Synthesis and Pharmacological Evaluation of Ring-Methylated Derivatives of 3,4-(Methylenedioxy)amphetamine (MDA)

Matthew A. Parker, Danuta Marona-Lewicka, Deborah Kurrasch, Alexander T. Shulgin,‡ and David E. Nichols*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

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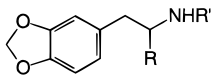
The three isomeric ring-methylated derivatives of the well-known hallucinogen and entactogen MDA (**1a**) were synthesized and evaluated for pharmacological activity as monoamine-releasing agents and as serotonin agonists. The 2-methyl derivative **2a** and the 5-methyl derivative **2b** were found to be more potent and more selective than the parent compound in inhibiting [^{3}H]-serotonin accumulation in rat brain synaptosomal preparations. Their activity in vivo was confirmed in rats trained to discriminate serotonin-releasing agents and hallucinogens from saline. The results indicate that compounds **2a**,**b** are among the most potent 5-HT-releasing compounds known and show promise as lead compounds in the search for antidepressant drugs that release serotonin rather than inhibit its uptake.

Introduction

MDA (3,4-(methylenedioxy)amphetamine, **1a**) is a ring-substituted phenethylamine derivative first synthesized in 1912 during early studies of phenylalkylamines.[1] It is of considerable interest in that it shares structural and pharmacological properties with both classical phenethylamine hallucinogens, the prototype of which is mescaline,[2] and entactogens, a class of potentially therapeutically useful compounds including **1b** (MDMA)[3] and **1c** (MBDB)[4] that facilitate communication and introspective states. These compounds exert their psychological effects by modulating monoamine neurotransmitter systems in the central nervous system (CNS). The hallucinogens are, in general, direct serotonin (5-HT) agonists acting primarily at 5-HT$_2$ receptor subtypes,[5] whereas the entactogens exert their effects indirectly by inducing the presynaptic release of 5-HT, dopamine (DA), and norepinephrine (NE). As MDA lies at the intersection of these two classes, it provides an excellent starting point for examining effects of variation in structure on the action of these compounds.



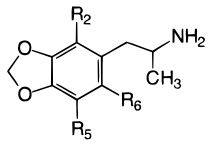
1a R = CH_3, R' = H

1b R = CH_3, R' = CH_3

1c R = C_2H_5, R' = CH_3

We have for the past several years been particularly interested in the activity of ring-substituted phenethylamines as selective serotonin-releasing agents.[6,7] We wished to explore this area further, especially in light of the recent demonstration in our laboratories that certain compounds which act as selective 5-HT releasers possess antidepressant properties in a rat model of depression.[8] Thus, our primary rationale for the synthesis of the current compounds was to examine the effects of ring methylation on their potency and selectivity as releasers of 5-HT versus DA and NE.

Four ring-methoxylated analogues of MDA were previously examined as 5-HT- and DA-releasing agents, but none surpassed the parent compound in potency, nor did any exhibit significant selectivity for neurotransmitter release.[9] However, as these compounds have several relatively polar ether substituents on the aryl ring, they are too hydrophilic to have optimal CNS activity.[10] It seemed, then, that ring methylation to give **2a**−**c** might lead to more active compounds by increasing the overall lipophilicity relative to that of the parent compound. It also seemed likely that selectivity might be increased, given our previous general observation that the structural requirements for activity are more stringent for the dopamine uptake carrier than for the serotonin carrier.[7]



2a R_2 = CH_3, R_5 = H, R_6 = H

2b R_2 = H, R_5 = CH_3, R_6 = H

2c R_2 = H, R_5 = H, R_6 = CH_3

In addition, we hoped to reinforce earlier evidence[11] that suggested the active conformation of the aminoethyl side chain in this class of molecules. In that study, we found that the 2-aminoindan derivative **3**, as well as the corresponding 2-aminotetralin analogue, substitutes for MDMA in rats trained to discriminate MDMA from saline, whereas **4** and its tetralin analogue lack activity. Later it was reported[12] that 2,*N*-dimethyl-4,5-(methylenedioxy)amphetamine (6-methyl-MDMA), the *N*-methyl derivative of **2c**, lacks entactogenic activity

* Address correspondence to: Dr. David E. Nichols. Phone: (765) 494-1461. Fax: (765) 494-1414. E-mail: drdave@pharmacy.purdue.edu.
‡ Present address: 1483 Shulgin Rd, Lafayette, CA 94549.

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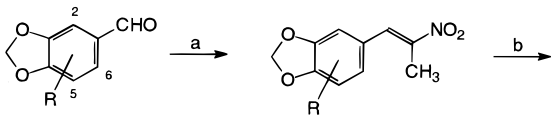
Scheme 1[a]

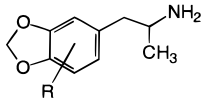


5a, R = 2-CH_3

5b, R = 5-CH_3

5c, R = 6-CH_3

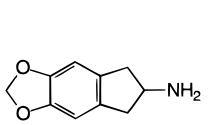
6a-6c



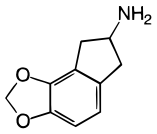
2a-2c

[a] (a) $EtNO_2$, NH_4OAc, reflux; (b) $LiAlH_4$, THF.

in humans. We conjecture that this compound lacks activity because the methyl group on the ring, through steric repulsion, forces the side chain into an unfavorable orientation like that in **4**. The 2-methyl compound **2a**, on the other hand, might be expected to retain activity because the methyl group would direct its side chain into a conformation similar to that found in **3**.



3



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We therefore describe here the synthesis of the three isomeric ring-monomethylated derivatives of MDA, an evaluation of their hallucinogen- and entactogen-like behavioral effects in the rat, and the determination of their potency in inhibiting the accumulation of tritiated 5-HT, DA, and NE into rat whole brain synaptosomal preparations.

Chemistry

The synthesis of the target compounds was straightforward starting from the appropriate substituted benzaldehydes. Two of the three required aldehydes were synthesized as described in the literature,[13,14] and the remaining aldehyde **5b** (5-methylpiperonal) was obtained by methylenating 4,5-dihydroxy-3-methylbenzaldehyde,[15] available in three steps from vanillin. The aldehydes were converted into the corresponding phenylisopropylamines by condensation with nitroethane followed by reduction with lithium aluminum hydride (Scheme 1), as described previously.[16]

Pharmacology

Compounds **2a**−**c** were evaluated in the two-lever drug discrimination assay in five groups of rats, each of which was trained to discriminate the effects of ip injections of saline from those of one of the following drugs: (+)-amphetamine, (+)-MBDB, MMAI, LSD, and DOI; the methods employed have been described previously.[16,17] For those compounds that completely substituted for a training drug, potencies were calculated as ED_{50} values with 95% confidence intervals.

In addition, the compounds were tested for their ability to inhibit the accumulation of tritiated 5-HT, DA, and NE into rat whole brain synaptosomes as also described previously,[6] and the data were used to calculate IC_{50} values.

Results and Discussion

Results of the [^{3}H]monoamine uptake inhibition studies in rats are shown in Table 1. Most apparent from the data is the fact that all three of the compounds are almost completely inert at the dopamine uptake carrier, being more than 10 times less potent than MDA in inhibiting uptake of [^{3}H]DA. They would therefore be expected to lack the serotonin neurotoxicity associated with the parent compound.[18] Compound **2c** shows reduced activity at the 5-HT carrier as well, being only about one-half as potent as the parent compound. Compounds **2a**,**b**, however, are quite potent at the serotonin carrier, being 4−5 times more active than MDA. All three compounds show modest activity in inhibiting [^{3}H]NE accumulation, with **2b** being the most potent.

The drug discrimination data for **2a**−**c** are presented in Table 2. As would be expected from an examination of the uptake inhibition data, none of the compounds substituted for the catecholamine releaser (+)-amphetamine. The data from uptake inhibition are also borne out in the behavioral data from rats trained using the selective serotonin releasers (+)-MBDB and MMAI. Compounds **2a**,**b** substituted completely for both (+)-MBDB and MMAI and were slightly more potent than MDA in the (+)-MBDB-trained rats, whereas **2c** exhibited only partial substitution in (+)-MBDB-trained rats and substituted in MMAI rats only at a relatively high dose.

From Table 2 it can also be seen that **2a**,**b** substitute for the hallucinogenic 5-HT_{2A} agonist DOI at doses comparable to those at which they substitute for the serotonin releasers (+)-MBDB and MMAI. Compound **2a** also substitutes in LSD-trained rats, albeit at more than twice the dose required in DOI-trained rats. Compound **2c** is once again only marginally active, showing partial substitution at high doses in DOI- and LSD-trained rats. Thus, **2a**,**b,** but not **2c,** retain the hallucinogen-like behavioral effects of the parent compound presumed to be mediated by the 5-HT_{2A} receptor.[19]

In summary, both the in vitro and in vivo data point to **2a**,**b** as serotonin-releasing agents more potent than MDA, with **2a** appearing to be slightly more potent than **2b** in most of the assays. These data indeed support our conjecture that in **2a**, as well as **2b**, the amine side chain can assume a favorable conformation for interacting with the serotonin carrier, whereas in **2c** the side chain is forced into an unfavorable position. Further support for this idea was provided by a conformational analysis employing semiempirical AM1 potential functions, which indicated that conformations where the side chain was directed toward the aryl methyl group in either **2a** or **2c** were about 8 kcal/mol above the minimum-energy conformation and about 6 kcal/mol less stable than conformations where the side chain was directed toward an aryl hydrogen atom.

Compounds **2a**,**b** are particularly interesting in that they are more potent as serotonin-releasing agents than

Table 1. IC_{50} for Monoamine Uptake Inhibition in Rat Synaptosomes[a]

compd	IC_{50} (nM)			selectivity	
	[^{3}H]-5-HT	[^{3}H]DA	[^{3}H]NE	5-HT/DA	5-HT/NE
1a (MDA)[b]	478 ± 40	890 ± 100	266 ± 28	2	<1
2a	93 ± 11	12000 ± 1260	1937 ± 329	129	21
2b	107 ± 12	11600 ± 970	1494 ± 411	108	14
2c	783 ± 68	28300 ± 7200	4602 ± 1390	36	6
PIA[c]	82 ± 8	589 ± 52	993 ± 86	7	12
MTA[d]	74 ± 10	3073 ± 407	2375 ± 121	42	32
IAP[e]	82 ± 6	1847 ± 213	849 ± 82	23	10

[a] The ability of the test compounds to inhibit accumulation of monoamines was examined in crude synaptosomes. The data from three or four experiments were combined, and the IC_{50} values ± SEM (nM) were calculated by curve fitting followed by appropriate statistics (see Experimental Section). [b] Values from Johnson et al.[6] included for comparison. [c] *p*-Iodoamphetamine; values from Nichols et al.[19] included for comparison. [d] *p*-(Methylthio)amphetamine; values from Huang et al.[20] included for comparison. [e] 1-(5-Indanyl)-2-aminopropane; values from Monte et al.[16] included for comparison.

Table 2. Results of the Drug Discrimination Studies in Rats

training drug	test drug	ED_{50} (μmol/kg)	95% CI[a] (μmol/kg)	*n*[b]
(+)-amphetamine	(+)-amphetamine	1.06	0.65−1.73	
	1a (MDA)	NS[c]		8−13
	2a	NS[c]		9−10
	2b	NS[c]		9
	2c	NS[c]		9−10
(+)-MBDB	(+)-MBDB	3.25	2.10−5.03	
	1a (MDA)	2.07	1.31−3.26	9−12
	2a	1.83	1.01−3.30	9−10
	2b	2.00	1.23−3.25	9−11
	2c	PS (17.4, 78%)[d]		8−11
MMAI	MMAI	1.83	1.15−2.91	
	2a	1.71	0.88−3.32	8−9
	2b	1.53	1.15−2.05	8−10
	2c	7.65	4.50−12.96	8−10
LSD	LSD	0.04	0.02−0.06	
	2a	4.10	2.82−5.98	11−18
	2b	PS (8.72, 75%)[d]		10−15
	2c	PS (17.4, 60%)[d]		10−16
DOI	DOI	0.30	0.20−0.47	
	2a	1.70	1.10−2.63	8−9
	2b	2.11	1.06−4.20	8
	2c	PS (8.72, 63%)[d]		7−8

[a] Range of 95% confidence interval for ED_{50}. [b] Number of animals tested at each dose. [c] NS, no substitution. [d] PS, partial substitution; with the dose at which highest substitution occurred followed by the percentage of rats selecting the drug lever at that dose given in parentheses.

most other compounds previously tested in these laboratories.[6,7,16] The only compounds that have shown higher activity than **2a**,**b** are *p*-iodoamphetamine (PIA),[20] *p*-(methylthio)amphetamine (MTA),[21] and 1-(5-indanyl)-2-aminopropane (IAP).[16] As can be seen in Table 1, although PIA, MTA, and IAP are slightly more potent than **2a**,**b**, the latter two compounds greatly surpass them in selectivity for 5-HT versus DA release, exhibiting a preference of more than 100-fold for the 5-HT carrier versus the DA carrier, while still retaining a moderate selectivity versus the NE carrier.

Indeed, the present pharmacological data point to **2a**,**b** as promising lead structures for therapeutically useful agents in the treatment of depression. However, the hallucinogen-like effects of these compounds would need to be eliminated before this clinical application could be considered. Several strategies to accomplish this goal are currently underway.

Experimental Section

Chemistry. Melting points were taken on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a 500-MHz Varian VXR-500S spectrometer. Chemical shifts are reported in δ values ppm relative to tetramethylsilane as an internal reference (0.03%, v/v). Elemental analyses were performed by the Purdue University Microanalysis Laboratory and are within 0.4% of the calculated values. Most reactions were carried out under an inert atmosphere of dry argon or nitrogen.

4-Methyl-1,3-benzodioxole-6-carboxaldehyde (5b). To a mechanically stirred suspension of 4,5-dihydroxy-3-methylbenzaldehyde[15] (12.84 g, 84.5 mmol) and cesium carbonate (41.28 g, 126.7 mmol) in anhydrous DMF (200 mL) was added $BrCH_2Cl$ (8.23 mL = 16.39 g, 126.7 mmol), and the resulting mixture was heated to 110 °C for 2 h. The reaction was then cooled to room temperature and filtered through a pad of Celite with EtOAc washing. The filtrate was concentrated almost to dryness, diluted with H_2O, and extracted three times with EtOAc. The extracts were washed with H_2O and brine, dried with $MgSO_4$, filtered, and evaporated, yielding 13.54 g (98%) of product as a tan solid of sufficient purity to be carried on to the condensation step. An analytical sample of 1.27 g gave after bulb-to-bulb distillation (120 °C, 0.25 Torr) 1.05 g of a white solid: mp 45−46 °C; ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3, CH_3), 6.07 (s, 2, OCH_2O), 7.19 (s, 1, ArH), 7.25 (s, 1, ArH), 9.78 (s, 1, CHO). Anal. ($C_9H_8O_3$) C, H.

4-Methyl-5-(2-nitro-1-propenyl)-1,3-benzodioxole (6a). To a solution of 0.527 g of 2-methylpiperonal[13] in 5 mL of nitroethane was added 60 mg of ammonium acetate. The mixture was stirred and heated at 85 °C for 22 h. The reaction mixture was cooled, diluted with Et_2O, washed twice with H_2O, dried with $MgSO_4$, and filtered. The Et_2O and excess nitroethane were evaporated, and the yellow crystalline product (0.69 g, 97%) was dried overnight under vacuum. An analytical sample was recrystallized from MeOH: mp 91−92 °C; ^{1}H

NMR ($CDCl_3$) δ 2.22 (s, 3, vinylic CH_3), 2.35 (s, 3, aryl CH_3), 6.02 (s, 2, OCH_2O), 6.73 (d, 1, ArH, J = 8.05 Hz), 6.80 (d, 1, ArH, J = 8.05 Hz), 8.11 (s, 1, vinylic H). Anal. ($C_{11}H_{11}NO_4$) C, H, N.

4-Methyl-6-(2-nitro-1-propenyl)-1,3-benzodioxole (6b). A mixture of 11.0 g (67.1 mmol) of aldehyde **5b**, 40 mL of nitroethane, 10.9 g (134 mmol) of dimethylamine hydrochloride, 0.58 g (10 mmol) of potassium fluoride, and 40 mL of toluene was placed in a flask equipped with a Dean−Stark trap and heated at reflux under N_2 for 24 h. Solvents were evaporated, and the residue was partitioned between Et_2O and H_2O. The Et_2O fraction was dried with $MgSO_4$ and evaporated, yielding 14.12 g (95%) of product as an orange-yellow solid. An analytical sample was recrystallized from MeOH to give pale-orange crystals: mp 97−98 °C; ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3, vinylic CH_3), 2.46 (s, 3, aryl CH_3), 6.03 (s, 2, OCH_2O), 6.80 (s, 1, ArH), 6.82 (s, 1, ArH), 8.00 (s, 1, vinylic H). Anal. ($C_{11}H_{11}NO_4$) C, H, N.

General Procedure for the Reduction of the Nitropropenes 6a−c to the Aminopropanes 2a−c. A solution of 10 mmol of the appropriate nitropropene in 20 mL of THF was added dropwise to a suspension of 70 mmol of $LiAlH_4$ in 35 mL of THF while stirring under N_2. The reaction mixture was stirred and heated under reflux for the specified time and cooled to room temperature, and the reaction was quenched carefully by the sequential addition of 2 mL of 2-propanol, 2 mL of 15% aqueous NaOH, and 7 mL of H_2O. The precipitate was removed by filtration, and the resulting solution was evaporated. The residue was suspended in H_2O, acidified with concentrated HCl, and washed three times with Et_2O. The resulting acidic solution was then made basic with aqueous NaOH and extracted three times with Et_2O. The Et_2O solution was evaporated, and the resulting oil was purified by bulb-to-bulb distillation. The distilled oil was dissolved in 10 mL of 2-propanol, neutralized with ethanolic HCl, and diluted with Et_2O to yield the hydrochloride salt of the aminopropane.

1-(4-Methyl-1,3-benzodioxol-5-yl)-2-aminopropane Hydrochloride (2a). This compound was obtained from **6a** in 71% yield as fine white crystals: mp 214−215 °C; ^{1}H NMR (D_2O) δ 1.13 (d, 3, $CHCH_3$, J = 7 Hz), 2.02 (s, 3, $ArCH_3$), 2.73 (m, 2, $ArCH_2$), 3.37 (sextet, 1, CH, J = 7 Hz), 5.79 (p, 2, OCH_2O, J = 1 Hz), 6.58 (s, 2, overlapping ArH). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

1-(4-Methyl-1,3-benzodioxol-6-yl)-2-aminopropane Hydrochloride (2b). This compound was obtained from **6b** in 65% yield as white crystals: mp 222−223 °C; ^{1}H NMR (D_2O) δ 1.11 (d, 3, $CHCH_3$, J = 7 Hz), 2.03 (s, 3, $ArCH_3$), 2.65 (m, 2, $ArCH_2$), 3.38 (sextet, 1, CH, J = 7 Hz), 5.79 (s, 2, OCH_2O), 6.48 (s, 1, ArH), 6.51 (s, 1, ArH). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

1-(5-Methyl-1,3-benzodioxol-6-yl)-2-aminopropane Hydrochloride (2c). This compound was obtained in 74% yield from **6c**.[14] The salt precipitated from 2-propanol/ether solution as a clear oil which crystallized overnight as white crystals: mp 157−158 °C; ^{1}H NMR (D_2O) δ 1.13 (d, 3, $CHCH_3$, J = 7 Hz), 2.07 (s, 3, $ArCH_3$), 2.70 (m, 2, $ArCH_2$), 3.39 (sextet, 1, CH, J = 7 Hz), 5.76 (tt, 2, OCH_2O, J = 1, 3 Hz), 6.59 (s, 1, ArH), 6.64 (s, 1, ArH). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

Pharmacology Methods. Animals. Male Sprague−Dawley rats (Harlan, Indianapolis, IN) weighing 175−200 g were used. Animals were group housed (for *in vitro* experiments) or individually caged (for drug discrimination experiments) in a temperature-controlled room with a 12-h day/night lighting schedule. Animals that were used for in vitro experiments were supplied with food (Lab Blox, Purina) and water ad libitum.

Drug Discrimination. The procedures for the drug discrimination assays were exactly as described previously.[16,17] Training drugs were (+)-amphetamine sulfate (1.0 mg/kg), (+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine hydrochloride ((+)-MBDB; 1.75 mg/kg), 5-methoxy-6-methyl-2-aminoindan hydrochloride (MMAI; 1.71 mg/kg), (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg), and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI; 0.40 mg/kg). All drugs were dissolved in 0.9% saline and were injected intraperitoneally in a volume of 1 mL/kg, 30 min before the sessions.

None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages, and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions to maintain approximately 80% of free-feeding weight. Lights were on from 0700 to 1900. The laboratory and animal facility temperature was 22−24 °C, and the relative humidity was 40−50%. Experiments were performed between 0830 and 1700 each day, Monday through Friday.

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

Briefly, a fixed ratio (FR) 50 schedule of food reinforcement (Bioserv, 45 mg of dustless pellets) in a two-lever paradigm was used. At least one drug and one saline session separated each test session. Rats were required to maintain an 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. Test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min, the session was ended and scored as a disruption. Treatments were randomized at the beginning of the study.

In Vitro [^{3}H]-5-HT and [^{3}H]DA Uptake Inhibition. The procedure of Steele et al.[22] was employed with minor modifications exactly as described previously.[16] Briefly, three rats were decapitated and their brains rapidly removed and dissected over ice. The cerebellums were removed and discarded, and the remaining brain tissue (ca. 4 g, wet weight) was pooled, diced, and homogenized in 20 mL of ice-cold 0.32 M sucrose. Homogenizations were done in a prechilled Potter-Elvehjem tissue grinder with a motor-driven Teflon pestle at 0 °C, for two periods of 1 min each, 6 strokes/min, with a 15-s interval between periods. The tissue homogenate was subjected to centrifugation (Beckman J2-21 with JA-20 rotor; 4 °C) at 1090*g* for 10 min. The pellet was discarded, and the supernatant was subjected to centrifugation at 17400*g* for 30 min. The resulting pellet was resuspended with a polytron (setting 5, 20 s; Kinematica) in 30−40 mL of ice-cold, aerated (5% CO_2 in O_2) modified Krebs-Ringer bicarbonate (KR) buffer containing the following (mM): NaCl (124.3), KCl (2.95), $MgSO_4$ (1.30), KH_2PO_4 (1.25), $NaHCO_3$ (26.0), $CaCl_2$ (2.41), *d*-glucose (10.4), Na_2EDTA (0.03), and Na ascorbate (0.06), pH 7.4−7.6. The synaptosomal suspension was stored on ice until use.

The ability of synaptosomes to accumulate tritiated serotonin ([^{3}H]-5-HT), dopamine ([^{3}H]DA), and norepinephrine ([^{3}H]NE) was measured in the absence and presence of various concentrations of test drugs as follows: a 200-μL aliquot of the synaptosomal suspension was added to test tubes containing 1.65 mL of ice-cold KR buffer, 50 μL of test drugs (dissolved in deionized water) or deionized water (for total and nonspecific determinations), and 50 μL of pargyline HCl solution (final concentration, 100 μM). The test tubes were preincubated in an aerated (5% CO_2 in O_2) 37 °C shaking water bath for 5 min. The tubes were then returned to the ice bath and chilled for 10−15 min. Tritiated neurotransmitter (New England Nuclear) was added (50 mL of stock solution; final concentration, 10 nM), giving a final incubation volume of 2 mL. All tubes except nonspecific assays were returned to the aerated 37 °C shaking water bath for 5 min to initiate neurotransmitter uptake.

Uptake was terminated by chilling the test tubes in an ice bath and then rapidly filtering them through glass fiber filters (Whatman GF/C) pretreated with 0.05% poly(ethylenimine) using a 24-well cell harvester (Brandel). Filters were washed with 2 × 3 mL of ice-cold KR buffer, allowed to air-dry for 10 min, and then placed in plastic liquid scintillation vials. Scintillation cocktail (10 mL of Ecolite; ICN Biomedicals) was added, and the vials were sealed, vortexed, and allowed to stand overnight. Radioactivity was measured using liquid scintillation spectroscopy (Packard model 4430). Specific uptake was defined as uptake at 37 °C minus uptake at 0 °C, in the absence of drugs. IC_{50} values were calculated from at least three experiments, each done in triplicate.

Statistical Analysis. Data from the drug discrimination studies were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (% SDL) for each dose of compound was determined. The degree of substitution was determined by the maximum % SDL for all doses of the test drug. No substitution (NS) is defined as 59% SDL or less, and partial substitution is 60−79% SDL. If the compound was one that completely substituted for the training drug (at least one dose resulted in a % SDL = 80% or higher), the ED_{50} values and 95% confidence intervals (95% CI) were then determined from quantal dose−response curves according to the procedure of Litchfield and Wilcoxon.[23] If the percentage of rats disrupted (% D) was 50% or higher, the ED_{50} value was not determined, even if the % SDL of nondisrupted animals was higher than 80%.

In vitro data were transformed from dpm to percent inhibition of specific uptake and analyzed using the computer program PRISM,[24] from which the IC_{50} values (nM) were calculated. The IC_{50} values reported are the mean ± SEM from three or four experiments run in triplicate with 9 or 10 concentrations of test drugs.

Materials. (*S*)-(+)-Amphetamine sulfate was purchased from Smith Kline & French Laboratories (Philadelphia, PA). Pargyline hydrochloride was purchased from Sigma (St. Louis, MO). (*S*)−(+)-MBDB, MMAI, and (±)-DOI, as their hydrochlorides, were synthesized in our laboratory.[4,25,26] (+)-LSD tartrate was obtained from NIDA. For drug discrimination experiments, all drugs were dissolved in 0.9% saline and injected intraperitoneally in a volume of 1 mL/kg, 30 min before the session.

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