Synthesis and Evaluation of Phenyl- and Benzoylpiperazines as Potential Serotonergic Agents

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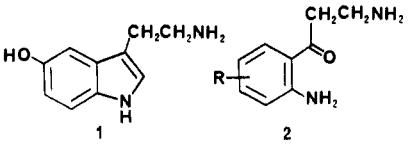
The binding of a series of phenylpiperazines (**3**) and benzoylpiperazines (**4**) to central serotonin (5-HT) sites was investigated. Several derivatives of **3** displayed nanomolar affinities for 5-HT_1 sites, whereas derivatives of **4** were essentially inactive both at 5-HT_1 and 5-HT_2 sites. 1-(2-Methoxyphenyl)piperazine (2-MPP, **3a**) was found to possess an affinity (K_i = 35 nM) for 5-HT_1 sites comparable to that of the recognized 5-HT agonist 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) (K_i = 20 nM); **3a** also displayed a 100-fold selectivity for 5-HT_1 sites (as compared to 8-fold for TFMPP). In tests of stimulus generalization using rats trained to discriminate TFMPP (ED_{50} = 0.17 mg/kg) from saline, **3a** was found to be nearly equipotent (ED_{50} = 0.22 mg/kg) with the training drug. These results suggest that **3a** may be a novel and more selective 5-HT_1 agonist than TFMPP.

With the recent discovery of two major populations of central serotonin (5-HT, **1**) binding sites has come a renewed interest in 5-HT research, particularly with respect to the development of site-selective agents. Serotonin binding sites labeled with high affinity by [^{3}H]-5-HT have been termed 5-HT_1 sites, whereas those in the frontal cortex that are labeled by [^{3}H]spiperone or [^{3}H]ketanserin are referred to as 5-HT_2 sites.[1,2]



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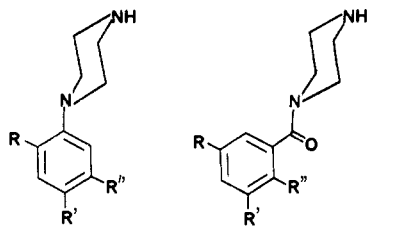

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Earlier studies employing peripheral 5-HT receptor (e.g., isolated tissue) preparations demonstrated that an intact indole nucleus was not necessary for serotonergic activity. For example, certain phenylalkylamine derivatives, including **2**, possess an affinity for the 5-HT receptors of the isolated rat fundus preparation.[3,4] Phenylpiperazine derivatives such as 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP; **3**, R = R′ = H, R″ = CF_3) and its corresponding chloro derivative mCPP (**3**, R = R′ = H, R″ = Cl) are also peripheral 5-HT agonists[5] and, in addition, have been shown to bind at central 5-HT sites.[6]

A feature common to a number of serotonin agonists is an aryl or heteroaryl ring separated from a terminal amine by a two- to four-atom spacer. In several instances, incorporation of aromatic methoxy groups or a benzylic carbonyl group has enhanced the receptor affinity of phenalkylamine derivatives;[4,7] this has been noted with 2-(phenylamino)ethanes and/or 3-(phenylamino)propanes. The ArCOXCCN moiety, where X = C or N, is also found imbedded in agents (including spiperone and ketanserin) that are known to bind at central serotonin sites. With the notable exceptions of TFMPP and mCPP, phenylpiperazines **3** have received relatively little attention, and simple benzoylpiperazines **4** have not been previously investigated. The purpose of this present study was to examine the central binding characteristics of several such derivatives that bear substituents already shown[8] to enhance the affinity of phenylalkylamines for peripheral 5-HT receptors.

Chemistry. The phenylpiperazines **3a**, **3c**, and **3d** (see Table II) were prepared, according to the general procedure of Brewster et al.,[9] by allowing the appropriately substituted aniline to react with bis(2-chloroethyl)amine. Attempts to prepare the bromo derivative **3e** (see Table II) from 2,5-dimethoxy-4-bromoaniline (**5**) were unsuccessful owing, perhaps, to the relative instability of **5**. Compound **3e** was prepared by the direct bromination of **3c**; the splitting patterns in the aromatic region of the proton NMR spectra of **3e** and **5** were very similar, suggesting that bromination had occurred para to the piperazine ring. Compound **3b** was prepared by treatment of **3a** with 48% HBr according to the method of Prelog and Blazek.[10]

Initial attempts to prepare the benzoylpiperazines involved the acylation of piperazine with the appropriately substituted benzoyl halides. Regardless of the reaction conditions or stoichiometry, only bis-substituted products were obtained (for example, see the preparation of the bis(2,5-dimethoxybenzoyl) derivative **7**), and attempts to partially hydrolyze these bis derivatives to **4** were unsuccessful. Subsequently, derivatives of **4** were prepared by using 1-formylpiperazine in place of piperazine in the above reaction. Thus, 1-formylpiperazine was acylated to afford the formylated intermediates **6a–g**, which, after Kugelrhor distillation, were hydrolyzed to **4a–g** (Table I).

Binding Studies. 5-HT_1 and 5-HT_2 binding data were

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Table I. Properties of Benzoylpiperazines 4

no.	R	R′	R″	mp, °C (solvent)[a]	% yield	formula[b]
4a	H	H	OCH_3	221–222 (E)	88	$C_{12}H_{16}N_2O_2$·HCl
4b	OCH_3	H	H	192–194 (A)	67	$C_{12}H_{16}N_2O_2$·HCl
4c	H	Cl	H	229–231 (E)	70	$C_{11}H_{13}ClN_2O$·HCl
4d	H	CH_3	H	227–229 (E)[c]	65	$C_{12}H_{16}N_2O$·HCl
4e	OCH_3	H	OCH_3	183–184 (E)	47	$C_{13}H_{18}N_2O_3$·$(COOH)_2$
4f	OCH_3	CH_3	OCH_3	232–234 (M)[d]	45	$(C_{14}H_{20}N_2O_3)_2$·$(COOH)_2$
4g	OCH_3	Br	OCH_3	185–187 (M)[e]	82	$C_{13}H_{17}BrN_2O_3$·$(COOH)_2$

[a] Recrystallization solvents: E = absolute EtOH, A = MeCN, M = MeOH. [b] Analytical data were obtained on each compound; values for C, H, and N are within 0.4% of calculated values. [c] Free base, mp 74–76 °C. [d] Free base, mp 112–114 °C. [e] Free base, mp 128–130 °C.

Table II. Results of Binding Studies

				5-HT_1		5-HT_2	
no.	R	R′	R″	K_i, nM	Hill slope	K_i, nM	Hill slope
3a	OMe	H	H	35 (±2)	0.60 (±0.02)	3500 (±100)	1.11 (±0.04)
3b	OH	H	H	150 (±10)	0.67 (±0.05)	17 200 (±1200)	0.99 (±0.09)
3c	OMe	H	OMe	1035 (±85)	0.70 (±0.07)	8430 (±50)	0.91 (±0.03)
3d	OMe	Me	OMe	680 (±70)	0.64 (±0.09)	4710 (±480)	0.86 (±0.03)
3e	OMe	Br	OMe	820 (±160)	0.64 (±0.09)	2430 (±170)	0.91 (±0.09)
4a	H	H	OMe	>100 000		>100 000	
4b	OMe	H	H	28 400 (±4000)	0.58 (±0.07)	64 300 (±3000)	1.03 (±0.02)
4c	H	Cl	H	17 100 (±4000)	0.57 (±0.04)	55 600 (±6000)	0.76 (±0.06)
4d	H	Me	H	20 700 (±3000)	0.82 (±0.10)	49 000 (±2500)	0.91 (±0.05)
4e	OMe	H	OMe	>100 000		>100 000	
4f	OMe	Me	OMe	>100 000		93 000 (±7000)	0.89 (±0.03)
4g	OMe	Br	OMe	>100 000		76 500 (±6000)	0.95 (±0.12)
2a				345 (±30)	0.64 (±0.05)	8480 (±700)	0.98 (±0.02)
TFMPP[a]				20 (±4)	0.57 (±0.05)	160 (±10)	0.73 (±0.01)

[a] TFMPP = 1-[3-(trifluoromethyl)phenyl]piperazine.

obtained for all derivatives of **3** and **4** (Table II). For the most part, derivatives of **3** displayed nanomolar affinities (i.e., K_i values) for 5-HT_1 sites and micromolar affinities for 5-HT_2 sites. Derivatives of **4** were essentially inactive at both sites. The 2-amino-5-methoxypropiophenone derivative **2a** (i.e., **2**, R = 5-OMe) displayed a moderate affinity for 5-HT_1 sites but a low affinity for 5-HT_2 sites.

With respect to 5-HT_1 binding, the affinity of 1-(2-methoxyphenyl)piperazine (2-MPP, **3a**; K_i = 35 nM) approaches that of the recognized 5-HT_1 agonist TFMPP (K_i = 20 nM). However, whereas TFMPP possesses only an 8-fold selectivity for 5-HT_1 sites, that for **3a** is 100-fold. Demethylation of the 2-methoxy group of **3a**, to give **3b**, results in a 5-fold decrease in affinity but in no loss of selectivity. Introduction of a second methoxy group, i.e., **3c**, with or without additional substituents, reduces both affinity and selectivity for 5-HT_1 sites.

Because certain phenalkylamines are known to interact at central dopamine sites, the binding of **3a–e** and **4a–g** at [^{3}H]spiperone-labeled dopamine sites was also examined. Each of the compounds was examined, in duplicate, at a concentration of 10^{-5} M, and several were found to be, at best, only weakly active. Reevaluation at 10^{-7} M revealed almost no displacement of specifically bound radioligand.

Behavioral Studies. Compounds **3a–d** were evaluated in tests of stimulus generalization in rats trained to discriminate 0.5 mg/kg of TFMPP from saline (Table III). The TFMPP stimulus generalized to **3a** (ED_{50} = 0.22 mg/kg, relative to 0.17 mg/kg for TFMPP). Compound **3b** produced saline-appropriate responding at doses of up to 10 mg/kg. Compounds **3c** and **3d** produced saline-appropriate responding at nearly 100 times the ED_{50} dose of TFMPP; a small increase in the dose of **3d** (i.e., to 12.5 mg/kg) resulted in disruption of behavior.

Compounds **4a–e** were evaluated in the TFMPP-trained animals at doses of 5 and 10 mg/kg; in each case, TFMPP-appropriate responding never exceeded 25% (data not shown). Selected compounds were also examined in rats trained to discriminate 1.0 mg/kg of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from saline. Compounds **3a** and **3c** produced saline-appropriate responding, followed, at higher doses, by disruption of behavior (Table III). Both **3d** and **3e** resulted in stimulus generalization (ED_{50} = 6.23 and 4.08 mg/kg, respectively). Ten doses (1.0–15.0 mg/kg) of **4f** were also evaluated (data not shown); **4f** produced saline-appropriate (i.e., 17% DOM-appropriate) responding at the highest dose tested.

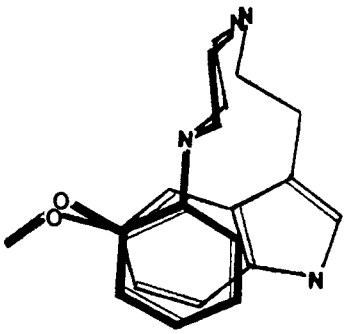

Figure 1. Computer-generated structure of 2-MPP (**3a**) (heavy lines) superimposed on 5-HT (**1**).

Molecular Modeling. The commonality of the behavioral response of 2-MPP (**3a**) and TFMPP, together with the binding data, suggests that these two agents may produce their effects via interaction at a common site. With the assumption that the oxygen and basic nitrogen atoms are involved in such an interaction, molecular modeling studies were conducted in order to determine if these atoms could be geometrically matched with those of 5-HT. Models of **3a** and 5-HT were generated, and two-point matches were developed by the COMPAR program (see Experimental Section) where two given atoms on each molecule were locked for least-squares fitting. The best match achieved (Figure 1) yields calculated N–N and O–O distances that are reasonable (i.e., 0.26 Å in both cases) and suggest that such an overlap is possible.

Table III. Results of Stimulus Generalization Studies

	dose, mg/kg	N^a	drug-appropriate responding % (±SEM)[b]	ED_{50}, mg/kg (95% CL)
		(A) TFMPP-Trained Animals[c]		
3a	0.05	4/4	23 (±11)	
	0.1	4/5	33 (±9)	
	0.4	3/4	63 (±18)	
	1.0	5/5	81 (±10)	
	1.2	5/5	94 (±4)	0.22 (0.08–0.60)
3b	0.3	3/3	17 (±7)	
	0.8	3/3	21 (±14)	
	2.0	4/4	12 (±6)	
	5.0	4/4	22 (±4)	
	10.0	5/5	24 (±8)	
3c	1.2	4/4	14 (±3)	
	3.0	4/4	6 (±3)	
	6.0	4/4	14 (±5)	
	10.0	3/3	19 (±7)	
	15.0	3/3	14 (±8)	
3d	1.5	3/3	11 (±6)	
	5.0	3/3	11 (±5)	
	8.0	3/3	7 (±4)	
	12.0	2/3	30 (±8)	
	12.5	0/3	*d*	
TFMPP	0.05	4/4	25 (±8)	
	0.10	4/4	8 (±4)	
	0.15	3/3	42 (±10)	
	0.20	4/4	51 (±14)	
	0.30	4/4	70 (±12)	
	0.35	5/5	90 (±5)	
	0.50	4/4	93 (±2)	0.17 (0.10–0.28)
saline (1.0 mL/kg)		6/6	9 (±3)	
		(B) DOM-Trained Animals		
3a	0.1	3/3	12 (±3)	
	0.5	2/3	20 (±6)	
	1.0	0/3	*d*	
3c	6.0	5/5	10 (±4)	
	12.0	4/4	28 (±15)	
	13.5	3/4	22 (±9)	
	14.0	1/5	*d*	
	15.0	1/4	*d*	
	18.0	0/4	*d*	
3d	4.0	5/5	21 (±10)	
	6.0	4/4	53 (±21)	
	8.0	5/5	68 (±22)	
	12.0	5/5	83 (±7)	6.23 (3.95–9.82)
3e	1.0	3/3	19 (±6)	
	3.0	3/3	31 (±8)	
	5.0	3/3	46 (±12)	
	8.0	3/3	72 (±10)	
	9.0	3/3	81 (±9)	4.08 (1.43–11.62)
DOM	1.0	6/6	95 (±4)	0.44 mg/kg
saline (1.0 mL/kg)		6/6	13 (±3)	

[a] Number of animals responding/number of animals receiving drug. [b] Data obtained during 2.5-min extinction session. Responding represents responses on the drug-appropriate lever (as a percent of total responses) for all animals (i.e., *N*) that meet criteria. [c] TFMPP = 1-[3-(trifluoromethyl)phenyl]piperazine. [d] Disruption of behavior; see Experimental Section for definition.

Discussion

With the exception of 2-MPP (**3a**) and **3b**, none of the compounds in Table II demonstrated significant potency and/or selectivity for 5-HT_1 or 5-HT_2 sites. Fuller et al.[6] had previously examined the 5-HT_1 binding properties of a small series of monosubstituted phenylpiperazines and found that 2-MPP was approximately one-fifth as potent as TFMPP; 5-HT_2 binding data were not reported. As can be seen from Table II, 2-MPP is somewhat less potent than TFMPP, but the difference in potencies is very small.

Perhaps more significant than the potency of 2-MPP is its selectivity for 5-HT_1 vs. 5-HT_2 sites. TFMPP possesses an 8-fold selectivity for 5-HT_1 sites (Table II); these results are midway between those reported by Martin and Sanders-Bush (i.e., 18-fold)[11] and Huff and co-worker (i.e., 3-fold).[12] Table II reveals that 2-MPP possesses a 100-fold selectivity for these sites.

With use of a two-lever operant procedure, animals can be trained to discriminate the stimulus effects of one agent from those of another or from saline vehicle. Once these animals have been trained, they can be administered novel agents (i.e., challenge drugs) in order to determine if these agents produce stimulus effects similar to those of the training drug (i.e., in tests of stimulus generalization).[13] TFMPP serves as an effective training drug and produces a stimulus that may be 5-HT_1 (and more specifically 5-$HT_{1\beta}$) mediated.[14,15] As shown in Table III, TFMPP-

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stimulus generalization occurred with 2-MPP, suggesting that both agents produce similar stimulus effects. Furthermore, these agents appear to be of comparable potency. Compounds **3b–d** did not result in stimulus generalization at the highest doses tested (higher doses of **3b** and **3c** may have resulted in generalization but were not evaluated). The relative inactivity of **3b** may be due to pharmacokinetic complications introduced by the polar hydroxyl group. Compound **3d** produced disruption of behavior at the highest dose tested, suggesting that it may be capable of producing a central effect other than one that is TFMPP-like. This prompted us to evaluate **3d** in DOM-trained animals.

Since this work was initiated, we have found that DOM displays a 30-fold selectivity for 5-HT_2 vs. 5-HT_1 sites;[16] we have also suggested that DOM may produce its stimulus effects primarily via a 5-HT_2-related mechanism.[15,17,18] As a consequence, compounds **3a**, **3c–e**, and **4f** were evaluated in tests of stimulus generalization in DOM-trained animals. Both **3d** and **3e** produced DOM-like effects. Thus, phenylpiperazines appear capable of producing either TFMPP-like (i.e., **3a**) or DOM-like (e.g., **3d**) stimulus effects depending upon their substitution pattern. Although too little data are available to formulate any structure–activity relationships, it might be noted that **3d** possesses the same aromatic substitution pattern as does DOM (and it is known that removal of the 4-methyl and 5-methoxy groups of DOM abolishes DOM-like stimulus effects[13]).

2-MPP (**3a**) is not a new agent. Early studies demonstrated that 2-MPP could produce a pronounced hypotensive effect in animals.[19] This led to its eventual evaluation in human subjects;[19] in addition to its hypotensive actions, this agent produced several central effects including drowsiness and lethargy. 2-MPP has also been demonstrated to be a metabolite of the antipsychotic agent millipertine.[20] Recently, Pawlowski reevaluated 2-MPP in animals and suggested that a number of its actions may be a direct consequence of serotonin agonism.[21] The results of the modeling studies (Figure 1) provide one suggestion as to how such an interaction might be viewed. These results coupled with the present finding that 2-MPP (**3a**) may be a selective 5-HT_1 (and, perhaps, a 5-HT_{1B}-selective) agonist suggest that further studies on phenylpiperazine derivatives are warranted.

Experimental Section

Proton magnetic resonance (^1H NMR) spectra were recorded on a Perkin-Elmer R-24 high-resolution spectrometer, and chemical shifts are reported relative to Me_4Si as an internal standard. Infrared spectra were obtained on a Perkin-Elmer 257 spectrophotometer. Spectral data were consistent with the assigned structures. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, and determined values are within 0.4% of theoretical.

1-(2,5-Dimethoxyphenyl)piperazine Dihydrochloride (3c). A mixture of bis(2-chloroethyl)amine hydrochloride (23.3 g, 131 mmol), anhydrous K_2CO_3 (18 g), freshly distilled 2,5-dimethoxyaniline (20.0 g, 131 mmol), and diglyme (75 mL) was heated at reflux for 48 h, allowed to cool to room temperature, and then poured into water (200 mL). The aqueous mixture was made basic (ca. pH 12) by the addition of saturated KOH solution and was extracted with EtOAc (3 × 200 mL). The combined organic portion was washed with water (3 × 200 mL), dried ($MgSO_4$), and evaporated to dryness under reduced pressure to yield a dark oil. Vacuum distillation afforded 18 g (62%) of the amine as a light-yellow liquid, bp 142–146 °C (0.18 mm) (lit.[9] bp 133 °C (0.2 mm)). A saturated solution of HCl gas in anhydrous Et_2O was added at room temperature to a solution of the amine in absolute EtOH to afford **3c**, mp 218–220 °C after recrystallization from absolute EtOH. Anal. ($C_{12}H_{18}N_2O_2 \cdot 2HCl$) C, H, N.

Compounds **3a** (as the monohydrochloride) and **3d** (as the dihydrochloride) were prepared in a similar manner: **3a**, mp 236–238 °C (lit.[10] mp 238 °C); **3d**, mp 241–243 °C (lit.[9] mp 242–243 °C). Treatment of **3a** with base liberated the free amine (bp 118–121 °C (0.12 mm)), which was converted to the monohydrobromide salt, mp 239–241 °C (lit.[10] mp 242.5 °C); this was done to confirm the identity of the product, but the monohydrochloride salt was used for testing purposes. Compound **3b** (as the dihydrobromide salt, mp 287–289 °C) was prepared from **3a** according to the method of Prelog and Blazek.[10]

1-(2,5-Dimethoxy-4-bromophenyl)piperazine Dihydrochloride (3e). A solution of Br_2 (3.2 g, 20 mmol) in glacial HOAc (20 mL) was added in a dropwise manner to a stirred solution of **3e** (as the free base; 4.0 g, 18 mmol) in 48% HBr (4 mL) and glacial HOAc (5 mL) at 0 °C, and after the addition was complete, the solution was stirred at room temperature for another 4 h. Solvent was removed under reduced pressure to afford a white solid material (mp 220–224 °C after recrystallization from absolute EtOH). A portion (2 g) of this solid material was dissolved in H_2O (10 mL); the solution was made basic (ca. pH 9) with 10% aqueous NaOH and extracted with EtOAc (3 × 50 mL). The combined organic portion was dried ($MgSO_4$) and evaporated to dryness under reduced pressure to afford a dark oil. The oil was distilled (Kugelrhor, 72–75 °C (0.08 mm)) and an ethanolic solution treated with HCl gas until salt formation ceased. Recrystallization from absolute EtOH gave 0.3 g of **3e** as small white crystals: mp 203–205 °C dec; IR (neat) 3456 (NH) cm^{-1}; ^1H NMR (free base, $CDCl_3$) 1.95 (br s, 1 H, NH), 3.05 (s, 8 H, NCH_2), 3.85 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 6.65 (s, 1 H, Ar H), 7.10 (s, 1 H, Ar H). Anal. ($C_{12}H_{17}BrN_2O_2 \cdot 2HCl$) C, H, N.

1-(2,5-Dimethoxy-3-bromobenzoyl)piperazine Hydrogen Oxalate (4g). Compound **6g** (1.5 g, 4 mmol) was dissolved in 120 mL of methanolic HCl (5.5 mL of concentrated HCl/60 mL of MeOH) with gentle heating. The solution was allowed to stir at room temperature for 18 h, and then the solvent was removed under reduced pressure to give 1.5 g of an off-white solid. This solid material (mp 191–194 °C from acetonitrile) was dissolved in H_2O (5 mL), and the solution was made basic (to pH 9) by the addition of 10% aqueous NaOH and extracted with Et_2O (3 × 25 mL). The combined Et_2O extracts were dried ($MgSO_4$) and evaporated to dryness under reduced pressure to afford 1.1 g of the amine as a white solid (mp 128–130 °C). The hydrogen oxalate salt was prepared and recrystallized from methanol to yield 1.4 g of a white crystalline solid, mp 185–187 °C. Anal. ($C_{13}H_{17}BrN_2O_3 \cdot C_2H_2O_4$) C, H, N.

Compounds **4a–f** were prepared in a similar manner and were isolated either as their hydrochloride or oxalate salts; see Table I.

1-(2,5-Dimethoxy-4-bromobenzoyl)-4-formylpiperazine (6g). A solution of 2,5-dimethoxy-3-bromobenzoic acid (2.5 g, 10 mmol) in thionyl chloride (20 mL) was heated at reflux for 3 h and allowed to cool to room temperature. Excess thionyl chloride was removed under reduced pressure, and the resultant crude product was distilled (Kugelrhor 54–56 °C (0.09 mm)) to afford 2.2 g (82%) of the acid chloride as a white solid, mp 57–59 °C. A solution of this acid chloride (2.8 g, 10 mmol) in $CHCl_3$ (20 mL) was added in a dropwise manner to a stirred mixture of $NaHCO_3$ (2.5 g) and 1-formylpiperazine (1.1 g, 10 mmol) in $CHCl_3$ (100 mL) at room temperature. After the addition was complete, the mixture was allowed to stir for 18 h; the mixture was washed with water (2 × 100 mL), and the organic portion was dried ($MgSO_4$) and evaporated to dryness to afford a dark

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yellow oil. Kugelrhor distillation (95 °C (0.06 mm)) afforded 1.9 g of the title compound as a viscous yellow oil, which solidified upon standing, mp 64–68 °C.

Intermediates **6a–f** were prepared in a similar manner and were used, generally, without characterization.

1,4-Bis(2,5-dimethoxybenzoyl)piperazine (7). A solution of 2,5-dimethoxybenzoyl chloride (2.0 g, 10 mmol) in $CHCl_3$ (10 mL) was added in a dropwise manner to a stirred solution of piperazine (1.7 g, 20 mmol) in $CHCl_3$ (50 mL) at 0 °C. After stirring for 1 h, the reaction mixture was filtered and the filtrate was evaporated to dryness to afford a beige solid. Recrystallization from absolute EtOH afforded 1.3 g (60%) of **7** as a white solid, mp 205–207 °C. Anal. ($C_{22}H_{26}N_2O_6$) C, H, N.

Binding Studies. The radioligand binding assay has already been described in detail.[16] By use of the method of Leysen et al.,[22] tissue homogenates were prepared from prefrontal cortex of female Sprague–Dawley (ca. 200 g) rats. The final suspension was in 50 mM Tris·HCl (pH 7.4) buffer at a tissue concentration of 16 mg wet weight/mL. The assays were performed in triplicate in 2.0-mL volumes of a 50 mM Tris, 5 mM $MgCl_2$, 0.5 mM EDTA Na_2 (pH 7.4 at 37 °C) buffer to which 4 mg wet weight of tissue was added. Competition experiments were performed with tritiated ligands obtained from New England Nuclear, i.e., either 0.4 nM [^{3}H]ketanserin (defined as 5-HT_2 binding) or 2 nM [^{3}H]-5-HT (defined as 5-HT_1 binding). Filtration was accomplished with glass fiber filters (Flow Laboratories), and filters were counted after buffer wash by liquid scintillation spectrometry with NEN 963. Nonlabeled 5-HT (1 μM) and cinanserin (1 μM) were used to measure nonspecific binding. Competition binding data were analyzed by a nonlinear least-squares curve-fitting procedure; IC_{50} values were determined in triplicate from a 23-point curve, and K_i values were calculated according to the equation $K_i = IC_{50} \div 1 + [D]/K_D$, where [D] = concentration of radioligand and K_D is the equilibrium dissociation constant of radioligand binding.

Behavioral Studies. Six male Sprague–Dawley rats were maintained at ca. 80% of their free-feeding body weights by partial food deprivation. Behavioral testing was conducted in standard two-lever operant chambers (Model E 10-10, Coulbourn Instruments) housed within light- and sound-attenuating outer chambers. Illumination of each chamber was provided by means of a 28-V overhead house light. One wall of each operant chamber was fitted with two levers and a dipper (housed equidistant between the levers) for delivery of reinforcement (0.01 mL of sweetened milk). Solid state and electromechanical programing and recording equipment were housed in the same room as the operant chambers.

The rats were initially trained to respond on both levers under a variable interval 15-s (VI-15s) schedule of reinforcement. After lever responding was established, each daily session was preceded by an intraperitoneal (ip) injection of either 1-[3-(trifluoromethyl)phenyl]piperazine hydrochloride (TFMPP, 0.5 mg/kg) or 0.9% saline (1.0 mL/kg). A presession injection interval (psii) of 15 min was employed; during the period following administration of TFMPP or saline, the animals were kept in their individual home cages. Training sessions were of 15-min duration. Responding on one of the levers was reinforced after administration of TFMPP, whereas responding on the opposite lever was reinforced after administration of saline. Saline and TFMPP were administered on a double-alternation schedule. On every fifth day, discrimination learning was assessed during an initial 2.5-min extinction session, followed by a 12.5-min training session. After 30 training sessions, discrimination performance was stable under each treatment condition, i.e., the animals made greater than 80% of their responses on the TFMPP-appropriate lever when administered the training dose of the training drug and less than 20% of their responses on the same lever after administration of saline.

A second group of rats was trained to discriminate 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM, 1.0 mg/kg) from saline by using a procedure identical with that used above; a detailed description of the training of this group of animals has already been reported.[18] Maintenance of the TFMPP/saline and DOM/saline discriminations was insured in the respective animals by continuation of the training sessions throughout the stimulus generalization studies. During the generalization studies, test sessions were interposed amongst the training sessions. The animals were allowed 2.5 min to respond under extinction conditions and were then returned to their home cages. An odd number of training sessions (not less than three) separated any two testing sessions. During these test sessions, doses of the challenge drugs were administered in a random sequence, using a 15-min psii. Stimulus generalization was said to occur when percent drug-appropriate responding exceeded 80%. Animals making less than five total responses during the entire 2.5-min extinction session were reported as being disrupted. Where stimulus generalization occurred, ED_{50} values (i.e., doses at which the animals would be expected to make approximately 50% of their responses on the drug-appropriate lever) were determined by the method of Finney.[23]

Modeling Studies. Modeling software supported by Molecular Design Ltd. (San Leandro, CA) was used to prepare and match three-dimensional models of 2-MPP (**3a**) and 5-HT (**1**). The structures of each were entered in the DRAW mode of the MACCS (Molecular Access) system on a Prime 9950 minicomputer through an Envision 230 graphics terminal. These two-dimensional files were processed through PRXBLD, a classical mechanics modeling program that generates reasonable low-energy structures in 3-D. Matching was accomplished using the COMPAR program. COMPAR brings two 3-D models onto the same screen and allows the selection of two or more atoms in each structure for a least-squares match. Once selected, the program generates an overlapped 3-D structure (e.g., Figure 1) and reports the average deviation of the selected atoms. At the graphics terminal, the matching process is facilitated by displaying each structure in a different color. Nitrogen, carbon, and oxygen atoms were selected for experimental matches until the best fit was found. As a measure of fit, the combined structures were transferred to DISP for measurement of the N–N and O–O distance by the LOOK program. These distances provide a numerical assessment of the match.

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Registry No. **3a**, 35386-24-4; **3a**·HCl, 5464-78-8; **3a**·HBR, 100939-96-6; **3b**, 1011-17-2; **3b**<<cet2HBr, 58260-69-8; **3c**, 1019-06-3; **3c**·2HCl, 100939-95-5; **3d**, 38869-11-3; **3d**·2HCl, 38869-44-2; **3e**, 100939-87-5; **3e**·2HCl, 100939-97-7; **4a**, 100939-88-6; **4a**·HCl, 100939-98-8; **4b**, 100939-89-7; **4b**·HCl, 100939-99-9; **jc**, 100939-90-0; **4c**·HCl, 100940-00-9; **4d**, 100939-91-1; **4d**·HCl, 100940-01-0; **4e**, 100939-92-2; 4e·oxalate, 100940-02-1; **4f**, 100939-93-3; **4f**·oxalate, 100940-03-2; **4g**, 100939-94-4; **4g**·oxalate, 100946-54-1; **6a**, 100940-04-3; **6b**, 100940-05-4; **6c**, 100940-06-5; **6d**, 100940-07-6; **6e**, 100940-08-7; **6f**, 100940-09-8; **6g**, 100940-10-1; **7**, 100940-14-5; *o*-$MEOC_6H_4NH_2$, 90-04-0; *o*-$MeOC_6H_4CO_2H$, 579-75-9; *m*-$MeOC_6H_4CO_2H$, 586-38-9; *m*-$ClC_6H_4CO_2H$, 535-80-8; *m*-$MeC_6H_4CO_2H$, 99-04-7; 2,5-$Me_2OC_6H_3CO_2H$, 2785-98-0; bis(2-chloroethyl)amine hydrochloride, 821-48-7; 2,5-dimethoxyaniline, 102-56-7; 2,5-dimethoxy-methylaniline, 34238-59-0; 2,5-dimethoxy-3-methylbenzoic acid, 100940-11-2; 3-bromo-2,5-dimethoxybenzoic acid, 100940-12-3; 3-bromo-2,5-dimethoxybenzoyl chloride, 100940-13-4; 1-formylpiperazine, 7755-92-2; piperazine, 110-85-0; 2,5-dimethoxybenzoyl chloride, 17918-14-8.

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