(38) Peroutka, S. J.; Hamik, A.; Harrington, M. A.; Hoffman, A. J.; Mathis, C. A.; Pierce, P. A.; Wang, S. S.-H. (*R*)-(−)-[^{77}Br]4-Bromo-2,5-dimethoxyamphetamine Labels a Novel 5-Hydroxytryptamine Binding Site in Brain Membranes. *Mol. Pharmacol.* **1988**, *34*, 537−542.
(39) Barfknecht, C. F.; Nichols, D. E.; Dunn, I., W. J. Correlation of Psychotomimetic Activity of Phenethylamines and Amphetamines with 1-Octanol−Water Partition Coefficients. *J. Med. Chem.* **1975**, *18*, 208−210.
(40) Nichols, D. E.; Shulgin, A. T.; Dyer, D. C. Directional Lipophilic Character in a Series of Psychotomimetic Phenethylamine Derivatives. *Life Sci.* **1977**, *21*, 569−576.
(41) Gupta, S. P.; Singh, P.; Bindal, M. C. QSAR Studies on Hallucinogens. *Chem. Rev.* **1983**, *83*, 633−649.
(42) Jung, M. E.; Abrecht, S. Improved Synthesis of 3-Substituted 7-Methoxybenzofurans. Useful Intermediates for Preparation of Morphine Analogues. *J. Org. Chem.* **1988**, *53*, 423−425.
(43) Monte, A. P.; Marona-Lewicka, D.; Cozzi, N. V.; Nichols, D. E. Synthesis and Pharmacological Examination of Benzofuran, Indan, and Tetralin Analogues of 3,4-(Methylenedioxy)amphetamine. *J. Med. Chem.* **1993**, *36*, 3700−3706.
(44) Short, J. H.; Dunnigan, D. A.; Ours, C. W. Synthesis of Phenethylamines from Phenylacetonitriles Obtained by Alkylation of Cyanide Ion with Mannich Bases from Phenols and Other Benzylamines. *Tetrahedron* **1973**, *29*, 1931−1939.
(45) Aboul-Enein, M. N.; Eid, A. I. A Novel Route for the Synthesis of Mescaline. *Acta Pharm. Suec.* **1979**, *16*, 267−270.
(46) Carvalho, C. F.; Sargent, M. V. Boron Trichloride as a Selective Demethylating Agent for Hindered Ethers. *J. Chem. Soc., Chem. Commun.* **1984**, 227−229.
(47) Caldwell, W. T.; Thompson, T. R. Nuclear Methylation of Phenols. A New Synthesis of Intermediates in the Preparation of Antisterility Factors. *J. Am. Chem. Soc.* **1939**, *61*, 765−767.
(48) Dallacker, F.; Meunier, E.; Limpens, J.; Lipp, M. Preparations of Derivatives of Trimethoxybenzene. *Monatsch.* **1960**, *91*, 1077−1088.
(49) Friedman, L.; Schecter, H. Preparation of Nitriles from Halides and Sodium Cyanide. An Advantageous Nucleophilic Displacement in Dimethyl Sulfoxide. *J. Org. Chem.* **1960**, *26*, 877−879.
(50) Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. Selective Reductions. XIX. The Rapid Reaction of Carboxylic Acids with Borane−Tetrahydrofuran. A Remarkably Convenient Procedure for the Selective Conversion of Carboxylic Acids to the Corresponding Alcohols in the Presence of Other Functional Groups. *J. Org. Chem.* **1973**, *38*, 2786−2792.
(51) Nichols, D. E.; Barfknecht, C. F.; Rusterholz, D. B.; Benington, F.; Morin, R. D. Asymmetric Synthesis of Psychotomimetic Phenylisopropylamines. *J. Med. Chem.* **1973**, *16*, 480−483.
(52) Lewin, A. H.; Parker, S. R.; Fleming, N. B.; Carroll, F. I. Formylation of Arenes by α,α-Dichloromethyl Methyl Ether. An Improved Experimental Procedure. *Org. Prep. Proc. Int.* **1978**, *10*, 201−204.
(53) Monte, A. P.; Marona-Lewicka, D.; Kanthasamy, A.; Sanders-Bush, E.; Nichols, D. E. Stereoselective LSD-like Activity in a Series of *d*-Lysergic Acid Amides of (*R*)- and (*S*)-2-Aminoalkanes. *J. Med. Chem.* **1995**, *38*, 958−966.
(54) Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Jacob, P., III; Shulgin, A. T. Derivatives of 1-(1,3-Benzodioxol-5-yl)-2-butanamine: Representatives of a Novel Therapeutic Class. *J. Med. Chem.* **1986**, *29*, 2009−2015.
(55) De Boer, T. J.; Backer, H. J. Diazomethane. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, pp 250−253.
(56) Black, T. H. The Preparation and Reactions of Diazomethane. *Aldrichimica Acta* **1983**, *16*, 3−10.

(57) Oberlender, R.; Nichols, D. E. Drug Discrimination Studies With MDMA and Amphetamine. *Psychopharmacology* **1988**, *95*, 71−76.

(58) Oberlender, R.; Nichols, D. E. (+)-*N*-Methyl-1-(1,3-Benzodioxol-5-yl)-2-Butanamine as a Discriminative Stimulus in Studies of 3,4-Methylenedioxymethamphetamine-Like Behavioral Activity. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 1098−1106.

(59) Litchfield, J. T.; Wilcoxon, F. A Simplified Method of Evaluating Dose-Effect Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.

(60) Johnson, M. P.; Mathis, C. A.; Shulgin, A. T.; Hoffman, A. J.; Nichols, D. E. [^{125}I]-2-(2,5-Dimethoxy-4-Iodophenyl) Aminoethane ([^{125}I]-2C-I) as a Label for the 5-HT_2 Receptor in Rat Frontal Cortex. *Pharmacol. Biochem. Behav.* **1990**, *35*, 211−217.

(61) Wainscott, D. B.; Cohen, M. L.; Schenk, K. W.; Audia, J. E.; Nissen, J. S.; Baez, M.; Kursar, J. D.; Lucaites, V. L.; Nelson, D. L. Pharmacological Characteristics of the Newly Cloned Rat 5-HT_{2F} Receptor. *Mol. Pharmacol.* **1993**, *4*, 419−426.

(62) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(63) Nelson, D. L.; Lucaites, V. L.; Audia, J. E.; Nissen, J. S.; Wainscott, D. B. Species Differences in the Pharmacology of the 5-Hydroxytryptamine$_2$ Receptor: Structurally Specific Differentiation by Ergolines and Tryptamines. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 1272−1279.

(64) Nelson, D. L.; Baez, M.; Kursar, J. D.; Sasso, D. A.; Wainscott, D. B. [^{3}H]Rauwolscine as an Antagonist Radioligand for the Cloned Human Serotonin$_{2B}$ (5-HT_{2B}) Receptor. *Soc. Neurosci. Abstr.* **1995**, *21*, Part 2: 1124.

(65) Pazos, A.; Hoyer, D.; Palacios, J. M. Mesulergine, a Selective Serotonin-2 Ligand in the Rat Cortex, Does not Label these Receptors in Porcine and Human Cortex: Evidence for Species Differences in Brain Serotonin-2 Receptors. *Eur. J. Pharmacol.* **1985**, *106*, 531−538.

(66) Cheng, Y.; Prusoff, W. H. Relationship Between the Inhibition Constant (Ki) and the Concentration of an Inhibitor that Causes 50% Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(67) Berridge, M. J.; Downes, C. P.; Hanley, M. R. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **1982**, *206*, 587−595.

(68) Grotowiel, M. S.; Sanders-Bush, E. Regulation of serotonin2A receptors in heterologous expression systems. *J. Neurochem.* **1994**, *63*, 1255−1260.

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