5-HT\textsubscript{1} and 5-HT\textsubscript{2} Binding Characteristics of 1-(2,5-Dimethoxy-4-bromophenyl)-2-aminopropane Analogues

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1-(2,5-Dimethoxy-4-bromophenyl)-2-aminopropane (DOB; 1a) is a purported serotonin (5-HT) agonist that binds selectively to central 5-HT\textsubscript{2} binding sites. Systematic removal of any or all of the aromatic substituents had relatively little effect on 5-HT\textsubscript{1} binding but reduced 5-HT\textsubscript{2} binding by approximately 2 or more orders of magnitude. Demethylation of the 2-methoxy group of 1a, or introduction of an N-n-propyl group, doubled 5-HT\textsubscript{1}-site affinity but decreased 5-HT\textsubscript{2}-site affinity by 3- and 30-fold, respectively. In tests of stimulus generalization, using rats trained to discriminate DOB from saline, the 2-demethyl and N-propyl derivatives were found to produce stimulus effects similar to those of DOB. In addition, the S-(+) isomer of the iodo analogue of 1a was found to possess one-third the affinity of its R-(−) enantiomer at 5-HT\textsubscript{2} sites and also resulted in DOM-stimulus generalization. Of the DOB analogues examined, DOB (1a) possesses optimal selectivity for 5-HT\textsubscript{2} binding.

There has long been evident evidence that certain 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropanes, such as DOB (1a, X = Br) and DOM (1b, X = CH\textsubscript{3}), produce their behavioral effects via a mechanism that involves the neurotransmitter serotonin (5-HT). It has also been suggested that these agents might be direct-acting serotonergic agonists. (For reviews, see ref 1-3.) The recent identification of two major populations of central 5-HT binding sites (i.e. 5-HT\textsubscript{1} and 5-HT\textsubscript{2}\textsuperscript{a,b}) coupled with our finding that the 5-HT\textsubscript{2} selective antagonists ketanserin and pirenperone potently antagonize the discriminative stimulus effects of, for example, DOM,\textsuperscript{6} prompted us to examine the affinities of such agents for 5-HT\textsubscript{2} and \textsuperscript{3}Hketanserin-labeled 5-HT\textsubscript{2} sites. It was determined that both DOM and DOB bind selectively (30- and 50-fold, respectively) at 5-HT\textsubscript{2} sites.\textsuperscript{7} The 4-iodo derivative DOI (1c, X = I) displays greater than a 100-fold selectivity for these sites.\textsuperscript{7} On the basis of these findings, we suggested that these agents constitute the first reported examples of 5-HT\textsubscript{2}-selective agonists. Two-site analysis of the binding of a series of derivatives of 1 to 5-HT\textsubscript{2} sites revealed that DOB possesses a significant affinity (K\textsubscript{i} = 2.4 nM) and selectivity for the high-affinity component of \textsuperscript{3}Hketanserin binding.\textsuperscript{7} Thus, this agent was selected as the basis for additional structure-activity studies. Also, because (S)-(+)DOI has not been previously reported, we wished to prepare and evaluate this compound for comparison with (R)-(−)-DOI.

Chemistry. Compounds 2-6 were available from earlier studies conducted in these laboratories. DOB (1a) was prepared by the direct bromination of 2,5-DMA (6) essentially according to the method of Aldous et al.\textsuperscript{9} The \textsuperscript{1}H NMR spectrum of the product (as was also the case with 8 and 11a) displayed singlets at 6 6.8 and 7.1, integrating for one proton each, suggesting that bromination had occurred at the 4-position. However, the melting point of the product was considerably higher (i.e. HBr salt, mp 175–177 °C) than that previously reported (i.e. 145–146 °C). Consequently, DOB was also prepared by using the procedure of Barknecht and Nichols\textsuperscript{9} (i.e. condensation of 2,5-dimethoxy-4-bromobenzaldehyde with nitroethane to afford the corresponding nitrostyrene), except that the nitrostyrene intermediate was reduced with AlH\textsubscript{3} instead of LiAlH\textsubscript{4} (in order to minimize debromination); the product, isolated as the HCl salt, was identical with that reported earlier. The DOB-HBr (prepared by the direct bromination route) was converted to its HCl salt and this salt was found to be identical with that prepared by the second method. The N-mono-n-propyl derivative 7 was prepared by acylation of 6 with propionyl chloride followed by reduction of the amide with LiAlH\textsubscript{4}. Direct bromination of 7 afforded 8.

The synthesis of 9 required the preparation of 2-methoxy-4-bromobenzaldehyde (12); however, attempts to directly formylate 3-methoxybromobenzene were unsuccessful. In one instance using dichloromethyl methyl ether under Lewis acid conditions, for example, the only product isolated was 2-bromo-4-methoxybenzaldehyde (identified as its p-nitrophenylhydrazonel). Vilsmeier formylation of 3-bromophenol was, likewise, unsuccessful, although formylation could be achieved in fairly low yield using Reimer-Tiemann conditions. The aldehyde was subsequently methylated to afford 12. Condensation of 12 with nitroethane followed by reduction of the nitrostyrene gave 9. Compound 10 was prepared from 4-bromo-3-methoxybenzaldehyde as previously reported,\textsuperscript{9} except that the nitrostyrene intermediate was reduced with AlH\textsubscript{3} instead of LiAlH\textsubscript{4}. The hydrochloride salt of this product was found to be quite hygroscopic; as a consequence, it was condensed with the corresponding nitrostyrene, except that the nitrostyrene intermediate was reduced with AlH\textsubscript{3} instead of LiAlH\textsubscript{4}. Direct bromination of 7 afforded 8.

Chemistry. Compounds 2-6 were available from earlier studies conducted in these laboratories. DOB (1a) was prepared by the direct bromination of 2,5-DMA (6) essentially according to the method of Aldous et al.\textsuperscript{9} The \textsuperscript{1}H NMR spectrum of the product (as was also the case with 8 and 11a) displayed singlets at 6 6.8 and 7.1, integrating for one proton each, suggesting that bromination had occurred at the 4-position. However, the melting point of the product was considerably higher (i.e. HBr salt, mp 175–177 °C) than that previously reported (i.e. 145–146 °C). Consequently, DOB was also prepared by using the procedure of Barknecht and Nichols\textsuperscript{9} (i.e. condensation of 2,5-dimethoxy-4-bromobenzaldehyde with nitroethane to afford the corresponding nitrostyrene), except that the nitrostyrene intermediate was reduced with AlH\textsubscript{3} instead of LiAlH\textsubscript{4} (in order to minimize debromination); the product, isolated as the HCl salt, was identical with that reported earlier. The DOB-HBr (prepared by the direct bromination route) was converted to its HCl salt and this salt was found to be identical with that prepared by the second method. The N-mono-n-propyl derivative 7 was prepared by acylation of 6 with propionyl chloride followed by reduction of the amide with LiAlH\textsubscript{4}. Direct bromination of 7 afforded 8.

Table I. Results of Binding Studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>R'</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; nM</th>
<th>Hill coeff</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; nM</th>
<th>Hill coeff</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-DOB</td>
<td>H</td>
<td>7660 (±1320)</td>
<td>0.91 (±0.07)</td>
<td>43000 (±3100)</td>
<td>0.89 (±0.03)</td>
</tr>
<tr>
<td>(R)-OMA</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3500 (±650)</td>
<td>0.80 (±0.03)</td>
<td>8130 (±880)</td>
<td>1.03 (±0.08)</td>
</tr>
<tr>
<td>(R)-MMA</td>
<td>H</td>
<td>2860 (±240)</td>
<td>0.75 (±0.04)</td>
<td>7860 (±230)</td>
<td>1.18 (±0.07)</td>
</tr>
<tr>
<td>(R)-2,5-DM</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2830 (±540)</td>
<td>0.75 (±0.04)</td>
<td>4650 (±300)</td>
<td>0.88 (±0.05)</td>
</tr>
<tr>
<td>(R)-DOB</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4100 (±800)</td>
<td>0.60 (±0.03)</td>
<td>25800 (±200)</td>
<td>1.00 (±0.06)</td>
</tr>
<tr>
<td>(R)-DOB</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3340&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 (±0.03)</td>
<td>1230 (±160)</td>
<td>0.92 (±0.10)</td>
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<tr>
<td>(R)-DOB</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1320 (±70)</td>
<td>0.89 (±0.03)</td>
<td>1230 (±160)</td>
<td>0.92 (±0.10)</td>
</tr>
<tr>
<td>(R)-DOB</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2900 (±200)</td>
<td>0.77 (±0.04)</td>
<td>4870 (±460)</td>
<td>1.06 (±0.03)</td>
</tr>
<tr>
<td>(R)-DOB</td>
<td>H</td>
<td>&gt;25000</td>
<td>&gt;25000</td>
<td>&gt;25000</td>
<td>&gt;25000</td>
</tr>
<tr>
<td>(R)-DOB</td>
<td>I</td>
<td>1710 (±250)</td>
<td>0.64 (±0.02)</td>
<td>210 (±30)</td>
<td>0.77 (±0.06)</td>
</tr>
<tr>
<td>(R)-DOB</td>
<td>I</td>
<td>2290&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96 (±0.03)</td>
<td>38 (±43)</td>
<td>0.66 (±0.06)</td>
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</table>

<sup>a</sup>Data are followed by SEM in parentheses. <sup>b</sup>Results previously reported<sup>11</sup> included for comparative purposes.

11 was prepared by bromination of 1-[2-(benzyloxy)-5-methoxyphenyl]-2-aminopropane<sup>11</sup> followed by demethylation of the hydroxyl group by treatment with acid. The synthesis of (S)-(+-DO1 [(S)-1c] paralleled our previously reported synthesis of racemic DOI (1c);<sup>12</sup> resolution was achieved with use of L- (+)-tartaric acid.

**Binding Studies.** The results of the competition studies, along with the slopes of their Hill plots, are shown in Table I. In general, there was relatively little variation with respect to 5-HT<sub>1</sub> binding; binding at 5-HT<sub>2</sub> sites was more responsive to molecular modification. Stripping DOB (1a) of all aromatic substituents affords the phenylisopropylamine amphetamine (2); 2 displays a 5-fold selectivity for 5-HT<sub>1</sub> sites, although in both instances the K<sub>i</sub> values are in the micromolar range. Introduction of a 2- or 3-methoxy group (i.e. 3 and 4, respectively) results in a slight enhancement in 5-HT<sub>1</sub>-site affinity and a 5-fold increase in 5-HT<sub>2</sub> binding. Similar results are obtained with the 4-bromo derivative 5. Overall, compounds 2-5 display a low degree of selectivity for 5-HT<sub>1</sub> sites. This general trend continues with the dimethoxy derivatives 6 and 7 and with the 2-methoxy-4-bromo derivative 9. Compound 10 appears relatively ineffective with respect to binding at either 5-HT<sub>1</sub> or 5-HT<sub>2</sub> sites. The effects of the methoxy and bromo groups are not additive. Optimal 5-HT<sub>2</sub>-site affinity and selectivity are associated with intact DOB (1a). In fact, simple transposition of the 4-bromo group of DOB to the 3-position, to afford 1-(2,5-dimethoxy-3-bromophenyl)-2-aminopropane<sup>14</sup> results in a greater than 100-fold decrease in affinity (i.e. K<sub>i</sub> = 10000 nM) for 5-HT<sub>2</sub> sites.

The 5-HT<sub>1</sub>-charateristic of certain serotonergic agents is known to be enhanced by N-alkyl and in particular N-n-propyl groups.<sup>14</sup> As shown in Table I, however, introduction of an N-propyl group (i.e. compound 8) reduces the affinity of DOB for 5-HT<sub>2</sub> sites. Demethylation of the 2-methoxy group of DOB (i.e. 11) results in approximately a twofold increase in affinity for 5-HT<sub>1</sub> sites and a corresponding decrease in affinity for 5-HT<sub>2</sub> sites. Overall, relatively little can be done to the DOB molecule that does not result in a significant reduction in affinity for 5-HT<sub>2</sub> sites. Furthermore, none of the compounds examined display the 5-HT<sub>2</sub>-selectivity noted for DOB; in fact, certain derivatives possess a slight selectivity for 5-HT<sub>1</sub> sites.

DOI (1c) is another potent and selective agent at 5-HT<sub>2</sub> sites; the R-(-) isomer seems to be responsible for this effect when data are compared with that obtained for the racemic mixture.<sup>7</sup> The S- (+) isomer has not been previously examined. The results shown in Table I are in agreement with these earlier findings in that the affinity of (S)-(+-1c is slightly less than that of (R)-(+-1c (i.e. 35 nM vs. 9.9 nM, respectively). Nevertheless, the enantiomeric potency ratio is only 3, and (S)-(+-1c still displays a significant affinity, although a somewhat reduced selectivity, for 5-HT<sub>2</sub> sites.

**Behavioral Studies.** We have previously demonstrated that DOM (1b) serves as a discriminative stimulus in animals when paired with saline.<sup>15</sup> In tests of stimulus generalization (i.e. transfer), the DOM stimulus generalizes to other agents that produce similar stimulus effects. That is, animals trained to press one of two levers in an operant training situation when administered DOM respond in like manner when administered other agents that produce stimulus effects similar to those produced by DOM.<sup>15</sup> Because of the relationship that exists between discrimination-derived ED<sub>50</sub> values and 5-HT<sub>2</sub>-site affinities, we have postulated that these effects might be 5-HT<sub>2</sub> mediated.<sup>16</sup> For example, DOM-stimulus generalization occurs with 6 and with DOB (1a), with the latter being 45 times more potent (on a molar basis) than 6. On the other hand, stimulus generalization does not occur with amphetamine (2), with either of its monoethoxy derivatives (i.e. 3 and 4) or with the positional isomer of DOB (i.e. 13), suggesting that these agents do not produce stimulus effects similar

that the intact molecule is necessary for optimal SHTp site of these agents. With respect to DOB and DOI, respectively, constitute the first examples of selective affinity and selectivity. Removal of any of the aromatic substituents resulted in a decrease in affinity for 5-HT2 sites; a dramatic decrease in affinity was also observed when the 4-bromo group was moved to the 3-position (i.e. (S)-1c). Introduction of an N-n-propyl group, and demethylation of the 2-methoxy group (i.e. 8 and 11, respectively), resulted in a 2-fold increase in affinity for 5-HT12 sites; however, in both cases, 5-HT2-site affinity was decreased (by ca. 30- and 3-fold, respectively).

Derivatives of 1 possess a chiral center; although stereochemistry appears to be involved in the binding of these agents to 5-HT2 sites, its role is rather small. In the present study, the enantiomeric (S/R) potency ratio for the isomers of DOI (S)-(-)-DOI and (R)-(+)-DOI is used as a radioligand for the identification and/or evaluation of novel 5-HT2-like agents. As a consequence, tests of stimulus generalization using DOM-trained animals might constitute a useful method for the identification and/or evaluation of novel 5-HT2-like agents. Thus, it is significant that the DOM stimulus generalizes to (S)-(-)-DOI and to those agents in the present study that possess the highest affinities for 5-HT2 sites (i.e. (S)-1c, 8, and 11) but did not generalize to agents such as 2-4.

Of the derivatives of DOB examined in the present study, DOB (la) itself was found to possess the greatest affinity and selectivity for 5-HT2 sites. As a consequence, [3H]DOB is currently being prepared as a radioligand for

<table>
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<th>Table II. Results of Stimulus Generalization Studies</th>
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<tr>
<td>agent</td>
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<tr>
<td>DOB (la) (S)-(+)-DOI ([(S)-1c)</td>
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<tr>
<td>N-Pr-DOB (8)</td>
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<td></td>
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<tr>
<td>DOM (1b) saline (1 mL/kg)</td>
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</table>

Number of animals responding/number to receive drug. Data obtained during the 2.5-min extinction session. 
Numbers in parentheses are 95% confidence limits.

Summary. 1-(2,5-Dimethoxy-4-X-phenyl)-2-amino-propanes, e.g. where X = Br, Me, I (i.e. DOB, DOM, and DOI, respectively), constitute the first examples of selective 5-HT2 agonists. The present study was undertaken, primarily, to further develop the structure-activity relationship and to better understand the binding characteristics of these agents. With respect to DOB (la), it was found that the intact molecule is necessary for optimal 5-HT2-site affinity and selectivity. Removal of any of the aromatic substituents resulted in a decrease in affinity for 5-HT2 sites; a dramatic decrease in affinity was also observed when the 4-bromo group was moved to the 3-position (i.e. 13).
use in future binding studies.

**Experimental Section**

Proton magnetic resonance spectra were recorded on a Perkin-Elmer R-24 spectrometer using Me$_4$Si as an internal standard. Infrared spectra were obtained on a Perkin-Elmer 257 spectrophotometer and mass spectra were determined on a Finnigan 4000 Series GC/MS data system. In those cases where final products were isolated as salts, spectral data were obtained on the corresponding free bases; all spectral data were consistent with the assigned structures. Elemental analysis was performed by Atlantic Microlab Inc. (Atlanta, GA) and determined values are within 0.4% of theoretical values. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected.

**DOB Hydrochloride**

A solution of propiony1 chloride (2.4 g, 26 mmol) in CHCl$_3$ (50 mL) was added in a dropwise manner to a stirred mixture of sodium bicarbonate (0.9 g, 21 mmol) and 1-(2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (5.0 g, 26 mmol) in CHCl$_3$ (50 mL) at 0 °C. After the addition was complete, the mixture was stirred at room temperature for 18 h. The reaction mixture was decomposed by the successive dropwise addition of water (1 mL), 10% aqueous NaOH solution (1 mL), and water (2 mL). The white precipitate was collected by filtration and this material was stirred with Et$_2$O (25 mL) for 30 min. The solid was removed by filtration and the Et$_2$O filtrate was combined with the THF filtrate, and the combined filtrates were dried (MgSO$_4$). The organic solvents were removed under reduced pressure to give 1.3 g of a light yellow liquid. Kugelrohr distillation [49–54 °C (0.13 mm)] afforded 1.2 (81%) of the amine as a clear oil. A sample of this oil was converted to the HBr salt and recrystallized twice from acetone triturating the salt as thin white plates, mp 124–126 °C. A second sample of the oil was converted to the HBr salt and recrystallized from MeCN/Et$_2$O to give a white solid: mp 112–113.5 °C; IR (neat) 3340 (NH) cm$^{-1}$; $^1$H NMR (CDCl$_3$) 0.75–1.35 (m, 7 H, 2 CH$_2$, NH), 1.25–1.90 (m, 5 H, Ar CH$_2$, NCH$_2$, NCH), 3.75, 3.80 (s, 6 H, OCH$_3$), 6.80 (m, 3 H, Ar H). Anal. (C$_{26}$H$_{24}$N$_2$O$_4$HCl) C, H, N.

**DOB Propionyl-Hydrochloride (8)**

A solution of 48% HBr (0.4 g) in glacial HOAc (5 mL) was added in one portion to a stirred solution of 7 (as free base) (0.5 g, 21 mmol) in glacial HOAc (5 mL) at 0 °C. At the same temperature, a solution of Br$_2$ (0.4 g, 23 mmol) in glacial HOAc (5 mL) was added in a dropwise fashion. After the addition was complete, the reaction mixture was collected by filtration, washed with anhydrous Et$_2$O (5 mL), and dried at room temperature (1 h) to afford 1.0 g of an off-white solid, mp 171–175 °C. This solid was recrystallized twice from MeCN to give 0.71 (85%) of the HBr salt, mp 184–186 °C. The free base of the amine was obtained by dissolving 0.6 g of this salt in water (20 mL), heating, and treating the cooled solution with 15% aqueous NaOH solution. This basic solution was extracted with Et$_2$O and evaporated to the Et$_2$O solution to dryness. Kugelrohr distillation [53–56 °C (0.1 mm)] afforded 0.19 g (53%) of the amine as a white solid, mp 63–65 °C.

**DOB Hydroxychloride (9)**

A solution of propiony1 chloride (2.4 g, 26 mmol) in CHCl$_3$ (50 mL) was added in a dropwise manner to a stirred mixture of sodium bicarbonate (0.9 g, 21 mmol) and 1-(2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (5.0 g, 26 mmol) in CHCl$_3$ (50 mL) at 0 °C. After the addition was complete, the mixture was stirred at room temperature for 18 h. The reaction mixture was decomposed by the successive dropwise addition of water (1 mL), 15% aqueous NaOH solution (1 mL), and water (2 mL). The white precipitate was collected by filtration and this material was stirred with Et$_2$O (25 mL) for 30 min. The solid was removed by filtration and the Et$_2$O filtrate was combined with the THF filtrate, and the combined filtrates were dried (MgSO$_4$). The organic solvents were removed under reduced pressure to give 1.3 g of a light yellow liquid. Kugelrohr distillation [49–54 °C (0.13 mm)] afforded 1.2 (81%) of the amine as a clear oil. A sample of this oil was converted to the HCl salt and recrystallized twice from acetone triturating the salt as thin white plates, mp 124–126 °C. A second sample of the oil was converted to the HBr salt and recrystallized from MeCN/Et$_2$O to give a white solid: mp 112–113.5 °C; IR (neat) 3340 (NH) cm$^{-1}$; $^1$H NMR (CDCl$_3$) 0.75–1.35 (m, 7 H, 2 CH$_2$, NH), 1.25–1.90 (m, 5 H, Ar CH$_2$, NCH$_2$, NCH), 3.75, 3.80 (s, 6 H, OCH$_3$), 6.80 (m, 3 H, Ar H). Anal. (C$_{26}$H$_{24}$N$_2$O$_4$HCl) C, H, N.

**DOB Propionyl-Hydrochloride (8)**

A solution of 48% HBr (0.4 g) in glacial HOAc (5 mL) was added in one portion to a stirred solution of 7 (as free base) (0.5 g, 21 mmol) in glacial HOAc (5 mL) at 0 °C. At the same temperature, a solution of Br$_2$ (0.4 g, 23 mmol) in glacial HOAc (5 mL) was added in a dropwise fashion. After the addition was complete, the reaction mixture was collected by filtration, washed with anhydrous Et$_2$O (5 mL), and dried at room temperature (1 h) to afford 1.0 g of an off-white solid, mp 171–175 °C. This solid was recrystallized twice from MeCN to give 0.71 (85%) of the HBr salt, mp 184–186 °C. The free base of the amine was obtained by dissolving 0.6 g of this salt in water (20 mL), heating, and treating the cooled solution with 15% aqueous NaOH solution. This basic solution was extracted with Et$_2$O and evaporated to the Et$_2$O solution to dryness. Kugelrohr distillation [53–56 °C (0.13 mm)] afforded 0.19 g (53%) of the amine as a white solid, mp 63–65 °C. Treatment of an Et$_2$O solution of this amine with HCl gas afforded 1a as white crystals after recrystallization from absolute EtOH/Et$_2$O, mp 195–196 °C.

(S)-(+)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane Hydrochloride ([S]-1c)

A solution of propiony1 chloride (2.4 g, 26 mmol) in CHCl$_3$ (25 mL) and the combined organic layers were dried (MgSO$_4$) and evaporated to dryness under reduced pressure to afford an oil. This oil was dried under high vacuum (0.1 mm) for 1 h and distilled to afford 0.4 g of the amine as a clear liquid [Kugelhoffer, 61–65 °C (0.11 mm)]. Dry HCl-saturated Et$_2$O (5 mL) was added, at room temperature (to a solution of this oil in anhydrous Et$_2$O (10 mL)). This solid product was collected by filtration, washed with anhydrous Et$_2$O (5 mL), and dried at room temperature (1 h) to afford 1.0 g of an off-white solid, mp 171–175 °C. This solid was recrystallized twice from MeCN to give 0.71 (85%) of the HBr salt, mp 184–186 °C. The free base of the amine was obtained by dissolving 0.6 g of this salt in water (20 mL), heating, and treating the cooled solution with 15% aqueous NaOH solution. This basic solution was extracted with Et$_2$O (2 × 25 mL) and CHCl$_3$ (25 mL), and the combined organic layers were dried (MgSO$_4$) and evaporated to dryness under reduced pressure to afford an oil. This oil was dried under high vacuum (0.1 mm) for 1 h and distilled to afford 0.4 g of the amine as a clear liquid [Kugelhoffer, 61–65 °C (0.11 mm)]. Dry HCl-saturated Et$_2$O (5 mL) was added, at room temperature (to a solution of this oil in anhydrous Et$_2$O (10 mL)). This solid product was collected by filtration, washed with anhydrous Et$_2$O (5 mL), and dried at room temperature to give 0.3 g of the HCl salt, mp 176–178 °C. This salt was recrystallized twice from MeCN resulting in white flakey crystals: mp 178–180 °C; IR (neat) 3400 (NH) cm$^{-1}$; $^1$H NMR (CDCl$_3$) 0.55–1.10 (m, 2 H, CH$_2$), 1.50–1.90 (m, 5 H, Ar CH$_2$, NCH$_2$, NCH), 3.75, 3.80 (s, 6 H, OCH$_3$), 6.80 (s, 3 H, Ar H). Anal. (C$_{26}$H$_{24}$N$_2$O$_4$HCl) C, H, N.

1-(2-Methoxy-4-bromophenyl)-2-aminopropane Hydrochloride (9)

2-Methoxy-4-bromobenzaldehyde (12: 4 g, 19 mmol) was added to a stirred solution of NH$_4$OAc (1.2 g, 15 mmol)
in nitroethane (75 mL) at room temperature. The solution was heated at reflux for 18 h and cooled to room temperature, and water (50 mL) was added; the mixture was extracted with 

CHCl₃ (3 × 50 mL), and the combined organic extracts were dried (MgSO₄). Evaporation of the solvent under reduced pressure gave a dark orange liquid. The liquid was further dried under high vacuum (0.08 mm) with heat (40 °C) for 2 h to afford 4.8 g (93%) of the crude nitrostyrene as a dark-orange solid.

Al, AlH₃, was prepared by the addition of a solution of 100% H₂SO₄ (1.1 g) to THF (50 mL) to a suspension of L-AlH₃ (0.8 g, 22 mmol) in dry THF (100 mL) at 0 °C under a nitrogen atmosphere. A solution of the nitrostyrene (50 mL) was added in a dropwise manner to the AlH₃ suspension at 0 °C. After the addition was complete, the mixture was allowed to stir for 6 h at room temperature. Excess AlH₃ was hydrolyzed by the addition of crushed ice (ca. 10 g) and 15% NaOH (20 mL). The mixture was filtered and the organic portion was separated and dried (MgSO₄). The solvent was removed under reduced pressure to give an orange liquid. Kugelrohr distillation [56-59 °C (0.02 mm)] afforded 1.1 g (61%) of the amine as a light-yellow liquid. Et₂O saturated with HCl gas was added to a solution of the amine (1.1 g) in absolute EtOH (5 mL) at room temperature until salt formation had ceased. The solid was collected by filtration and recrystallized thrice from heptane (ca. pH 11). The basic solution was extracted with Et₂O (3 × 50 mL), and the combined organic extracts were dried (MgSO₄), and the solvent was removed under reduced pressure to give a viscous, light-brown oil. The oil was dried under high vacuum (0.13 mm) with heat (40 °C) for 2 h to afford 2.9 g (74%) of crude 11a, which was used without further purification for the synthesis of 11.

2-Methoxy-4-bromobenzaldehyde (12). Chloroform (42 g) was added in a dropwise manner to a suspension of 3-bromophenol (30.0 g, 173 mmol) in an aqueous solution of NaOH (55.4 g in 75 mL of water) with the temperature maintained between 70 and 75 °C. After the addition was complete, the temperature was kept between 70 and 75 °C until evidence of refluxing had ceased (ca. 15 min). The mixture was cooled to 0 °C, made acidic with 1 N HCl solution (ca. pH 3), and extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried (MgSO₄), and the solvent was removed under reduced pressure to give a liquid. A solution of this liquid in Et₂O (200 mL) was layered over a saturated NaHSO₄ solution and allowed to stand at room temperature for 5 days. The bisulfite adduct was collected by filtration and washed with Et₂O (50 mL) and the solid suspended in a 0.5 N HCl solution (200 mL). The stirred mixture was heated at 50 °C for 2 h and extracted with EtOAc (3 × 100 mL), and combined organic extracts were dried (MgSO₄), and the solvent was removed under reduced pressure to yield a yellow liquid. Kugelrohr distillation [48-52 °C (0.04 mm)] afforded 6.2 g (18%) of the hydroxy aldehyde, which solidified on standing at room temperature, mp 50-52 °C. Subsequent recrystallization from absolute EtOH afforded the aldehyde as long, colorless needles, mp 61-63 °C (p-nitrophenyl)hydrazine derivative, mp 254-256 °C (lit.17 mp 258 °C).

In a separate experiment, solid K₂CO₃ (5.6 g, 40 mmol) and methyl iodide (11.4 g 80 mmol) were added to a solution of the 2-hydroxy-4-bromobenzaldehyde (8.0 g, 40 mmol) in acetonitrile (150 mL) at room temperature. The mixture was heated at reflux for 48 h and cooled to room temperature where solids were removed by filtration and the solvent was removed under reduced pressure to give a crude product. The oily product was suspended in Et₂O (50 mL), and the insoluble solids were removed by filtration. The Et₂O was evaporated under reduced pressure to yield a light-yellow liquid [bp 52-54 °C (0.04 mm)] which solidified on standing to afford 4.3 g (50%) of 12, mp 66-68 °C (lit.18 mp 71 °C) (p-nitrophenyl)hydrazine derivative, mp 213-214 °C; lit.18 mp 215 °C). Binding Studies. The radioligand binding assay has already been described in detail.17 In brief, tissue preparation was performed as described by Lysen et al.19 using prefrontal cortex of female Sprague–Dawley rats (ca. 200 g). A homogenate was prepared and the final suspension was in 50 mM Tris-HCl (pH 7.4) buffer at a tissue concentration of 15 mg wet weight/mL. The assay was performed in triplicate in 50 mM Tris, 5 mM MgCl₂, 0.5 mM EDTA Na₂ (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, Le., either 0.4 nM [3H]ketanserin (defined [5-HT] binding) or 2 nM [3H]-5-HT (defined as 5-HT binding). Filtration was accomplished with glass fiber filters (Flow Laboratories), and filters were counted after buffer wash by liquid scintillation spectrometry using 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; IC₅₀ values were determined according to the equation IC₅₀ = IC₀ + 1/D/Kₐ, where [D] = concentration of radioligand and Kₐ is the equilibrium dissociation constant of radioligand binding.

Behavioral Studies. Male Sprague–Dawley rats were maintained at approximately 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 26-V overhead house light. One wall of each operant chamber was fitted with two openings (15 cm in diameter, housed equidistant between the levers) for delivery of reinforcement (0.01 mL of sweetened milk). Solid-state and electronic engineering and recording equipment were housed in the attenuating outer chambers. Illumination of each chamber was provided by means of a 26-V overhead house light. One wall of each operant chamber was fitted with two openings (15 cm in diameter, housed equidistant between the levers) for delivery of reinforcement (0.01 mL of sweetened milk).

in the same room as the operant chambers. The rats were initially trained to respond on both of two 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 (4)-DOM hydrochloride (1.0 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 DOM 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 DOM, whereas responding on the opposite lever was reinforced after administration of saline. Saline and DOM 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 26 training sessions, discrimination performance was stable under each treatment condition, i.e. the animals made greater than 80% of their responses on the DOM-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. Maintenance of the DOM/saline discrimination was insured in all six animals by continuation of the training sessions throughout the stimulus generalization studies. During the generalization studies, test sessions were interposed among 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 DOM-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, ED50 values (i.e. doses at which the animals would be expected to make approximately 50% of their responses on the DOM-appropriate lever) were determined by the method of Finney.20

Note Added in Proof: Results of a preliminary study using [3H]DOB as a radioligand for S-HT2 sites have just been published as a rapid communication (Eur. J. Pharmacol. 1985, 117, 145).

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**Synthesis, Structure, and Antitumor Activity of N-Salicyloyl-N'-(2-furylthiocarbonyl)hydrazine and Its Copper(II) Complex**

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N-Salicyloyl-N’-(2-furylthiocarbonyl)hydrazine (Hsfth) and its Cu(II) complex [Cu(sfth)] were prepared and characterized by physicochemical studies. The IR and ESR spectral studies imply dibasic tetradentate behavior of the ligand bonding through “thiolo” sulfur, enolic oxygen, and hydrazinic nitrogens in a polymeric structure. The electronic spectrum of the complex indicates a square-planar geometry around Cu(II). Maximum antitumor activity was observed when 25 mg/kg dose levels of Hsfth and Cu(sfth) were injected intraperitoneally in mice bearing either solid fibrosarcoma or ascites Dalton’s lymphoma. However, Hsfth appeared to possess better antitumor activity as demonstrated by higher T/C (percent) values than those observed for Cu(sfth). The appearance of lymphocytes, leukocytes, and macrophages within the tumor mass 2-6 days after treatment are indicative of involvement of the host’s immune system.

Brockman et al. discovered the antitumor activity of 2-formylpyridine thiosemicarbazone against L1210, L52T, and L4946 leukemia in mice.1 Furthermore, a number of derivatives of thiosemicarbazones have been shown to possess strong antineoplastic activity against a number of transplanted, spontaneous murine tumors2 and in human tumor.3 Role of metal chelation in the mechanism of action of these compounds has been discussed by French and his co-workers.4-6 It has been established that copper ion chelation plays a definite role in the antineoplastic activity of 3-ethoxy-2-oxobutyr aldehyde bis(thiosemicarbazone).7 In fact, copper(II) complexes of substituted thiosemicarbazones and copper(II) and iron(II) complexes of 5-substituted 2-formylpyridine and 1-formylisoquinoline thiosemicarbazones8-10 have been found to be cytotoxic to the tumor cells in vivo and in vitro. These complexes are strong inhibitors of the enzyme ribonucleotide reductase, an obligatory enzyme in the pathway of synthesis of precursors of DNA.12-15

Platinum, iron, copper, palladium, and zinc complexes of 2-formylpyridine thiosemicarbazone have been proved to be significant antitumor agents against Ehrlich ascites carcinoma and L1210 leukemia in mice.13-15 Bis(2-}

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