5-HT_1 and 5-HT_2 Binding Characteristics of 1-(2,5-Dimethoxy-4-bromophenyl)-2-aminopropane Analogues

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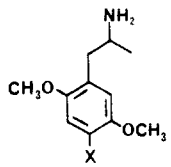
1-(2,5-Dimethoxy-4-bromophenyl)-2-aminopropane (DOB; **1a**) is a purported serotonin (5-HT) agonist that binds selectively to central 5-HT_2 binding sites. Systematic removal of any or all of the aromatic substituents had relatively little effect on 5-HT_1 binding but reduced 5-HT_2 binding by approximately 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_1-site affinity but decreased 5-HT_2-site affinity by 3- and 30-fold, respectively. In tests of stimulus generalization, using rats trained to discriminate DOM 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_2 sites and also resulted in DOM-stimulus generalization. Of the DOB analogues examined, DOB (**1a**) possesses optimal selectivity for 5-HT_2 binding.

There has long been evidence that certain 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropanes, such as DOB (**1a**, X = Br) and DOM (**1b**, X=CH_3), produce their behavioral effects via a mechanism that involves the neurotransmitter serotonin (5-HT). It has also been suggested that these



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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_1 and 5-HT_2),[4,5] coupled with our finding that the 5-HT_2-selective antagonists ketanserin and pirenperone potently antagonize the discriminative stimulus effects of, for example, DOM,[6] prompted us to examine the affinities of such agents for 5-HT_1 and [^{3}H]ketanserin-labeled 5-HT_2 sites. It was determined that both DOM and DOB bind selectively (30- and 50-fold, respectively) at 5-HT_2 sites.[7] The 4-iodo derivative DOI (**1c**, X = I) displays greater than a 100-fold selectivity for these sites.[7] On the basis of these findings, we suggested that these agents constitute the first reported examples of 5-HT_2-selective agonists. Two-site analysis of the binding of a series of derivatives of **1** to 5-HT_2 sites revealed that DOB possesses a significant affinity (K_i = 2.4 nM) and selectivity for the high-affinity component of [^{3}H]ketanserin binding.[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.[8] The ^{1}H NMR spectrum of the product (as was also the case with **8** and **11a**) displayed singlets at δ 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 Barfknecht and Nichols[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_3 instead of $LiAlH_4$ (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 $LiAlH_4$. 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*-nitrophenylhydrazone[10]). 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,[9] except that the nitrostyrene intermediate was reduced with AlH_3 instead of $LiAlH_4$. The hydrochloride salt of this product was found to be quite hygroscopic; as a consequence, it was converted to its free base and used as such (after neutralization with acid) in the binding studies. Compound

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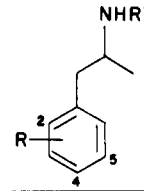
Table I. Results of Binding Studies



	R_2	R_4	R_5	R'	5-HT_1 K_i,[a] nM	5-HT_1 Hill coeff[a]	5-HT_2 K_i,[a] nM	5-HT_2 Hill coeff[a]
(±)-Amph (2)	H	H	H	H	7660 (±1320)	0.91 (±0.07)	43000 (±3100)	0.99 (±0.03)
(±)-OMA (3)	OCH_3	H	H	H	3500 (±650)	0.80 (±0.03)	8130 (±880)	1.03 (±0.08)
(±)-MMA (4)	H	H	OCH_3	H	2660 (±240)	0.75 (±0.04)	7850 (±230)	1.18 (±0.07)
5	H	Br	H	H	2830 (±540)	0.59 (±0.04)	4650 (±300)	0.88 (±0.05)
(±)-2,5-DMA (6)	OCH_3	H	OCH_3	H	1020[b]		5200[b]	
7	OCH_3	H	OCH_3	*n*-Pr	4100 (±600)	0.60 (±0.03)	25600 (±200)	1.00 (±0.06)
(±)-DOB (1a)	OCH_3	Br	OCH_3	H	3340[b]		63[b]	
8	OCH_3	Br	OCH_3	*n*-Pr	1320 (±70)	0.89 (±0.03)	1930 (±160)	0.92 (±0.10)
9	OCH_3	Br	H	H	2900 (±200)	0.77 (±0.04)	4870 (±460)	1.06 (±0.03)
10	H	Br	OCH_3	H	>25000		>25000	
11	OH	Br	OCH_3	H	1710 (±250)	0.64 (±0.02)	210 (±30)	0.77 (±0.06)
(*R*)-(−)-DOI((*R*)-1c)	OCH_3	I	OCH_3	H	2290[b]		9.9[b]	
(*S*)-(+)-DOI ((*S*)-1c)	OCH_3	I	OCH_3	H	920 (±160)	0.73 (±0.06)	35 (±3)	0.66 (±0.06)

[a] Data are followed by SEM in parentheses. [b] Results previously reported[7] included for comparative purposes.

11 was prepared by bromination of 1-[2-(benzyloxy)-5-methoxyphenyl]-2-aminopropane[11] followed by deprotection of the hydroxyl group by treatment with acid. The synthesis of (*S*)-(+)-DOI [(*S*)-1c] paralleled our previously reported synthesis of racemic DOI (1c);[12] 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 lttle variation with respect to 5-HT_1 binding; binding at 5-HT_2 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_1 sites, although in both instances the K_i 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_1-site affinity and a 5-fold increase in 5-HT_2 binding. Similar results are obtained with the 4-bromo derivative 5. Overall, compounds 2–5 display a low degree of selectivity for 5-HT_1 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_1 or 5-HT_2 sites. The effects of the methoxy and bromo groups are not additive. Optimal 5-HT_2-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 (13),[13] results in a greater than 100-fold decrease in affinity (i.e. K_i = >10000 nM) for 5-HT_2 sites.

The 5-HT_1 character of certain serotonergic agents is known to be enhanced by *N*-alkyl and in particular *N*-*n*-propyl groups.[14] As seen in Table I, however, introduction of an *n*-propyl group (i.e. compound 8) reduces the affinity of DOB for 5-HT_2 sites. Demethylation of the 2-methoxy group of DOB (i.e. 11) results in approximately a twofold increase in affinity for 5-HT_1 sites and a corresponding decrease in affinity for 5-HT_2 sites. Overall, relatively little can be done to the DOB molecule that does not result in a significant reduction in affinity for 5-HT_2 sites. Furthermore, none of the compounds examined display the 5-HT_2 selectivity noted for DOB; in fact, certain derivatives possess a slight selectivity for 5-HT_1 sites.

DOI (1c) is another potent and selective agent at 5-HT_2 sites; the *R*-(−) isomer seems to be responsible for this effect when data are compared with that obtained for the racemic mixture.[7] 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_2 sites.

Behavioral Studies. We have previously demonstrated that DOM (1b) serves as a discriminative stimulus in animals when paired with saline.[15] 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.[15] Because of the relationship that exists between discrimination-derived ED_{50} values and 5-HT_2-site affinities for a small series of agents that produce DOM-like effects, we have postulated that these effects might be 5-HT_2 mediated.[16] 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 monomethoxy derivatives (i.e. 3 and 4) or with the postiional isomer of DOB (i.e. 13), suggesting that these agents do not produce stimulus effects similar

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Table II. Results of Stimulus Generalization Studies

agent	dose, mg/kg	N^a	DOM-appropriate responding, % (± SEM)[b]	responses per min (± SEM)[b]
DOB (1a)	ED_{50} = 0.20 mg/kg[c]			
(S)-(+)DOI ((S)-1c)	0.5	3/3	14 (±2)	12.2 (±3.6)
	1.2	3/3	43 (±18)	16.9 (±5.7)
	1.8	3/3	93 (±6)	7.8 (±3.4)
	ED_{50} = 0.99 (0.41-2.42)[d] mg/kg			
N-Pr-DOB (8)	4.0	4/4	24 (±7)	10.2 (±2.7)
	5.0	6/6	63 (±14)	6.3 (±2.2)
	6.0	4/5	79 (±12)	10.2 (±5.5)
	7.0	3/5	88 (±12)	5.2 (±2.0)
	8.0	3/3	92 (±6)	5.9 (±1.7)
	ED_{50} = 4.76 (3.69 – 6.15)[d] mg/kg			
9	2.0	4/4	14 (±7)	16.3 (±2.4)
	5.0	3/4	15 (±2)	14.9 (±2.5)
	10.0	3/3	8 (±7)	4.5 (±2.1)
	11.0	3/3	17 (±11)	3.3 (±0.4)
	12.0	2/3	65 (±21)	2.8 (±0.0)
	13.0	1/3	[e]	
	13.0[f]	1/3	[e]	
11	0.1	4/4	19 (±6)	15.4 (±3.7)
	0.2	3/3	55 (±19)	12.1 (±6.1)
	0.5	3/3	96 (±3)	11.9 (±3.7)
	ED_{50} = 0.18 (0.09-0.34)[d] mg/kg			
DOM (1b)	1.0	6/6	92 (±4)	13.1 (±3.0)
saline (1 mL/kg)		6/6	13 (±4)	13.9 (±2.5)

[a] Number of animals responding/number to receive drug. [b] Data obtained during the 2.5-min extinction session. [c] ED_{50} value previously reported;[12] included for comparative purposes. [d] Numbers in parentheses are 95% confidence limits. [e] Disruption of responding, i.e. majority of animals did not respond. [f] Same dose administered to a second group of three animals.

to those of DOM.[15] Several of the derivatives prepared in this present investigation were evaluated in DOM-trained animals (Table II). The DOM stimulus generalized to the *n*-propyl derivative 8 and to the 2-hydroxy derivative 11, providing evidence that all three agents are capable of producing similar stimulus effects. The 2-methoxy-4-bromo derivative 9 produced only partial generalization (65% DOM-appropriate responding at 12 mg/kg); however, the animals response rates were severely depressed at doses greater than 10 mg/kg. Administration of 13 mg/kg of 9 resulted in disruption of behavior (i.e. no responding) in two of the three animals tested; interestingly, the one animal that did respond made greater than 90% of its responses on the DOM-appropriate lever. In a second group of three animals, similar results were obtained. These results suggest that, at doses of greater than 10 mg/kg, 9 may produce a central effect other than (or in addition to) a DOM-like effect that tends to disrupt the animals.

The DOM stimulus also generalized to (*S*)-(+)-DOI (Table II), although it was only one-third as potent (ED_{50} = 0.99 mg/kg) as its *R*-(−) enantiomer (ED_{50} = 0.26 mg/kg[12]).

Summary. 1-(2,5-Dimethoxy-4-X-phenyl)-2-aminopropanes, e.g. where X = Br, Me, I (i.e. DOB, DOM, and DOI, respectively), consitute the first examples of selective 5-HT_2 agonists. The present sudy was undertaken, primarily, to further develop the structure–activity relationship and to better understand the binding characteristics of these agents. With respect to DOB (1a), it was found that the intact molecule is necessary for optimal 5-HT_2-site affinity and selectivity. Removal of any of the aromatic substituents resulted in a decrease in affinity for 5-HT_2 sites; a dramatic decrease in affinity was also observed when the 4-bromo group was moved to the 3-position (i.e. 13). 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-HT_1 sites; however, in both cases, 5-HT_2-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-HT_2 sites, its role is rather small. In the present study, the enantiomeric (*S*/*R*) potency ratio for the isomers of DOI (1c) is only 3. Although the optical purity of (*S*)-(+)-DOI was not determined, it might be noted that the optically pure isomers of DOB (1a) also display a small potency ratio (*S*/*R* = 2).[15] In both cases, however, the *R*-(−) isomers are more potent than their (*S*)-(+) enantiomers.

We have reported that a significant correlation exists between discrimination-derived ED_{50} values (when DOM is used as the training drug) and 5-HT_2-site affinities.[16] As such, tests of stimulus generalization using DOM-trained animals might constitute a useful in vivo method for the identification and/or evaluation of novel 5-HT_2-like agents. Thus, it is significant that the DOM stimulus generalized to DOB and (*R*)-(−)-DOI and to those agents in the present study that possess the highest affinities for 5-HT_2 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 (1a) itself was found to possess the greatest affinity and selectivity for 5-HT_2 sites.[17] As a consequence, [^{3}H]DOB is currently being prepared as a radioligand for

(17) J. E. Leysen (Janssen Pharmaceutica, Beerse, Belgium) examined the binding of racemic DOB (1a) and DOI (1c) to D_2-dopamine, α_1-adrenergic, α_2-adrenergic, β-adrenergic, H_1-histaminic, μ-opiate, and muscarinic binding sites. In each case, IC_{50} > 5000 nM, except for the interaction of DOI with [^{3}H]-pyrilamine-labeled H_1 sites (i.e. IC_{50} = 2000 nM).

use in future binding studies.

Experimental Section

Proton magnetic resonance spectra were recorded on a Perkin-Elmer R-24 spectrometer using Me_4Si as an internal standard. Infrared spectra were obtained on a Perkin-Elmer 257 spectrophotomer 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 Mircolab 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.

1-(2,5-Dimethoxy-4-bromophenyl)-2-aminopropane (DOB; 1a). Alane, AlH_3, was prepared by the dropwise addition of a solution of H_2SO_4 (100%, 0.19 g) in dry THF (10 mL) to a stirred suspension of $LiAlH_4$ (0.15 g, 4 mmol) in THF (75 mL) at 0 °C under a nitrogen atmosphere. This mixture was stirred for 30 min, and a solution of 1-(2,5-dimethoxy-4-bromophenyl)-2-nitropropene[9] (0.4 g, 1 mmol) in THF (50 mL) was added in a dropwise fashion at 0 °C. After the addition was complete, the lime-green mixture was stirred for 6 h at room temperature. Excess AlH_3 was decomposed by the careful addition of small chips of ice to the cooled (0 °C) suspension until no further reaction was evident. The mixture was made basic by the addition of 15% NaOH (10 mL), and the precipitated solids were collected by filtration. The solid material was suspended in THF (25 mL) and the suspension was allowed to stir at room temperature for 15 min. The solid material was collected by filtration, and the combined THF filtrates were dried ($MgSO_4$). The solvent was removed under reduced pressure to give a thick oil. Kugelrohr distillation [50–57 °C (0.1 mm)] afforded 0.19 g (53%) of the amine as a white waxy solid. A sample of this material (0.1 g) was dissolved in small volume of absolute EtOH and treated with a saturated solution of HCl gas in anhydrous Et_2O. The resultant solution was diluted with anhydrous Et_2O to give 0.04 g of DOB as the HCl salt, mp 195–198 °C (lit.[9] mp 198–199 °C, lit.[18] mp 207–208 °C).

The title compound, **1a**, was also prepared according to the procedure of Aldous et al.[8] via the bromination of 1-(2,5-dimethoxyphenyl)-2-aminopropane. The initial product was isolated as the hydrobromide salt, mp 175–177 °C (lit.[8] mp 144–146 °C) after recrystallization from absolute EtOH. The melting point was unchanged after repeated recrystallizations from absolute EtOH or MeCN. Anal. ($C_{11}H_{16}BrNO_2$·HBr) C, H, N.

A solution of this salt in water was made basic (pH 9) by the addition of 15% aqueous NaOH; the free base was obtained by extraction with Et_2O and evaporation of the Et_2O solution to dryness. Kugelrohr distillation [53–56 °C (0.13 mm)] afforded the free base of **1a** as a white solid, mp 63–65 °C. Treatment of an Et_2O solution of this amine with HCl gas afforded **1a** as white crystals after recrystallization from absolute EtOH/Et_2O, mp 195–198 °C.

(*S*)-(+)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane Hydrochloride [(*S*)-1c]. Racemic *N*-acetyl-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane was prepared, as previously described,[12] by the direct iodination of *N*-acetyl-1-(2,5-dimethoxyphenyl)-2-aminopropane with I_2 and silver trifluoroacetate; mp 164–166 °C (lit.[12] mp 162–163 °C) after recrystallization from 95% EtOH. Hydrolysis of the product afforded **1c** as the free base. A small sample was converted to DOI·HCl (**1c**), mp 198–199 °C (lit.[12] mp 198–200 °C); the remainder of the free base (10.0 g, 31 mmol) was dissolved in warm (40 °C) absolute EtOH (25 mL), and to this was added a solution of (*L*)-(+)-tartaric acid (4.67 g, 31 mmol) in absolute EtOH at 40 °C. The solution was allowed to cool to room temperature, and the solid product was collected by filtration and was washed with anhydrous Et_2O (10 mL). After seven recrystallizations from MeOH, this salt had a constant melting point (mp 167–168 °C) and optical rotation ($[\alpha]^{25}_D$ +15.8°; water). An aqueous solution of the tartrate salt was neutralized by treatment with 10% aqueous Na_2CO_3, and extracted with Et_2O, and the dried ($MgSO_4$) Et_2O extract was treated with HCl gas to afford the title product as a white solid, mp 224–225 °C, $[\alpha]^{25}_D$ +12.6° (MeOH), after three recrystallizations from absolute EtOH/Et_2O. Anal. ($C_{11}H_{16}INO_2$·HCl) C, H, N.

N-n-Propyl-1-(2,5-dimethoxyphenyl)-2-aminopropane Hydrochloride (7). A solution of propionyl chloride (2.4 g, 26 mmol) in $CHCl_3$ (50 mL) was added in a dropwise manner to a stirred mixture of sodium bicarbonate (3.0 g, 38 mmol) and 1-(2,5-dimethoxyphenyl)-2-aminopropane (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 mixture was filtered and the filtrate was washed with 0.5 N HCl (2 × 50 mL). The organic portion was dried ($MgSO_4$) and evaporated to dryness under reduced pressure to afford a solid. Recrystallization from absolute ethanol and then from carbon tetrachloride/hexane afforded 1.8 g (27%) of the acylated product as a white solid, mp 95–96.5 °C.

A solution of *N*-propionyl-1-(2,5-dimethoxyphenyl)-2-aminopropane (1.5 g, 6 mmol) in freshly distilled THF (20 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ (0.9 g, 24 mmol) in THF (30 mL) at 0 °C under a nitrogen atmosphere. After the addition was complete, the mixture was heated at reflux for 4 h and then stirred at room temperature for 18 h. The reaction mixture was cooled to 0 °C and excess $LiAlH_4$ 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 stirred with Et_2O (25 mL) for 30 min. The solid was removed by filtration and the Et_2O 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 acetonitrile to give 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_2O to give a white solid: mp 112–113.5 °C; IR (neat) 3340 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–1.25 (m, 7 H, 2 CH_3, NH), 1.25–1.90 (m, 2 H, CH_2), 2.45–3.10 (m, 5 H, Ar CH_2, NCH_2, NCH), 3.75 (s, 6 H, OCH_3), 6.80 (m, 3 H, Ar H). Anal. ($C_{14}H_{23}NO_2$·HCl) C, H, N.

N-n-Propyl-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane 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 was allowed to stir at room temperature for 3 h. The solvent was removed under reduced pressure to give a light orange solid. This solid was suspended in anhydrous Et_2O (10 mL), collected by filtration, washed with anhydrous Et_2O (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 g (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 (30 mL), with heating, and treating the cooled solution with a 15% aqueous NaOH solution. This basic solution was extracted with Et_2O (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 [Kugelrhor, 61–65 °C (0.11 mm)]. Dry HCl-saturated Et_2O (5 mL) was added, at room temperature, to a solution of this oil in anhydrous Et_2O (10 mL). This solid product was collected by filtration, washed with anhydrous Et_2O (5 mL), and dried at room temperature to give 0.3 g of as 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}; ^1H NMR ($CDCl_3$) δ 0.55–1.10 (m, 6 H, CH_3), 1.15 (s, 1 H, NH), 1.20–180 (m, 2 H, CH_2), 2.45–3.40 (m, 5 H, Ar CH_2, NCH_2, NCH), 3.80 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.80 (s, 1 H, Ar H), 7.10 (s, 1 H, Ar H). Anal. ($C_{14}H_{22}BrNO_2$·HCl) C, H, N.

1-(2-Methoxy-4-bromophenyl)-2-aminopropane Hydrochloride (9). 2-Methoxy-4-bromobenzaldehyde (12; 4.0 g, 19 mmol) was added to a stirred solution of NH_4OAc (1.2 g, 15 mmol)

(18) Shulgin, A. T.; Sargent, T.; Naranjo, C. *Pharmacology* 1981, *5*, *103*.

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 $CHCl_3$ (3 × 50 mL), and the combined organic extracts were dried ($MgSO_4$). 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.

Alane, AlH_3, was prepared by the addition of a solution of 100% H_2SO_4 (1.1 g) and THF (50 mL) to a suspension of $LiAlH_4$ (0.8 g, 22 mmol) in dry THF (100 mL) at 0 °C under a nitrogen atmosphere. A solution of the nitrostyrene (2.0 g, 7 mmol) in THF (50 mL) was added in a dropwise manner to the alane suspension at 0 °C. After the addition was complete, the mixture was allowed to stir for 6 h at room temperature. Excess AlH_3 was decomposed 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 ($MgSO_4$). 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. Et_2O saturated with HCl gas was added to a solution of the amine (1.1 g) in absolute EtOH (5 mL) and anhydrous Et_2O (10 mL) at room temperature until salt formation had ceased. The solid was collected by filtration and recrystallized thrice from absolute ethanol to yield 0.2 g of **9** as a white solid: mp 176–177 °C; IR (neat) 3380 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (d, 3 H, J = 6 Hz, CH_3), 1.40 (br s, 2 H, NH_2), 2.80 (d, 2 H, J = 6 Hz, Ar CH_2), 3.00–3.40 (m, 1 H), 3.65 (s, 3 H, OCH_3), 6.45–7.10 (m, 3 H, Ar H). Anal. ($C_{10}H_{14}BrNO$·HCl) C, H, N.

1-(2-Hydroxy-4-bromo-5-methoxyphenyl)-2-aminopropane Hydrochloride (11). A solution of **11a** (2.9 g, 8 mmol) in 20% v/v concentrated HCl in glacial HOAc (100 mL) was heated at reflux for 6 h and allowed to cool to room temperature. The solvent was removed under reduced pressure to give a viscous, dark oil. This oil was further dried under high vacuum (0.16 mm), with heat (40 °C), for 1 h. A suspension of the oil in anhydrous Et_2O (25 mL) was stirred until crystallization occurred. The solid material was collected by filtration and was decolorized by treatment of an ethanolic solution with activated charcoal. The warm filtrate was allowed to stand overnight at room temperature and was then diluted by the addition of anhydrous Et_2O (5 mL) until a precipitate began to form. After standing for 2 h, the suspension was filtered, and the solids were washed with anhydrous Et_2O (5 mL) and recrystallized from 2-propanol/ether to afford 0.6 g (26%) of 11 as a white solid, mp 238–239 °C dec. The free base of 11 was obtained by neutralization of a solution of 0.6 of the crude salt (isolated from the recrystallization filtrates) in water (5 mL) by the addition of 10% NaOH. This solution was extracted with EtOAc (3 × 25 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated to dryness under reduced pressure to give a dark oil. Kugelrohr distillation [84–86 °C (0.08 mm)] afforded 0.2 g of a viscous, light-brown oil which solidified upon standing to give an off-white solid: mp 84–86 °C; IR (neat) 3340 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (d, 3 H, J = 6 Hz, CH_3), 2.80 (m, 2 H, Ar CH_2), 3.30–3.70 (m, 1 H, CH), 3.95 (s, 3 H, OCH_3), 5.75 (br s, 3 H, Ar OH, NH_2), 6.85 (s, 1 H, Ar H), 7.40 (s, 1 H, Ar H). Treatment of an ethanolic solution of the amine with HCl gas afforded additional 11. Anal. ($C_{10}H_{14}BrNO_2$·HCl) C, H, N.

1-[2-(Benzyloxy)-4-bromo-5-methoxyphenyl]-2-aminopropane (11a). 1-[2-(Benzyloxy)-5-methoxyphenyl]-2-aminopropane[11] (3.0 g) was brominated, using conditions analogous to those used for the synthesis of **8**, to yield a crude oily product. The oil was dissolved in EtOAc (some warming was required) and the solution was allowed to stand at room temperature (18 h). The precipitate was collected by filtration and washed with anhydrous Et_2O (10 mL) to give 3.9 g of an off-white solid. A portion of this solid (0.3 g) was dissolved in warm absolute, EtOH, treated with charcoal, and filtered hot, and the filtrate was layered with anhydrous Et_2O to precipitate the salt. Recrystallization from MeCN gave 0.1 g of **11a**·HBr as a white solid, mp 167–169 °C. The remaining crude solid was dissolved in water (25 mL) and the aqueous solution was made basic with a 10% NaOH solution (ca. pH 11). The basic solution was extracted with Et_2O (3 × 50 mL), and the combined Et_2O extracts were dried ($MgSO_4$), and the ether 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 ($MgSO_4$), and the solvent was removed under reduced pressure to give a liquid. A solution of this liquid in Et_2O (200 mL) was layered over a saturated $NaHSO_4$ solution and allowed to stand at room temperature for 5 days. The bisulfite adduct was collected by filtration and washed with Et_2O (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. Kugelrhor 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.[10] mp 258 °C).

Solid K_2CO_3 (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 acetone (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 Et_2O (50 mL), and the insoluble solids were removed by filtration. The Et_2O 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.[10] mp 215 °C).

Binding Studies. The radioligand binding assay has already been described in detail.[7] In brief, tissue preparation was performed as described by Leysen 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 nM 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 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; 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 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 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

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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 reinformcement. After lever responding was established, each daily session was preceded by an intraperitoneal (ip) injection of either (±)-DOM hydrochloride (1.0 mg/kg) or 0.9% saline (1.0 mL/kg). A pressession 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, ED_{50} 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 [^{3}H]DOB as a radioligand for 5-HT_2 sites have just been published as a rapid communication (*Eur. J. Pharmacol.* **1985**, *117*, 145).

Acknowledgment. This work was supported, in part, by PHS Grant DA-01642. We also express our appreciation to Amy Hauck, Betsy Mack, and Mary Tocarz for their assistance with the discrimination studies and to Dr. J. Leysen for her evaluation of racemic DOB and DOI.

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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 (H_2sfth) 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 H_2sfth and Cu(sfth) were injected intraperitoneally in mice bearing either solid fibrosarcoma or ascites Dalton's lymphoma. However, H_2sfth 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, L82T, 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 tumors[2] 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-oxobutyraldehyde 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 thiosemicarbazones[8-10] 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.[10-12]

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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