A Structure–Affinity Study of the Binding of 4-Substituted Analogues of 1-(2,5-Dimethoxyphenyl)-2-aminopropane at 5-HT<sub>2</sub> Serotonin Receptors

Mark R. Seggel, Mamoun Y. Yousif, Robert A. Lyon, Milt Titeler, Bryan L. Roth, Eva A. Suba, and Richard A. Glennon*

Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298-0581, Department of Pharmacology and Toxicology, Albany Medical College, Albany, New York 12208, and Naval Medical Research Institute, Bethesda, Maryland 20814. Received November 11, 1988.

With [3H]ketanserin as the radioligand, structure–affinity relationships (SAFIRs) for binding at central 5-HT<sub>2</sub> serotonin receptors (rat frontal cortex) were examined for a series of 27 4-substituted 1-(2,5-dimethoxyphenyl)-2-aminopropanes (2,5-DMAs). The affinity (K<sub>i</sub>) values ranged over a span of several orders of magnitude. It appears that the lipophilic character of the 4-position substituent plays a major role in determining the affinity of these agents for 5-HT<sub>2</sub> receptors, 2,5-DMAs with polar 4-substituents (e.g., OH, NH<sub>2</sub>, COOH) display a very low affinity (K<sub>i</sub> > 25000 nM) for these receptors, whereas those with lipophilic functions display a significantly higher affinity. The results of these studies prompted us to synthesize and evaluate examples of newer lipophilic derivatives and several of these (e.g., n-hexyl, n-octyl) bind with very high (K<sub>i</sub> values = 2.5 and 3 nM, respectively) affinities at central 5-HT<sub>2</sub> sites. Although, 2,5-DMAs are generally considered to be 5-HT<sub>2</sub> agonists, preliminary studies with isolated rat thoracic aorta suggest that some of the more lipophilic derivatives (e.g., the n-hexyl and n-octyl derivatives) are 5-HT<sub>2</sub> antagonists.

Central serotonin (5-HT) receptors can be categorized as belonging to one of three major classes: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>3</sub>. Serotonin itself is nonselective and binds at each of these neurotransmitter sites. One of the goals of our work is to develop site-selective 5-HT agonists and antagonists and, to this extent, we have demonstrated that certain 4-substituted 1-(2,5-dimethoxyphenyl)-2-aminopropane (i.e., 2,5-DMA) derivatives bind at 5-HT<sub>2</sub> receptor sites. We have also found that the affinity and selectivity of these agents are significantly influenced by the nature of the 4-substituent. For example, 2,5-DM(A)1a binds at 5-HT<sub>2</sub> sites with little selectivity and with rather low affinity (K<sub>i</sub> = 5200 nM), whereas its 4-bromo derivative DOB (1g) binds selectively and with considerably greater affinity (K<sub>i</sub> = 40 nM). Indeed, [3H]DOB is now commercially available as a radioligand for use in binding studies. In order to determine the influence of the 4-substituents on binding at 5-HT<sub>2</sub> sites, several years ago we conducted a Hansch analysis on a series of 13 derivatives of 2,5-DMAs that varied in structure only at the 4-position. Preliminary results suggested that lipophilic substituents are important for affinity. We have now prepared and examined a total of 27 4-substituted derivatives of 2,5-DMAs with a broader range of substituents in order to further challenge this hypothesis. We report here the synthesis and 5-HT<sub>2</sub> binding data for the new compounds and the structure–affinity relationships (SAFIR) for the entire series. We have also found that some of the new, more lipophilic 2,5-DMAs derivatives act as 5-HT<sub>2</sub> antagonists.

### Chemistry

A total of 27, 2,5-DMAs derivatives were examined in the present study; some of these compounds (i.e., 1a–e,g,k,l,m–o) are the 13 included in the original study and their syntheses have been previously reported. The 4-chloro derivative 1f was synthesized according to the method of Coutts and Malicky. Amine 1t was prepared by catalytic reduction of the corresponding 4-nitro compound by a literature procedure.

### Table I. Structures and 5-HT<sub>2</sub> Binding Data for 1-(2,5-Dimethoxyphenyl)-2-aminopropanes

<table>
<thead>
<tr>
<th>R</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;, nM</th>
<th>R</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>H</td>
<td>5200</td>
<td>1o</td>
</tr>
<tr>
<td>1b</td>
<td>OMe</td>
<td>1250</td>
<td>1p</td>
</tr>
<tr>
<td>1c</td>
<td>OEt</td>
<td>2200</td>
<td>1q</td>
</tr>
<tr>
<td>1d</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>300</td>
<td>1r</td>
</tr>
<tr>
<td>1e</td>
<td>F</td>
<td>1100</td>
<td>1s</td>
</tr>
<tr>
<td>1f</td>
<td>Cl</td>
<td>218</td>
<td>1t</td>
</tr>
<tr>
<td>lg</td>
<td>Br</td>
<td>41</td>
<td>1u</td>
</tr>
<tr>
<td>lh</td>
<td>I</td>
<td>19</td>
<td>1v</td>
</tr>
<tr>
<td>lI</td>
<td>Me</td>
<td>100</td>
<td>1w</td>
</tr>
<tr>
<td>lj</td>
<td>Et</td>
<td>100</td>
<td>1x</td>
</tr>
<tr>
<td>lk</td>
<td>Pr</td>
<td>67</td>
<td>1y</td>
</tr>
<tr>
<td>ll</td>
<td>i-Pr</td>
<td>76</td>
<td>1z</td>
</tr>
<tr>
<td>lm</td>
<td>Bu</td>
<td>58</td>
<td>1aa</td>
</tr>
<tr>
<td>ln</td>
<td>t-Bu</td>
<td>19</td>
<td>1aa</td>
</tr>
</tbody>
</table>

*Affinities (K<sub>i</sub>) values for [3H]ketanserin-labeled 5-HT<sub>2</sub> sites. Some of the K<sub>i</sub> values were reported earlier. K<sub>i</sub> values are followed by ±SEM only for those data not previously published. SEM not determined for 1z and 1aa.

Analogues of 1-(2,5-Dimethoxyphenyl)-2-aminopropane


Nitrile 1u was prepared by treatment of an N-phthalimido-protected 4-bromo derivative of 2,5-DMA (i.e., 5) with cuprous cyanide followed by deprotection with hydrazine. Esters 1v and 1w were obtained by esterification of acid 1aa, and amide 1y was obtained by treatment of 1v with n-propylamine. Although 4-OH analogue 1z has been mentioned in the literature on several occasions (e.g., see ref 9), there is no evidence of its preparation or characterization. Attempts to prepare 1z via hydrolysis of the diazonium salt generated from N-acetyl 1t were unsuccessful. However, 1x was prepared in low yield by Baeyer-Villiger oxidation of the 4-formyl derivative of 5 followed by deprotection.

Results

Radioligand binding data for the entire set of 27 4-substituted 2,5-DMA analogues are shown in Table I; Kᵢ values range from 2.5 to >50,000 nM. Substitution at the 4-position by polar/hydrophilic substituents such as NH₂, OH, and COOH (i.e., 1t, 1z, 1aa, respectively) result in compounds with a lower affinity (Kᵢ > 25,000 nM) for 5-HT₂ sites than that of the 4-unsubstituted 2,5-DMA itself (1a, Kᵢ = 5200 nM). Compounds with hydrophobic substituents display high affinity for 5-HT₂ sites. The 4-aryl (Kᵢ = 7 nM), 4-hexyl (Kᵢ = 2.5 nM), 4-ocetyl (Kᵢ = 3 nM), 4-[3-(phenyl)propyl] (Kᵢ = 10 nM), and 4-benzyl (Kᵢ = 7 nM) derivatives of 2,5-DMA (i.e., derivatives 1o–s, respectively) all possess affinities (Kᵢ values) for the 5-HT₂ sites of 10 nM or less (Table I). Surprisingly, esters 1v and 1w, though fairly lipophilic (4-substituent π values = 1.07 and 1.68, respectively), display a low affinity for 5-HT₂ sites.

Evaluation of 2,5-DMA Analogues as Potential Antagonists. Overall, it seems that affinity is explained primarily by the lipophilicity of the 4-substituent. But, previous studies have shown that certain of these compounds behave as agonists whereas others do not (see ref 12 for a review). For example, using rats trained to discriminate DOM from saline, ED₅₀ values for stimulus generalization are significantly correlated with 5-HT₂ affinity. However, some of the compounds included in the present study (e.g., 1n and 1o) possess a high affinity for the 5-HT₂ sites but do not result in stimulus generalization. If the lipophilic contribution of the 4-substituent is predictive of affinity and if all agents with high affinity are not necessarily agonists, the possibility exists that some of these agents might serve as antagonists (or at least as partial agonists). We examined this possibility in an isolated tissue assay. In rat thoracic aorta, serotonin receptors

![Figure 1](https://example.com/figure1.png)

Figure 1. Antagonism of 5-HT-induced contractions of rat thoracic aorta by the 4-benzyl (1s; A), 4-hexyl (1p; B), 4-phenylpropyl (1r; C), and 4-ocetyl (1q; D) derivatives of 2,5-DMA. A, B, C, and D show the concentration–response curves of 5-HT (10⁻⁶ to 10⁻³ M) in the presence of antagonists.

Discussion

Our preliminary studies suggested that the 5-HT₂ receptor affinity of 2,5-DMA analogues is related to the lipophilicity of the 4-substituent and that as lipophilicity

---

(11) A quantitative structure–activity relationship analysis of the binding data shown in Table I has been conducted. There is a significant correlation between 5-HT₂ affinity (Kᵢ values) and the lipophilicity (π values) of the 4-position substituents (n = 25, r = 0.858; the 4- OH and 4-COOH derivatives 1a and 1aa, respectively, could not be included in the analysis due to their low affinity and lack of specific Kᵢ values). If esters 1v and 1w are neglected for reasons discussed in the text, the correlation between affinity and π values is still significant (n = 23, r = 0.903). For more detail, see: Glennon, R. A.; Seggel, M. R. In Probing Bioactive Mechanisms; Magee, P. S., Henry, D. Block, J., Eds.; American Chemical Society: Washington, DC, 1998; pp 264–280.
increases, affinity increases. Although the initial series of compounds was small (n = 13), the results in Table I are consistent with these preliminary findings in that those compounds with the highest affinity are those with lipophilic 4-substituents. On the other hand, esters 1v and 1w, which are also quite lipophilic, possess a relatively low affinity. This low affinity might be explained by the adjacency of the 4-position carbonyl group to the 5-position methoxy group (i.e., a possible alteration in substituent orientation might result from an electrostatic repulsion between the two oxygen atoms), or it may be a result of partial hydrophobic under the conditions of the binding assay to the low-affinity 4-COOH derivative 1aa. Preliminary data showed that acid 1aa was of low affinity (i.e., Ki > 5000 nM); however, subsequent examination of this agent at higher concentrations revealed that the acid displays minimal affinity even at concentrations of up to 50,000 nM. Hansch analysis of the binding data show that there is a significant correlation between affinity and the lipophilicity of the 4-position substituent.

Other than for our preliminary studies, an analysis of the role of the 4-position substituents of these types of agents on 5-HT2 receptor affinities has not been previously reported. However, we have demonstrated that the hallucinogenic potencies of phenylalkylamines are significantly correlated with their affinities for 5-HT2 receptors and that such agents are most likely acting as 5-HT2 agonists. In 1975, Barfknecht and co-workers reported that the octanol/water partition coefficients of phenylalkylamines may be an important, though not necessarily exclusive, determinant of their hallucinogenic potency. Using a somewhat larger data set, we later reported that the overall lipophilicity of these agents might be important, but that (a) by itself, lipophilicity could not account for the potencies of all of these agents, and (b) that the overall lipophilicities of these agents might simply reflect the large lipophilic contribution of the 4-position substituents of the more potent agents. Furthermore, we provided evidence for a direct receptor interaction of the 4-position substituents. Shulgin and Dyer also found, for a limited set of 4-substituted 2,5-DMAs, that hallucinogenic potency could be explained on the basis of the lipophilicity of the 4-position substituent. Several other studies (e.g., refs. 21–24) have also addressed the possible relationships between the activities of related agents and serotonin receptor interactions; however, because the 5-HT receptors involved in those studies may not be of the 5-HT2 type, the significance of these results is unknown.

This study represents the first comprehensive investigation of the structure–affinity requirements for the binding of 4-substituted phenylalkylamine derivatives at 5-HT2 receptors. It was determined that the lipophilic character of the 4-position substituents of the 2,5-DMAs appears to be important in determining their affinity for central 5-HT2 receptors. It might be hypothesized that the receptors possess a hydrophobic area in this region that can accommodate these 4-substituents. The nature of this hydrophobic site needs to be investigated in greater detail, as does its ramifications for receptor selectivity. Nevertheless, it is apparent that not all agents with high affinity for these sites behave as agonists. For example, in rat thoracic aorta, DOB (1g) is a potent 5-HT2 agonist whereas compounds 1p–s act as antagonists. Furthermore, the affinities of some of these agents at [3H]ketanserin-labeled 5-HT2 sites rivals that of the currently most popular 5-HT2 antagonist ketanserin (Ki = 1.2 nM) and are several orders of magnitude greater than that of serotonin (Ki = 500 nM). Thus, the possibility exists for the development of an entirely new structural class of 5-HT2 antagonists. Because [3H]ketanserin binds at neurotransmitter sites other than 5-HT sites and because it apparently binds to a tetrabenzine-sensitive site in brain and in the periphery, a new structural class of 5-HT2 antagonists may not share some of these disadvantages of ketanserin.

Experimental Section
Melting points were determined on a Thomas-Hoofer melting point apparatus and are uncorrected. Microanalysis were performed by Atlantic Microlab (Atlanta, GA) and determined values are within 0.4% of theoretical values. Proton NMR spectra were recorded on a JEOL FX90Q spectrometer, operating at 90 MHz and using tetramethylsilane as an internal standard. Infrared spectra were obtained on a Nicolet 5DX FT-IR spectrophotometer. All spectral data are consistent with the assigned structures. The 4-isopropyl compound 11 as its HCl salt, was a gift from Drs. F. Benington and R. D. Morin (Neurosciences Program, Department of Psychiatry, University of Alabama, Birmingham, AL).

1-(2,5-Dimethoxy-4-hexylphenyl)-2-aminopropane Hydrochloride (1p). A suspension of 4a (0.15 g, 0.4 mmol) in 15% aqueous NaOH (5 mL), H2O (5 mL), and MeOH (10 mL) was heated at reflux for 2.5 h. The solution was allowed to cool to room temperature and was extracted with EtO (5 × 20 mL). The combined extracts were dried (Na2SO4) and ethereal HCl (ca. 20 mL) was added. After removal of the solvent under reduced pressure, the residue was recrystallized from EtOH/Et2O to give 0.09 g (71%) of the title compound: mp 133–135 °C. Anal. (C19H33N02·HCl) C, H, N.

1-(2,5-Dimethoxy-4-octylphenyl)-2-aminopropane Hydrochloride (1q). Compound 1q was prepared from 1b in a manner similar to that used for 1p. The residue was recrystallized from EtOH/Et2O to give 78% of compound 1q: mp 138–140 °C. Anal. (C19H33N02·HCl) C, H, N.

1-(2,5-Dimethoxy-4-(3-phenylpropyl)phenyl)-2-aminopropane Hydrochloride (1r). Compound 1r was prepared from 4c in a manner similar to that used for 1p. Excess HCl and solvent were removed in vacuo and the residue was recrystallized from EtOH/Et2O to give 81% of the title compound: mp 160–162 °C. Anal. (C25H37N02·HCl) C, H, N.

1-(4-Benzyl-2,5-dimethoxyphenyl)-2-aminopropane Hydrochloride (1s). Compound 1s was prepared from 4d in a manner similar to that used for 1p. Removal of the EtOH under reduced pressure left a white solid which was recrystallized from EtOH/Et2O, yielding 85% of the title compound: mp 181–183 °C. Anal. (C19H22N02·HCl) C, H, N.

1-(4-Cyano-2,5-dimethoxyphenyl)-2-aminopropane Hydrochloride (1u). The title compound was prepared by using the general procedure described by Cheng and Castagnoli. Cuprous cyanide (0.81 g, 1.6 mmol) and 5 (0.83 g, 1.57 mmol) were heated at reflux in DMF (10 mL) for 5 h. After cooling, the reaction mixture was poured into a solution of FeCl3/H2O (0.48 g) in aqueous HCl (0.12 mL of concentrated HCl in 0.72 mL of H2O). The mixture was heated to 60 °C and allowed to cool. After dilution with H2O (60 mL) and extraction with CH2Cl2 (3 × 25 mL).

1-(4-Benzyl-2,5-dimethoxyphenyl)-2-aminopropane Hydrochloride (1s). Compound 1s was prepared from 4d in a manner similar to that used for 1p. Removal of the EtOH under reduced pressure left a white solid which was recrystallized from EtOH/Et2O, yielding 85% of the title compound: mp 181–183 °C. Anal. (C19H22N02·HCl) C, H, N.

1-(4-Cyano-2,5-dimethoxyphenyl)-2-aminopropane Hydrochloride (1u). The title compound was prepared by using the general procedure described by Cheng and Castagnoli. Cuprous cyanide (0.81 g, 1.6 mmol) and 5 (0.83 g, 1.57 mmol) were heated at reflux in DMF (10 mL) for 5 h. After cooling, the reaction mixture was poured into a solution of FeCl3/H2O (0.48 g) in aqueous HCl (0.12 mL of concentrated HCl in 0.72 mL of H2O). The mixture was heated to 60 °C and allowed to cool. After dilution with H2O (60 mL) and extraction with CH2Cl2 (3 × 25 mL).


heating a solution of Analogues 0.15 g (58%) of the title compound: mp 236-238 °C. Anal. washed with aqueous Na₂CO₃ (10% w/v, 3 phase was dried (Na₂SO₄) and evaporated. The oily residue was suspended in H₂O (15 mL) and the suspension was extracted with CH₂Cl₂ (3).

The resulting product was recrystallized from EtOH/EθO to give 0.15 g (58%) of the title compound: mp 236-238 °C. Anal.

N-Propyl 2,5-Dimethoxy-4-(2-aminomethyl)benzamide Hydroxide Oxalate (iv). The title compound was prepared by following the procedure for the synthesis of n-butyl ester 1w. Thus, a mixture of 1-propanol (300 mg, 5.0 mmol), concentrated H₂SO₄ (50 mg, 0.5 mmol), and 1aa (150 mg, 0.5 mmol) in benzene, after heating at reflux and extraction, gave 80 mg (60%) of the propyl ester: mp 85-90 °C. The hydrogen oxalate salt was prepared as for 1w: mp 169-170 °C. Anal. (C₁₆H₁₉N₂O₄·(COOH)₂) C, H, N.

N-Butyl 2,5-Dimethoxy-4-(2-aminomethyl)benzamide Hydroxide Oxalate (iv). 1-Butanol (650 mg, 10.0 mmol) and concentrated H₂SO₄ (100 mg, 1.0 mmol) were added to a suspension of 1aa (as the free base; 0.24 g, 1.0 mmol) in benzene (30 mL). The reaction mixture was mixed at room temperature in a Dean-Stark trap. After reflux, the reaction mixture was extracted with H₂O (2 × 50 mL). The aqueous extract was made alkaline (pH 10) with 10% Na₂CO₃ solution and extracted with EtO (3 × 50 mL). The combined organic extracts were washed with H₂O (2 × 50 mL) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the oily residue was dissolved in absolute EtOH and layered with ethereal HCl. The precipitate was recrystallized from EtOH/H₂O to afford 1.33 g (49%) of 3a: mp 105-107 °C.

I-(Carboxy-2,5-dimethoxyphenyl)-2-aminopropane Hydrochloride (1a). A solution of Ig (as the free base; 1.4 g, 5.0 mmol) in anhydrous EtOH (20 mL) was added dropwise to a stirred solution of n-butyl lithium (1.3 g, 20 mmol; 12.5 mL of 1.6 M solution) in anhydrous EtOH (20 mL) at 0 °C. The reaction mixture was heated at room temperature for 2 h, after which the mixture was poured over solid CO₂ (50 g). The solvent was removed under reduced pressure and the residue was washed with EtO. The residue was dissolved in water (50 mL) and washed with CHCl₃ (2 × 30 mL). The aqueous solution was acidified with concentrated HCl and washed with CHCl₃ (2 × 30 mL). The aqueous portion was evaporated under reduced pressure to give a solid residue, which was recrystallized twice from MeCN to afford 0.25 g (30%) of a very light tan amorphous solid: mp 194-196 °C (lit. mp 196-198 °C).

N-(Trifluoroacetyl)-1-(2,5-dimethoxyphenyl)-2-aminopropane (2). Trifluoroacetic anhydride (20 mL) was slowly added to 1-(2,5-dimethoxyphenyl)-2-aminopropane (1a; 2.7 g, 13.8 mmol) while cooling in an ice bath. The reaction mixture was allowed to warm to room temperature, was stirred for 2 h, and was poured over crushed ice (200 g). The white precipitate was collected by filtration and washed with a large volume of cold H₂O. Recrystallization from EtOH/H₂O afforded 3.0 g (74%) of the title compound as a flocculent, white solid: mp 101-103 °C. Anal. (C₁₆H₁₇N₂O₃·(COOH)₂·1/2H₂O) C, H, N.

N-N-Prolyl 2,5-dimethoxy-4-(2-aminomethyl)benzamide Hydroxide Oxalate (1y). A solution of n-prolyl 2,5-dimethoxy-4-(2-aminomethyl)benzamide (1v; 120 mg, 0.43 mmol) in n-propylamine (5 mL) was heated under reflux for 5 h. Excess n-propylamine was removed in vacuo and the resulting solid residue was washed with EtO to yield 60 mg (66%) of the amide as a pale, buff solid: mp 110-115 °C. A solution of the amide (50 mg) in absolute EtOH (1 mL) was added to a solution of acetic anhydride in anhydrous EtO (5 mL). The oxalate salt was collected by filtration and recrystallized from 2-PrOH/EθO: mp 188-184 °C. Anal. (C₁₆H₁₉N₂O₄·(COOH)₂) C, H, N.

N-Trimethyl-2,5-dimethoxy-4-hydroxyphenyl)-2-aminopropane Hydroxide Oxalate (1z). To a solution of N-naphtholyl-1-(2,5-dimethoxy-4-hydroxyphenyl)-2-aminopropane (0.5 g, 1.42 mmol) in CHCl₃ (25 mL) was added 0.42 g (2.07 mmol) of 85% 3-chloroformic acid in anhydrous EtO (5 mL). The mixture was stirred at reflux and extraction, gave 80 mg (60%) of the title compound as a flocculent, white solid mp 101-103 °C. Anal. (C₁₆H₁₇N₂O₃·(COOH)₂·1/2H₂O) C, H, N.


N-(Trifluoroacetyl)-1-(4-benzoyl-2,5-dimethoxyphenyl)-2-aminopropane (3c). Compound 3c was prepared in a manner similar to that used for the synthesis of 3a. Recrystallization from EtOH/H2O gave 48% of the title compound: mp 111-112°C. Anal. (C21H30F3N03) C, H, N.

N-(Trifluoroacetyl)-1-(2,5-dimethoxy-4-hydroxyphenyl)-2-aminopropane (3d). Compound 3d was prepared by using the same method used for the preparation of 3a. The material remaining after removal of the solvent under reduced pressure was recrystallized from EtOH/H2O to give 1.3 g (44%) of the title compound: mp 131-133°C. Anal. (C21H30F3N03) C, H, N.

N-Phthaloyl-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (5). A solution of 48% HBr (0.80 g) in glacial acetic acid (10 mL) was added to a stirred solution of N-phthaloyl-1-(2,5-dimethoxyphenyl)-2-aminopropane 4a (1.5 g, 4.0 mmol) in glacial acetic acid (10 mL) at 0°C. A solution of Br2 (0.8 g, 4.2 mmol) in glacial acetic acid (10 mL) was then added dropwise to the reaction mixture. The reaction mixture was then allowed to stir at room temperature for 3 h. The solvent was removed under reduced pressure and the residue was recrystallized from EtOH/H2O to give 61% of compound 4c: mp 147-149°C. Anal. (C34H32BrNO4) C, H, Br.

N-(Trifluoroacetyl)-1-(2,5-dimethoxy-4-propionylphenyl)-2-aminopropane (4d). The title compound was prepared by using the procedure described for 4a. Recrystallization of the residue from 95% EtOH gave 63% of the title compound: mp 125-126.5°C. Anal. (C34H32F3N03) C, H, N.

N-(Trifluoroacetyl)-1-(2,5-dimethoxy-4-propionylphenyl)-2-aminopropane (7). Titanium tetrachloride (1.4 mL, 12.8 mmol) was added dropwise under a N2 atmosphere to a solution of 2 (2.0 g, 6.9 mmol) in CH2Cl2 (70 mL) cooled to ca. -30°C. Propionyl chloride (0.88 g, 9.5 mmol) in CH2Cl2 (5 mL) was added dropwise to the dark brown solution while the reaction mixture was maintained at ca. -30°C. The mixture was stirred at ca. -30°C for an additional 30 min and was allowed to warm to room temperature. Stirring was continued for 3 days and the reaction mixture was cautiously poured over 300 g of crushed ice. The deep red disspitated upon vigorous mixing. After separating the products from the aqueous portion was extracted with CH2Cl2 (ca. 175 mL) and the organic portion was washed successively with H2O (200 mL), 5% HCl (100 mL), H2O (200 mL), saturated aqueous NaHCO3 (200 mL), and saturated aqueous NaCl (200 mL). After drying (Na2SO4), the solvent was removed under reduced pressure. Recrystallization from EtOH/H2O gave 1.63 g (68%) of 7: mp 143-145°C. Anal. (C34H32F3N03) C, H, N.

Radioligand Binding Studies. Radioligand binding assays were conducted in essentially the same manner as previously reported. Briefly, Taconic Farms male Sprague-Dawley rats (ca. 220 g) were decapitated and the brains were immediately placed in ice-cold 0.9% saline. Dissecting over ice, the frontal corticies were removed with the anterior border of the corpus callosum as a landmark. Tissue was either used immediately or stored at -30°C until needed (no differences were noted between preparations). Membrane homogenates were prepared in a 50 mM (pH 7.4) Tris buffer (pH 7.4) containing 10 mM MgCl2 and 0.5 mM NaN3EDTA. Assays were performed in 2.0 mL of this same buffer to which was added to 0.1% ascorbate and 10 μM pargyline; membranes (3-mg wet weight) were added last. Displacement experiments at 11 concentrations of nonlabeled drug were performed in triplicate with tritiated ketanserin (90.4 Ci/mmol) (New England Nuclear). Specific binding was defined with 1 μM cinanserin. Solutions of all test compounds were made fresh daily. Following incubation at 37°C for 15 min, membranes were rapidly filtered over glass-fiber filters that had been presoaked in 0.1% polyethyleneimine, followed by a 10-mL wash with ice-cold buffer. Following a 6-h equilibration in Scintiverse (Fisher), samples were counted in a Beckman 3801 counter with an efficiency of 45%. IC50 values were determined and Ki values were calculated with the Cheng-Prusoff equation.15

Isolated Tissue Studies. The isolated tissue studies with rat aorta were performed as previously described in detail.14 In brief, using male Sprague-Dawley rats (250-300 g), 4-mm rings of rat thoracic aorta were dissected and suspended between two stainless steel hooks connected to a force displacement transducer. The organ bath was continuously bubbled with 95% O2/5% CO2 in a Krebs-bicarbonate buffer maintained at 37°C. Resting tension was set at 2.5 g and, after a 1-h equilibration period, concentration-response studies were performed as previously described.14 The aortic segments were incubated with varying concentrations of antagonist for 10 min at 37°C prior to titration with graded concentrations of 5-HT. All results represent the mean ± standard error of the mean, of five or six individual experiments.

Acknowledgment. This work was supported in part by PHS grants DA 01642 and NS 23520. BLR and EAS are members of the Naval Medical Research Command Work Unit #MRO 4120.05-1004. The opinions and assertions contained herein are private ones and are not to be construed reflecting the views of the Navy Department, the Naval Service at large, or the Department of Defense. The experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animal Resources; National Research Council. Department of Health, Education, and Welfare. U. S. Government Printing Office: Washington, DC, 1985, Publication No. (NIH) 78-23.