Synthesis and Biological Evaluation of Meperidine Analogues at Monoamine Transporters

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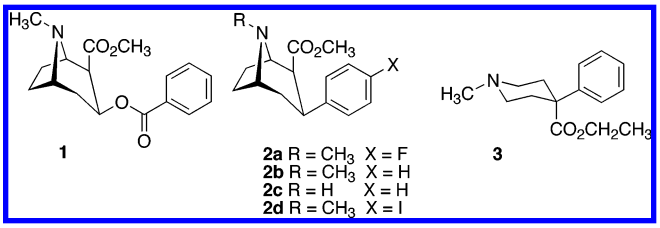
A series of aryl-substituted meperidine analogues was synthesized, and the binding affinities were determined at the DAT, SERT, and NET as well as at μ-opioid receptors. Generally the analogues exhibited increased affinity for the DAT and SERT relative to meperidine but exhibited low binding affinity for the NET. The 2-naphthyl derivative **7f** was the most potent ligand at the SERT ($K_i = 0.0072$ μM) and was the most selective ligand for the SERT over the DAT (DAT/SERT = 158) and μ-opioid receptors (μ/SERT = 281). The 3,4-dichlorophenyl derivative **7e** was the most potent ligand at the DAT ($K_i = 0.125$ μM) and was the most selective ligand for the DAT over μ-opioid receptors (μ/DAT = 16.3) but remained slightly more selective for the SERT over the DAT(DAT/SERT = 6.68). Three compounds, the 3,4-dichlorophenyl derivative **7e** and the 2-naphthyl analogues **6f** and **7f**, were identified that were more potent at the DAT than meperidine and that exhibited well-defined biphasic dopamine uptake inhibition similar to meperidine. However, none of the analogues tested produced locomotor effects or substituted for cocaine in drug discrimination studies, suggesting that the μ-opioid effects of these analogues may contribute to the poor efficacy observed in vivo.

Introduction

Cocaine (**1**) binding at the dopamine transporter (DAT) has been well established.[1−11] Initially, specific binding sites were identified in rodent brain that bind [^{3}H]cocaine at a single saturable site.[1,2] Subsequent studies using rodent,[3] human,[4] and nonhuman primate[5,6] brain tissue preparations (striatum and caudate putamen) later provided evidence that suggests that cocaine binding at the dopamine transporter is heterogeneous. Two distinct binding sites on the dopamine transporter have been proposed—a high affinity and a low affinity site.[3−6,10,13] These sites are termed high and low based upon the relative affinity of cocaine for these sites. The phenyltropane analogues of cocaine, specifically WIN 35,428 (**2a**), WIN 35,065-2 (**2b**), and WIN 35,-981 (**2c**), in addition to several other cocaine-like compounds, also have binding curves that are better fit with a two-site than a one-site model.[10,13] However, it is uncertain if these compounds interact at the high and low affinity site in a fashion similar to that of cocaine. It may be possible for compounds other than cocaine to have low affinity for the high affinity site and vice versa. Alternatively, DAT inhibitors such as mazindol[13,14] and GBR 12909[15,16] exhibit only single site affinity for the DAT.

A strong correlation between binding affinity at the dopamine transporter (DAT) and potency for reinforcing effects among cocaine (**1**) and its analogues has been reported.[17,18] For other effects of cocaine and its analogues, the correlation between affinity for the dopamine transporter and potency can depend on whether high- or low-affinity sites are examined. For example, in comparison of DAT binding affinity with the cocaine-like discriminative-stimulus (subjective) effects, the correlation between substitution potencies for cocaine analogues and DAT affinity is greater for the high-affinity constants than for the corresponding low-affinity constants.[19]



Currently there are no known dopamine uptake inhibitors that discriminate between high-affinity and low-affinity sites on the dopamine transporter. However, it has been reported that meperidine (**3**, an atypical μ-opioid agonist with some stimulant effects) is a potent inhibitor of [^{3}H]dopamine uptake when examined in a chopped tissue rather than synaptosomal preparation.[20] Further, the concentration response curve exhibited a plateau at approximately 20% inhibition over a broad range of low concentrations of meperidine. This high-affinity component to the curve resembled the high-affinity component of the inhibition of dopamine uptake produced by cocaine. The maximal inhibition of dopamine uptake produced by meperidine at low concentrations (20%) was also consistent with the amount of total binding of [^{3}H]WIN 35,428 (**2a**) that is attributable to high-affinity sites (18%).[6,10] In addition, when the opioid-

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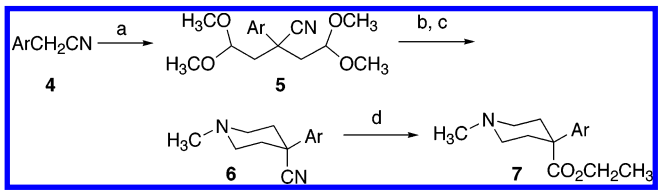
Scheme 1[a]



[a] Reagents and conditions: (a) $BrCH_2CH(OCH_3)_2$, $NaNH_2$, toluene. (b) 3 N HCl, 50 °C, 4.5 h. (c) CH_3NH_2·HCl, CH_3OH, $NaBH_3CN$. (d) H_2SO_4/H_2O 120 °C; then EtOH, azeotropic distillation.

agonist actions of meperidine were blocked by naltrexone, meperidine substituted for cocaine in squirrel monkeys trained to discriminate cocaine from saline.[20] These results suggested that the high-affinity inhibition of dopamine uptake by cocaine may underlie its subjective effects.

Upon the basis of these previous studies, it was of interest to explore the structure−activity relationships of a series of meperidine derivatives to develop a more potent dopamine transporter ligand with selectivity for the high-affinity cocaine binding site. Compounds that allow a differentiation of high- and low-affinity actions of cocaine would undoubtedly be significant pharmacological probes to explore the relationship between those actions and the abuse liability of cocaine. In our preliminary studies, a series of meperidine analogues were prepared that exhibited increased affinity for dopamine and serotonin transporters over that observed for meperidine.[21] Herein we report the synthesis and biological evaluation of a series of meperidine analogues at dopamine, serotonin, and norepinephrine transporters as well as at μ-opioid receptors.

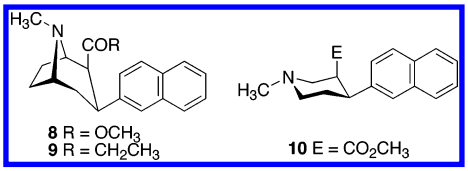
Results and Discussion

Chemistry. A general synthetic approach was developed that could be easily modified to explore a broad spectrum of aryl-substituted derivatives of meperidine. As illustrated in Scheme 1, the dialkylation of readily available[22] and commercially available 2-aryl acetonitriles **4** with bromoacetaldehyde dimethyl acetal afforded the corresponding diacetals **5** in good yields (75−90%). Hydrolysis of the acetals in 3 N hydrochloric acid generated the corresponding dialdehydes which upon concomitant reductive amination with methylamine hydrochloride and sodium cyanoborohydride in methanol gave the *N*-methyl-4-aryl-4-piperidine-4-carbonitriles **6** in moderate overall yields (26−45%) for the two-step process. Careful control of the reaction temperature of the acetal hydrolysis proved to be critical for reasonable yields of the desired piperidines. Hydrolysis reaction temperatures that exceeded 50 °C typically gave intractable mixtures of pyran derivatives. The nitriles **6** were then converted into the corresponding ethyl esters **7** via a two-step sequence that furnished the ethyl esters **7** in good overall yields (52−76%).

Biology. The transporter binding affinities were determined for the meperidine analogues **6** and **7** by their ability to displace bound radiolabeled ligands from rat caudate-putamen tissue.[23] The K_i values that are reported in Table 1 are inhibition constants derived for the unlabeled ligands. The binding affinities of the meperidine analogues were determined at DAT by inhibition of [^{3}H]WIN 35,428, at SERT by inhibition of [^{3}H]paroxetine, and at NET by inhibition of [^{3}H]nisoxetine binding as previously described.[24,25] In addition, the inhibition of [^{3}H]dopamine uptake was determined for each compound in the same manner as that originally described for meperidine.[20] Moreover, μ-opioid receptor binding affinities were also determined using a [^{3}H]-DAMGO binding assay.[26] The results of these in vitro binding studies are summarized in Table 1.

In general, the esters **7** were significantly more potent than the corresponding nitriles **6** at the DAT, SERT, and NET. Like meperidine, all of the analogues exhibited a greater affinity for the SERT than for the DAT. The 2′-naphthyl analogue **7f** (K_i = 0.0072 μM) was the most potent ligand of the series at the SERT and was 59-fold more potent than meperidine (**3**). The 4-iodophenyl derivative **7c** (K_i = 0.021 μM) and the 3,4-dichlorophenyl derivative **7e** (K_i = 0.019 μM) also exhibited low nanomolar affinities for the SERT. The increased SERT affinity of the 4-iodophenyl- **7c** (20-fold) and 2′-naphthyl- **7f** (60-fold) derivatives relative to **3** followed a similar trend observed for the SAR of the phenyltropane analogue, WIN 35,065-2 (**2b**). The 3β-(4-iodophenyl) derivative **2d**[27] and 3β-(2′-naphthyl) analogue **8**[28] exhibited a 390-fold and a 2500-fold, respectively, increase in potency at SERT relative to **2b**.

The DAT/SERT ratios (Table 1) illustrate that the meperidine analogues exhibited 3- to 158-times greater affinity for SERT than for DAT. The high selectivity of the 4-iodophenyl analogue **7c** (DAT/SERT = 155) was unexpected since DAT/SERT selectivity is modest for the corresponding 3β-(4-iodophenyl)tropane **2d** DAT/SERT = 3.0).[27] In addition, the high selectivity of the 2′-naphthyl analogue **7f** (DAT/SERT = 158) was quite surprising since the SAR previously described for the 3β-(2-naphthyl)tropane derivative **8** and the corresponding 2β-acyl analogue **9** have been reported to exhibit moderate DAT selectivity.[28,29] Moreover, the 3β-carbomethoxy-1-methyl-4β-(2′-naphthyl)piperidine (**10**) has been reported to exhibit only slight SERT selectivity (DAT/SERT = 2.8).[30]



The SAR of the meperidine analogues at the DAT exhibited a similar trend to that of the 3β-phenyltropanes, albeit the meperidine derivatives were 160−4000 times less potent. Nevertheless, substitution of the phenyl ring with halogen or a methyl group affected the DAT binding affinity of the meperidine derivatives in a fashion similar to substitution on the phenyl ring of **2b** (K_i or IC_{50}: H > F > CH_3, > Cl ≈ I > 3,4-Cl_2).[31,32] The 3,4-dichlorophenyl derivative **7e** (DAT K_i = 0.125 μM) was found to be the most potent ligand of the series for the DAT and was over 100-fold more potent than meperidine (**3**). In addition, **7e** was the least selective of the ester analogues for the SERT (DAT/SERT = 6.68). From the SAR of the meperidine derivatives, it is clear the 3,4-dichlorophenyl group is an important moiety for molecular recognition at the DAT. Similar effects of the 3,4-dichlorophenyl group on DAT affinity have been

Table 1. In Vitro Data for Meperidine Analogues

compd	Ar[a]	[3H]WIN 35,428 (DAT) K_i (μM)[b]	[3H]paroxetine (SERT) K_i (μM)[b]	[3H]nisoxetine (NET) K_i (μM)[b]	[3H]DAMGO K_i (μM)[b]	[3H]DA uptake inhibition IC_{50} (μM)[b,c]	DAT/ SERT	μ/DAT	μ/SERT
1		32 ± 5[d] 388 ± 221[d]				405 ± 91[d]			
3	Ph	17.8 ± 2.7	0.413 ± 0.044		0.92	0.61 ± 2.2 nM (22%)[f] 12.6 ± 1.2	43.1	0.052	2.13
6a	4-F-Ph	45% @ 100 μM[c]	10.1 ± 0.4	(101.4)[e]	(15)[c]	(8)[c]			
6b	4-Cl-Ph	22.0 ± 10.1	5.11 ± 0.59	(106.2)[e]	36.8 (51)[c]	(36)[c]	4.31	1.67	0.18
6c	4-I-Ph	8.34 ± 0.67	0.430 ± 0.034	(93.6)[e]	17.3 (61)[c]	36.7 ± 1.3	19.4	2.07	40.2
6d	4-Me-Ph	41.8 ± 6.1	13.7 ± 0.4	(102.9)[e]	40.6 (46)[c]	(22)[c]	3.05	0.97	2.96
6e	3,4-Cl_2-Ph	2.67 ± 0.24	0.805 ± 0.12	(94.1)[e]	40.0 (46)[c]	11.1 ± 1.2	3.32	15.0	49.7
6f	2-naphthyl	2.36 ± 0.66	0.125 ± 0.022	(88.1)[e]	15.4 (61)[c]	0.37 ± 2.0 nM (11%)[f] 21.8 ± 1.2	18.9	6.53	123
7a	4-F-Ph	10.7 ± 2.3	0.308 ± 0.026	(71.9)[e]	1.47	(47)[c]	34.7	0.14	4.77
7b	4-Cl-Ph	4.10 ± 1.27	0.277 ± 0.040	601.2 ± 45.9	4.41	26.9 ± 1.2	14.8	1.08	15.9
7c	4-I-Ph	3.25 ± 0.20	0.0211 ± 0.0024	519.3 ± 51.1	2.35	11.1 ± 1.2	155	0.72	111
7d	4-Me-Ph	12.4 ± 5.2	1.61 ± 0.11	(57.6)[e]	2.67	76.2 ± 1.2	7.69	0.22	1.66
7e	3,4-Cl_2-Ph	0.125 ± 0.015	0.0187 ± 0.0026	74.5 ± 5.1	2.04	6.01 ± 6.26 nM (22%)[f] 1.40 ± 1.25	6.68	16.3	109
7f	2-naphthyl	1.14 ± 0.38	0.0072 ± 0.0001	71.1 ± 9.7	2.03	3.19 ± 7.41 nM (16%)[f] 11.6 ± 1.3	158	1.78	282

[a] All compounds were tested as the HCl salt. [b] All values are the mean ± SEM of three experiments performed in triplicate. [c] Percent inhibition at highest dose tested (100 μM). [d] The K_i and IC_{50} values are reproduced from ref 39 and were collected under identical conditions. [e] Percent specific binding at highest dose tested (10 μM). [f] Percent of total uptake inhibition.

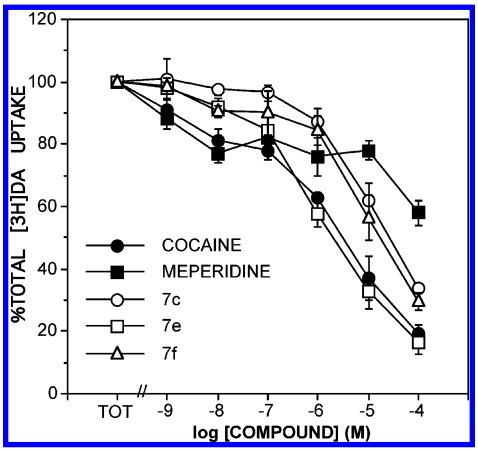


Figure 1. Meperidine-like biphasic dopamine uptake inhibition of meperidine derivatives **7e** and **7f**.

observed for other tropane derivatives,[32−34] non-nitrogen tropane analogues,[35,36] and piperidine derivatives.[37] Although, the 3,4-dichlorophenyl group also increased the binding affinity of **7e** for the SERT, the overall effect was only 15% of that observed for the DAT.

None of the meperidine analogues **7a**−**f** exhibited significant affinity for the NET. The meperidine ester analogues **7b**, **7c**, **7e**, and **7f** exhibited only low micromolar affinity for the NET, while all the other analogues exhibited only partial, if any, inhibition of [3H]nisoxetine at the highest dose tested (10 μM).

All of the meperidine derivatives exhibited weak inhibition of dopamine uptake (Table 1). The dopamine uptake inhibition produced by most of the meperidine compounds modeled better for a single site (e.g. **7c**, Figure 1). However, several of the compounds (**6f**, **7e**, **7f**) modeled better for two sites, a high-affinity site and a low-affinity site, similar to that observed for meperidine. As illustrated in Figure 1, the esters, **7e** and **7f** exhibited biphasic dopamine uptake inhibition with a high-affinity uptake plateau similar to that for meperidine (**3**) of approximately 20% of the total inhibition.[20] The percent of total inhibition of uptake that is accounted for by the high affinity component is based upon a nonlinear model of the data. The graphs do not show this model, but show the actual data points connected by lines. Each of the compounds that modeled better for two sites than for one site had a high-affinity uptake plateau component of approximately 20% of the total inhibition, which is similar to both meperidine and cocaine. This is consistent with the percentage (18%) of the total binding that corresponds to the high-affinity component of WIN 35 428 (**2a**).[6,10] This suggests that modification of the phenyl group of **3** can increase DAT binding affinity with the retention of an ability to differentiate between the high-affinity and low-affinity components of the inhibition of DA uptake. Of the phenyl substitutions examined, the 3,4-dichlorophenyl (**7e**) and the 2-naphthyl (**7f**) groups were identified as important substructures for differentiating between the high- and low-affinity components, while significantly increasing binding affinity relative to meperidine. Alternatively, the 4-chlorophenyl and 4-iodophenyl derivatives **7b** and **7c** exhibited increased DAT binding affinity relative to **3**, but were unable to discriminate the between the high- and low-affinity components.

All of the meperidine nitrile analogues **6a**−**f** prepared in this study exhibited significantly diminished μ-opioid receptor affinity relative to meperidine (**3**). The ester analogues **7a**−**f** exhibited only slightly reduced affinity for μ-opioid receptors relative to **3**, but all of the analogues exhibited a marked increase in DAT and SERT selectivity over μ-opioid receptors compared to meperidine. The 3,4-dichlorophenyl analogue **7e** (μ/DAT = 16) was found to exhibit the greatest selectivity for the DAT over that of μ-opioid receptors (Table 1) while the 2-naphthyl analogue **7f** (μ/SERT = 282) exhibited the greatest selectivity for the SERT over the μ-opioid receptors (Table 1).

Selected compounds **7c**, **7e**, and **7f** were evaluated for their locomotor-stimulant activity in mice (Figure 2) using experimental conditions previously reported.[24] Upon the basis of the high selectivity of these compounds for the dopamine and serotonin transporters, it was believed initially that any behavioral effects medi-

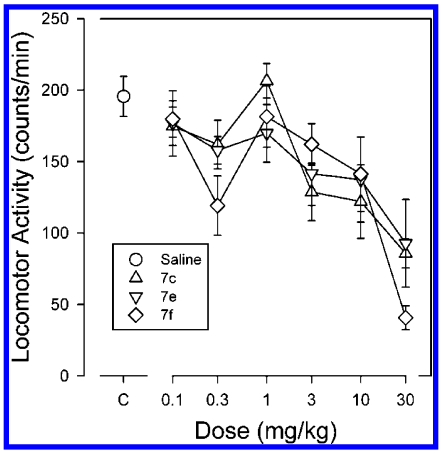


Figure 2. Dose-dependent effects of **7c**, **7e**, and **7f** on locomotor activity in mice. Ordinates: horizontal locomotor activity counts after drug administration in counts per second. Abscissae: dose of drug in μmol/kg, log scale. Each point represents the average effect determined in six mice. The data are from a 30-min period starting 30 min after drug administration. Note that none of the compounds produced a stimulation of activity.

ated by μ-opioid receptors would be diminished relative to the cocaine-like effects mediated by the transporters. Naive mice were injected ip and locomotor activity (horizontal activity counts) was assessed immediately after injection for a 1-h period. Each data point is a mean of eight subjects. None of the compounds tested produced locomotor stimulant effects similar to cocaine. Instead each of the compounds decreased locomotor activity across a range of doses from those having no effects to those that virtually eliminated activity.

The compounds **7c** (SERT selective) and **7e** (DAT selective) also were tested in rats trained to discriminate injections of cocaine from saline. Rats were trained to discriminate ip injections of cocaine (10 mg/kg) from saline injections until reliable performances were maintained according to procedures previously outlined.[24] Compounds were then tested for their effectiveness in substituting for cocaine immediately after injection during sessions that lasted for a maximum of 20 min. Each data point is typically a mean of six subjects; however, lever selection data was excluded if more than half of the subjects tested did not respond. This only occurred at the highest doses tested. Neither compound **7c** nor **7e** substituted for cocaine in rats trained to discriminate between cocaine and saline (Figure 3). In addition, efficacy observed for these compounds closely resembled that of meperidne (**3**). The low efficacy observed for **7c** and **7e** may be due to their μ-opioid activity, which may not be as diminished as originally envisaged. It is unlikely that actions at the SERT interfered with the discriminative stimulus effect as these effects do not do so for cocaine and other drugs that have affinity for both the DAT and SERT.[38]

In summary, a series of aryl-substituted meperidine analogues were synthesized and the binding affinities determined at the DAT, SERT, and NET as well as at μ-opioid receptors. Generally the analogues exhibited increased affinity for the DAT and SERT relative to meperidine but exhibited low binding affinity for the NET. The 2-naphthyl derivative **7f** was the most potent ligand at the SERT and was the most selective ligand for the SERT over the DAT and μ-opioid receptors. The

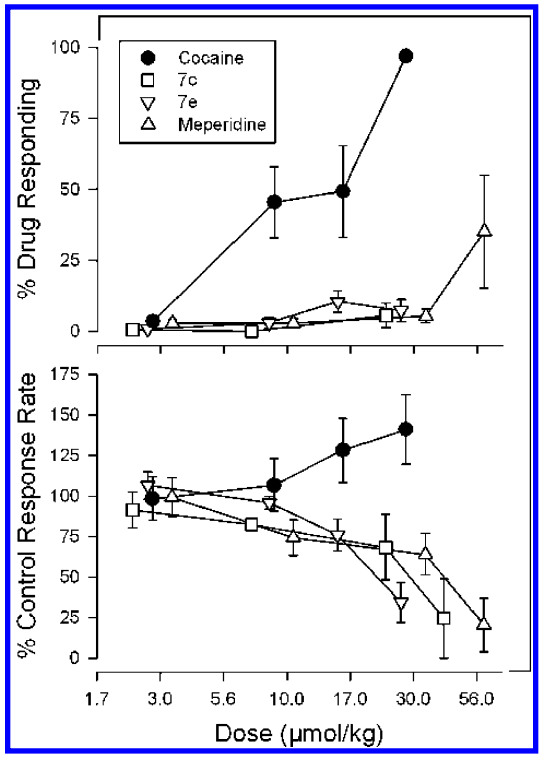


Figure 3. Effects of **7c** and **7e** in rats trained to discriminate injections of cocaine from saline. Ordinates for top panels: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panels: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissa: drug dose in μmol/kg (log scale). Each point represents the effect in six to nine rats. The percentage of responses emitted on the cocaine-appropriate key was considered unreliable, and not plotted, if fewer than half of the subjects responded at that dose. Note that neither compound substituted for cocaine.

3,4-dichlorophenyl derivative **7e** was the most potent ligand at the DAT and was the most selective ligand for the DAT over μ-opioid receptors but remained slightly more selective for the SERT over the DAT. Three compounds, the 3,4-dichlorophenyl derivative **7e** and the 2-naphthyl analogues **6f** and **7f**, were identified that were more potent at the DAT than meperidine and that exhibited dopamine uptake inhibition curves that resembled the biphasic dopamine uptake inhibition of meperidine. However, none of the analogues tested produced locomotor effects or substituted for cocaine in drug discrimination studies, suggesting that the μ-opioid effects may still be significant. While the monoamine transporter affinity SAR of these meperidine analogues is somewhat reminiscent of the 3β-phenyltropanes, it is apparent from the dopamine uptake inhibition that the 3,4-dichlorophenyl and 2-naphthyl moieties are important in the meperidine system for differentiating between the high- and low-affinity components of this effect as well as between the DAT and the SERT. Additional studies directed toward the development of DAT and SERT selective meperidine derivatives with diminished μ-opioid receptor affinity are underway and will be reported in due course.

Experimental Section

All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, unless otherwise noted. THF, CH_2Cl_2, and MeOH were dried under the argon system. Toluene and Et_2O

were dried by distillation over Na/benzophenone. Chromatography refers to column chromatography on silica gel (Silica Gel 60, 230−400 mesh). Petroleum ether refers to pentanes with a boiling point range of 30−60 C. Reported melting points are uncorrected. NMR spectra were recorded on a Varian-Gemini 400 MHz spectrometer. Chemical shifts are reported as δ values with tetramethylsilane (TMS), employed as the internal standard. Elemental analyses were obtained from Atlantic Microlabs, Inc., Norcross, GA.

General Method A. Preparation of Hydrochloride Salts. Some of the compounds were converted into the hydrochloride salts for biological testing as well as for storage and handling purposes. The base (100 mg) was dissolved in the minimum amount of Et_2O (1−2 mL) and added to a saturated ethereal solution (10 mL) of anhydrous hydrogen chloride. The hydrochloride salts crystallized and were washed with Et_2O (3 × 2 mL) and purified by tritration with Et_2O and ethyl acetate. Fractional moles of water could not be prevented despite vigorous drying (110 °C, 1h) under vacuum (0.01 mmHg). All compounds were homogeneous by thin-layer chromatography ($CHCl_3$:MeOH:NH_4OH, 90:9:1).

(4-Iodophenyl)acetonitrile (4c).[22] A mixture of KCN (2.7 g, 41 mmol) and deionized water (4.0 mL) was heated in an oil bath to dissolve the KCN. A solution of the 4-iodobenzyl bromide (9.8 g, 33 mmol) in MeOH (190 mL) was added to the reaction flask via addition funnel and allowed to stir at reflux for 4 h. The reaction mixture was allowed to cool to room temperature. The salts were filtered, and the filtrate was partially concentrated under reduced pressure. The newly formed salts were then filtered, and the remaining filtrate was concentrated under reduced pressure. The resulting residue was purified by bulb-to-bulb distillation. The impurities were collected from 80 to 100 °C, and the product was collected from 100−120 °C. This compound was obtained as a white solid (4.8 g, 60% yield). mp 56−58 °C [lit.:[22] mp 56−57C]. ^{1}H NMR: δ 7.71 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 3.70 (s, 2H). ^{13}C NMR: δ 138.1, 129.7, 129.5, 117.2, 93.5, 23.2. ^{1}H NMR: δ 7.73−7.68 (m, 2H), 7.10−7.05 (m, 2H), 3.70 (s, 2H). ^{13}C NMR: δ 138.2, 129.7, 129.5, 117.2, 93.5, 23.2.

General Method B: 2-Aryl-2-(2,2-dimethoxyethyl)-4,4-dimethoxybutyronitrile (5). To a round-bottom flask of arylacetonitrile **4** (5.4 mmol) and bromoacetaldehyde dimethyl acetal (1.6 mL, 13 mmol) in dry toluene (7 mL) under an atmosphere of nitrogen was added portionwise (2 to 4 × 1 mL) a suspension of $NaNH_2$ (50wt % in toluene). The reaction was allowed to stir at reflux for 15 min before each portion of $NaNH_2$ was added. This was repeated until the monosubstituted product was no longer visible by TLC (Et_2O/hexanes, 1:1). The reaction mixture was cooled to room temperature and quenched with water (20 mL). The aqueous layer was extracted with ether (4 × 25 mL). The combined organic fractions were dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography using an elution gradient of 25% Et_2O/hexanes to 50% Et_2O/hexanes to afford the diacetals **5**.

2-(4-Fluorophenyl)-2-(2,2-dimethoxyethyl)-4,4-dimethoxybutyronitrile (5a). General Method B**.** This compound was obtained as an orange oil (1.2 g, 66%). ^{1}H NMR: δ 7.44 (m, 2H), 7.10 (m, 2H), 4.27 (dd, J = 4.8, 4.2 Hz, 2H), 3.23 (s, 6H), 3.17 (s, 6H), 2.41 (dd, J = 14.2, 6.6, 2H), 2.14 (dd, J = 14.4, 4.0, 2H). ^{13}C NMR: δ 162.0 (J_{C-F} = 246 Hz), 133.6, 127.7, 121.1, 115.7, 101.9, 53.6, 53.1, 43.7, 41.4. Anal. ($C_{16}H_{22}NO_4F$) C, H, N.

2-(4-Chlorophenyl)-2-(2,2-dimethoxy-ethyl)-4,4-dimethoxybutyronitrile (5b). General Method B. This compound was obtained as an orange solid (1.2 g, 66%). ^{1}H NMR: δ 7.40 (m, 4H), 3.32 (s, 6H), 3.17 (s, 6H), 2.40 (dd, J = 6.8, 14.4 Hz, 2H), 2.13 (dd, J = 4.0, 14.4 Hz, 2H). ^{13}C NMR: δ 136.4, 133.7, 128.9, 127.4, 120.8, 101.9, 53.5, 53.1, 43.4, 41.5. Anal. ($C_{16}H_{22}NO_4Cl$) C, H, N.

2-(4-Iodophenyl)-2-(2,2-dimethoxyethyl)-4,4-dimethoxybutyronitrile (5c). General Method B. The compound was obtained as an orange solid (1.7 g, 77%), mp 73−75 °C. ^{1}H NMR: δ 7.74 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 4.27 (m, J = 4.0 Hz, 2H), 3.32 (s, 6H), 3.17 (s, 6H), 2.40 (dd, J = 7.2, 14.4 Hz, 2H), 2.13 (dd, J = 4.4, 14.6 Hz, 2H). ^{13}C NMR: δ 137.8, 137.6, 127.8, 120.7, 101.7, 93.3, 53.5, 53.1, 43.2, 41.3. Anal. ($C_{16}H_{22}NO_4I$) C, H, N.

2-(4-Methylphenyl)-2-(2,2-dimethoxyethyl)-4,4-dimethoxybutyronitrile (5d). General Method B. This compound was obtained as an orange oil (1.3 g, 80%). ^{1}H NMR: δ 7.34 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 4.26 (m, 2H), 3.31 (s, 6H), 3.17 (s, 6H), 2.40 (dd, J = 6.8, 14.4 Hz, 2H), 2.36 (s, 3H), 2.14 (dd, J = 3.6, 14.4 Hz, 2H). ^{13}C NMR: δ 137.7, 134.8, 129.6, 125.8, 121.4, 102.1, 53.7, 53.0, 43.6, 41.6, 20.9. Anal. ($C_{17}H_{25}NO_4$) C, H, N.

2-(3,4-Dichlorophenyl)-2-(2,2-dimethoxyethyl)-4,4-dimethoxybutyronitrile (5e). General Method B. This compound was obtained as an orange oil (1.2 g, 81%). ^{1}H NMR: δ 7.56 (d, J = 2.4 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.31 (dd, J = 2.4, 8.4 Hz, 1H), 4.30 (m, J = 4.4 Hz, 2H), 3.33 (s, 6H), 3.19 (s, 6H), 2.40 (dd, J = 6.4, 14.4 Hz, 2H), 2.13 (dd, J = 4.4, 14.4 Hz, 2H). ^{13}C NMR: δ 138.3, 133.0, 132.1, 130.6, 128.1, 125.4, 120.4, 101.8, 53.6, 53.3, 43.3, 41.5. Anal. ($C_{16}H_{21}NO_4Cl_2$) C, H, N.

2-(2,2-Dimethoxyethyl)-4,4-dimethoxy-2-naphthalen-2-yl-butyronitrile (5f). General Method B. This compound was obtained as an orange oil (1.5 g, 82%). ^{1}H NMR: δ 8.05 (s, 1H), 7.87 (m, 3H), 7.52 (m, 3H), 4.28 (dd, J = 4.0, 7.0 Hz, 2H), 3.31 (s, 6H), 3.13 (s, 6H), 2.50 (dd, J = 7.2, 14.2 Hz, 2H), 2.26 (dd, J = 4.0, 14.6 Hz, 2H). ^{13}C NMR: δ 135.0, 133.1, 132.6, 128.9, 128.2, 127.5, 126.7, 126.6, 125.6, 122.9, 121.3, 102.1, 53.7, 53.2, 43.5, 42.1. Anal. ($C_{20}H_{25}NO_4$) C, H, N.

General Method C: 4-Aryl-1-methylpiperidine-4-carbonitrile (6). To a 250 mL three-neck round-bottom flask of 3 N HCl (120 mL) at 50 °C was added the diacetal **5** (7.5 mmol), and the mixture was allowed to stir overnight. The acid mixture was allowed to cool to room temperature and then extracted with Et_2O (300 mL). The ether layer was washed with $NaHCO_3$ (100 mL) and dried (Na_2SO_4). The Et_2O was removed under reduced pressure. The resulting residue was dissolved in dry MeOH (37 mL), the *N*-methylamine hydrochloride (7.5 mmol) was added followed by $NaBH_3CN$ (6.7 mmol), and the mixture was allowed to stir 48 h under an atmosphere of nitrogen. The solvent was removed under reduced pressure. The resulting residue was treated with saturated $NaHCO_3$ (120 mL) and extracted with Et_2O (3 × 100 mL). The combined organic fractions were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The resulting crude product was purified by chromatography ($CHCl_3$:MeOH:NH_4OH, 90:9:1) to afford the 4-arylpiperidine-4-carbonitriles **6**.

4-(4-Fluorophenyl)-1-methylpiperidine-4-carbonitrile (6a). General Method C. This compound was obtained as an orange solid (0.63 g, 39%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 215−216 °C (HCl salt). ^{1}H NMR (free base): δ 7.47 (m, 2H), 7.09 (m, 2H), 2.96 (m, 2H), 2.45 (m, 2H), 2.39 (s, 3H), 2.09 (m, 4H). ^{13}C NMR (free base): δ 162.2 (J_{C-F} = 246 Hz), 135.9, 127.3, 121.7, 115.8, 52.6, 45.9, 41.6, 36.7. Anal. ($C_{13}H_{15}N_2F$·HCl) C, H, N.

4-(4-Chlorophenyl)-1-methylpiperidine-4-carbonitrile (6b). General Method C. This compound was obtained as an orange solid (0.46 g, 26%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 211−212 °C. ^{1}H NMR (free base): δ 7.44 (d, J = 7.6 Hz, 2H), 7.37 (d, J = 9.2 Hz, 2H), 2.96 (m, 2H), 2.48 (m, 2H), 2.38 (s, 3H), 2.09 (m, 4H). ^{13}C NMR (free base): δ 138.6, 133.9, 129.1, 126.9, 121.4, 52.5, 45.9, 41.7, 36.4. Anal. ($C_{13}H_{15}N_2Cl$·HCl) C, H, N.

4-(4-Iodophenyl)-1-methylpiperidine-4-carbonitrile (6c). General Method C. This compound was obtained as a yellow oil (0.76 g, 31%). The compound was converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 267−270 °C. ^{1}H NMR (free base): δ 7.73 (d, J = 8.8 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 2.97 (d, J = 14.8 Hz, 2H), 2.48 (m, 2H), 2.39 (s, 3H), 2.09 (m, 4H). ^{13}C NMR (free

base): δ 139.8, 138.1, 127.5, 121.4, 93.7, 52.6, 46.0, 42.0, 36.4. Anal. ($C_{13}H_{15}N_2I$·HCl) C, H, N.

4-(4-Methylphenyl)-1-methylpiperidine-4-carbonitrile (6d). General Method C. This compound was obtained as an oil (0.63 g, 39%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 198−200 °C (HCl salt). ^{1}H NMR (free base): δ 7.38 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 2.96 (m, 2H), 2.48 (m, 2H), 2.38 (s, 3H), 2.35 (s, 3H), 2.10 (m, 4H). ^{13}C NMR (free base): δ 137.8, 137.2, 129.6, 125.4, 122.0, 52.7, 46.0, 41.7, 36.6, 20.8. Anal. ($C_{14}H_{18}N_2$·HCl·$^1/_3H_2O$) C, H, N.

4-(3,4-Dichlorophenyl)-1-methylpiperidine-4-carbonitrile (6e). General Method C. This compound was obtained as an orange oil (0.79 g, 39%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 266−268 °C. ^{1}H NMR (free base): δ 7.56 (d, J = 2.0 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.31 (dd, J = 2.4, 8.4 Hz, 1H), 2.95 (m, 2H), 2.46 (m, 2H), 2.36 (s, 3H), 2.07 (m, 4H). ^{13}C NMR (free base): δ 140.2, 133.2, 132.4, 130.9, 127.9, 125.0, 121.0, 52.4, 45.8, 41.6, 36.3. Anal. ($C_{13}H_{14}N_2Cl_2$·HCl·$^1/_4$ H_2O) C, H, N.

1-Methyl-4-naphthalen-2-yl-piperidine-4-carbonitrile (6f). General Method C. This compound was obtained as an orange oil (1.0 g, 45%). The compound was converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 256−258 °C. ^{1}H NMR (free base): δ 7.97 (d, J = 1.6 Hz, 1H), 7.85 (m, 3H), 7.57 (dd, J = 2.0, 8.4 Hz, 1H), 7.50 (m, 2H), 2.99 (d, J = 12.0, 2H), 2.53 (m, 2H), 2.40 (s, 3H), 2.22 (m, 4H). ^{13}C NMR (free base): δ 137.3, 133.1, 132.7, 128.9, 128.1, 127.5, 126.6, 126.5, 124.6, 123.3, 121.9, 52.7, 46.0, 42.3, 36.5. Anal. ($C_{17}H_{18}N_2$·HCl·$^1/_3H_2O$) C, H, N.

General Method D. 4-Aryl-1-methylpiperidine-4-carboxylic Acid Ethyl Ester (7). The nitriles **6** (5.2 mmol) in an aqueous solution of sulfuric acid (6.5 mL H_2SO_4:H_2O, 1:1) was heated in an oil bath at 120 °C for 1.5 h. The flask was then equipped with a Dean−Stark trap with a stopcock. The water was azeotropically removed by distillation with continual drainage of the solvent collected in the trap over 4.5 h, and alcohol was added as needed. The reaction was then heated at reflux overnight. The reaction was allowed to cool to room temperature. The solvent was removed under reduced pressure. The residue was then cooled in an ice bath, and the acid was neutralized to a pH of 10 with 1 N NaOH. The aqueous layer was extracted with Et_2O (3 × 75 mL). The combined organic fractions were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by chromatography (MeOH:$CHCl_3$, 2.5:97.5) to afford the esters **7**.

4-(4-Fluorophenyl)-1-methylpiperidine-4-carboxylic Acid Ethyl Ester (7a). General Method D. This compound was obtained as a white solid (1.0 g, 75%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 137−138 °C (HCl salt). ^{1}H NMR (free base): δ 7.36 (m, 2H), 7.00 (m, 2H), 4.12 (q, J = 6.8 Hz, 2H), 2.78 (br s, 2H), 2.57 (d, J = 10.2 Hz, 2H), 2.26 (s, 3H), 2.13 (t, J = 10.8 Hz, 2H), 1.94 (m, 2H), 1.18 (t, J = 7.2 Hz, 3H). ^{13}C NMR (free base): δ 174.1, 161.7 (J_{C-F} = 245 Hz), 138.7, 127.5, 115.3, 60.8, 53.4, 48.1, 46.2, 34.0, 14.0. Anal. ($C_{15}H_{20}NO_2F$·HCl·$^1/_3H_2O$) C, H, N.

4-(4-Chlorophenyl)-1-methylpiperidine-4-carboxylic Acid Ethyl Ester (7b). General Method D. This compound was obtained as a white solid (1.0 g, 69%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 168−169 °C (HCl salt). ^{1}H NMR (free base): δ 7.31 (m, 4H), 4.12 (m, J = 6.8 Hz, 2H), 2.77 (br s, 2H), 2.56 (d, J = 12.8 Hz, 2H), 2.27 (s, 3H), 2.13 (t, J = 11.2 Hz, 2H), 1.93 (m, J = 10.4 Hz, 2H), 1.18 (t, J = 7.2 Hz, 3H). ^{13}C NMR (free base): δ 174.0, 141.5, 132.9, 128.6, 127.3, 60.9, 53.4, 48.3, 46.2, 33.9, 14.0. Anal. ($C_{15}H_{20}NO_2Cl$·HCl) C, H, N.

4-(4-Iodophenyl)-1-methylpiperidine-4-carboxylic Acid Ethyl ester (7c). General Method D. This compound was obtained as a white solid (1.3 g, 68%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 247−249 °C. ^{1}H NMR (free base): δ 7.65 (d, J = 8.8 Hz, 2H), 7.13 (d, J = 8.4 Hz, 2H), 4.12 (m, J = 7.2 Hz, 2H), 2.78 (d, J = 8.4 Hz, 2H), 2.54 (d, J = 13.2 Hz, 2H), 2.27 (s, 3H), 2.14 (t, J = 10.8 Hz, 2H), 1.94 (t, J = 10.4 Hz, 2H), 1.18 (t, J = 7.6 Hz, 3H). ^{13}C NMR (free base): δ 173.7, 137.5, 127.8, 92.7, 60.9, 53.2, 48.3, 46.1, 33.6, 14.0. Anal. ($C_{15}H_{20}NO_2I$·HCl) C, H, N.

4-(4-Methylphenyl)-1-methylpiperidine-4-carboxylic Acid Ethyl Ester (7d). General Method D. This compound was obtained as a white solid (0.75.g, 52%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 196−197 °C (HCl salt). ^{1}H NMR (free base): δ 7.27 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 2.78 (br s, 2H), 2.57 (d, J = 13.2 Hz, 2H), 2.30 (s, 3H), 2.26 (s, 3H), 2.13 (t, J = 11.2 Hz, 2H), 1.96 (t, J = 11.2 Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H). ^{13}C NMR (free base): δ 174.3, 136.4, 129.1, 125.5, 60.6, 53.4, 48.2, 46.1, 33.8, 20.7, 13.9. Anal. ($C_{16}H_{23}NO_2$·HCl) C, H, N.

4-(3,4-Dichlorophenyl)-1-methylpiperidine-4-carboxylic Acid Ethyl Ester (7e). General Method D. This compound was obtained as a white solid (1.3 g, 76%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 195−196 °C. ^{1}H NMR (free base): δ 7.47 (d, J = 2.0 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.23 (dd, J = 2.4, 8.4 Hz, 1H), 4.14 (m, J = 6.8 Hz, 2H), 2.80 (d, J = 8.4 Hz, 2H), 2.55 (d, J = 12.8 Hz, 2H), 2.28 (s, 3H), 2.16 (t, J = 11.6 Hz, 2H), 1.94 (m, J = 11.2 Hz, 2H), 1.20 (t, J = 6.8 Hz, 3H). ^{13}C NMR (free base): δ 173.4, 132.6, 131.2, 130.4, 128.2, 125.4, 61.2, 53.2, 48.2, 46.0, 33.6, 14.0. Anal. ($C_{15}H_{19}NO_2Cl_2$·HCl) C, H, N.

1-Methyl-4-naphthalen-2-yl-piperidine-4-carboxylic Acid Ethyl Ester (7f). General Method D. This compound was obtained as a white solid (1.1 g, 73%) and converted into a hydrochloride salt (General Method A), which was obtained as a white solid, mp 191−193 °C. ^{1}H NMR (free base): δ 7.80 (m, 4H), 7.55 (dd, J = 2.0, 8.6 Hz, 1H), 7.44 (m, 2H), 4.12 (m, J = 6.8 Hz, 2H), 2.83 (d, J = 9.2 Hz, 2H), 2.70 (d, J = 12.4 Hz, 2H), 2.28 (s, 3H), 2.21 (t, J = 11.6 Hz, 2H), 2.10 (m, 2H), 1.16 (t, J = 6.8 Hz, 3H). ^{13}C NMR (free base): δ 174.3, 133.3, 132.3, 128.1,128.0, 127.3, 126.0, 125.9, 124.6, 124.0, 60.8, 53.5, 48.7, 46.2, 33.9, 14.0. Anal. ($C_{19}H_{23}NO_2$·HCl) C, H, N.

[^{3}H]WIN 35,428 Binding Assay. Male Sprague−Dawley rats (200−250 g, Taconic, Germantown, NY) were decapitated and their brains removed to an ice-cooled dish for dissection of the caudate-putamen. The tissue was homogenized in 30 volumes of ice-cold modified Krebs-HEPES buffer (15 mM HEPES, 127 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4, 10 mM glucose, pH adjusted to 7.4) using a Teflon/glass homogenizer and centrifuged at 20 000*g* for 10 min at 4 °C. The resulting pellet was then washed two more times by resuspension in ice-cold buffer and centrifugation at 20,000*g* for 10 min at 4 °C. Fresh homogenates were used in all experiments. Binding assays were conducted in modified Krebs-HEPES buffer on ice, essentially as previously described.[23] The total volume in each tube was 0.5 mL, and the final concentration of membrane after all additions was approximately 0.3% (w/v) corresponding to 150−300 g of protein/sample. Increasing concentrations of the drug being tested were added to triplicate samples of membrane suspension. Five minutes later, [^{3}H]WIN 35 428 (final concentration 1.5 nM) was added and the incubation was continued for 1 h on ice. The incubation was terminated by the addition of 3 mL of ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.1% BSA in water to reduce nonspecific binding) using a Brandel Cell Harvester (Gaithersburg, MD). After filtration, the filters were washed with three additional 3 mL washes and transferred to scintillation vials. Absolute ethanol (0.5 mL) and Beckman Ready Value Scintillation Cocktail (2.75 mL) were added to the vials which were counted the next day at an efficiency of about 36%. Under these assay conditions, an average experiment yielded approximately 6000 dpm total binding per sample and approximately 250 dpm nonspecific binding. Nonspecific binding was defined as binding in the presence of 100 μM cocaine. K_i

values were derived from 14 point competition assays using increasing concentrations of unlabeled compounds (0.05 nM to 100 μM) against 1.5 nM [^{3}H]WIN 35 428. Data were analyzed with GraphPad Prism software (San Diego, California).

[^{3}H]Dopamine Uptake Inhibition Studies: Rats were sacrificed by decapitation and their brains removed to an ice-cooled dish for dissection of the caudate-putamen. [^{3}H]Dopamine uptake was measured in a chopped tissue preparation as described previously.[39] Briefly, the tissue was chopped into 225 μm slices on a McIllwain tissue slicer with two successive cuts at an angle of 90°. The strips of tissue were suspended in oxygenated modified Krebs-HEPES buffer (see above), which was pregassed with 95% O_2/5% CO_2 and warmed to 37 °C. After rinsing, aliquots of tissue slice suspensions were incubated in buffer in glass test tubes at 37 °C to which either the drug being tested or no drug was added, as appropriate. After a 5 min incubation period in the presence of drug, [^{3}H]-dopamine (final concentration 15 nM) was added to each tube. After 5 min the incubation was terminated by the addition of 2 mL of ice-cold buffer to each tube and filtration under reduced pressure over glass fiber filters (presoaked in 0.1% polyethylenimine in water). The filters were rinsed and placed in scintillation vials to which 1 mL methanol and 2 mL 0.2 M HCl were added to extract the accumulated [^{3}H]dopamine. Radioactivity was determined by liquid scintillation spectrometry at an efficiency of approximately 30%. The reported values represent specific uptake from which nonspecific binding to filters was subtracted. Data were analyzed with GraphPad Prism software (San Diego, CA).

[^{3}H]Paroxeting Binding Assay. Brains from male Sprague−Dawley rats weighing 200−225 g (Taconic Labs) were removed, and midbrain was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25 °C), using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 50,000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 15 mg/mL. Ligand binding experiments were conducted in assay tubes containing 4.0 mL buffer for 90 min at room temperature. Each tube contained 0.2 nM [^{3}H]paroxetine (NEN) and 1.5 mg midbrain tissue (original wet weight). Nonspecific binding was determined using 1 μM citalopram. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, MD). The filters were washed twice with 5 mL cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Data were analyzed by using GraphPad Prism software (San Diego, CA).

[^{3}H]Nisoxetine Binding Assay. Frontal cortex of male Sprague−Dawley rats was removed and frozen. Membranes were prepared by homogenizing tissues in 50 mM Tris (120 mM NaCl, 5 mM KCl; pH 7.4 at 25 °C) and centrifuging (50 000*g* for 10 min at 4 °C. The resulting pellet was then washed and centrifuged two more times. The final pellet was resuspended to a concentration of 80 mg/mL (original wet weight). Assays were conducted in the above Tris buffer. Volume totaled 0.5 mL with tissue concentration of 8 mg/tube. [^{3}H]nisoxetine (specific activity 80 Ci/mmol; final concentrated 0.5 nM, New England Nuclear, Boston, MA) was added and the incubation continued for 1 h on ice. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine (PEI). Nonspecific binding was defined using 1 M desipramine. For these assays, an initial screen was conducted to assess displacement of nisoxetine at a concentration of 1 M of the unknown compound. If there was greater than 50% displacement of nisoxetine, a K_i value was determined in subsequent studies.

Cocaine Discrimination Studies. Sessions were conducted daily, 5 days per week. Five minutes before sessions ip injections were given and the subjects were placed in experimental chambers. The chambers contained two response levers, a device for delivering food pellets, and stimulus lights. Session start was indicated by illumination of the lights. Subjects were trained to press the response levers, with responses on one lever reinforced with food presentation after a presession injection of cocaine and responses on the other lever reinforced after an injection of saline. Over the course of several sessions, the number of responses required for each food pellet was gradually increased to 20. "Cocaine" and "saline" sessions alternated in a sequence of double alternations. Sessions ended after 20 food presentations or 15 min, whichever occurred first. Test sessions began once performances met a criterion for stability (greater than 85% correct responses prior to the first food presentation and throughout the entire session). Test sessions were identical to training sessions with the exception that 20 or 30 consecutive responses on either lever were reinforced.

Locomotor Activity Studies. Subjects were experimentally naive group-housed male Swiss Webster mice approximately 14 weeks old. They were studied in 40 cm^3 clear acrylic chambers which counted ambulatory activity with photoelectric detectors placed 2.56 cm apart along the walls. One activity count was registered at each instance in which the subject crossed a beam. Multiple interruptions of the same beam (e.g. grooming, head bobbing) were not counted. Mice were injected and placed into the apparatus for 60 min. Each dose was studied in six mice.

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Supporting Information Available: Microanalysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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