Expansion of Structure−Activity Studies of Piperidine Analogues of 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935) Compounds by Altering Substitutions in the N-Benzyl Moiety and Behavioral Pharmacology of Selected Molecules

Aloke K. Dutta,*,† Matthew C. Davis,†,‡ Xiang-Shu Fei,† Patrick M. Beardsley,§ Charles D. Cook,§ and Maarten. E. A. Reith||

Department of Pharmaceutical Sciences, Wayne State University, Detroit, Michigan 48202, Virginia Commonwealth University, Richmond, Virginia, and Department of Biomedical and Therapeutic Sciences, College of Medicine, University of Illinois, Peoria, Illinois 61605

Received July 10, 2001

A series of substituted N-benzyl analogues of the dopamine transporter (DAT) specific compound, 4-[2-(diphenylmethoxy)ethyl]-1-benzylpiperidine were synthesized and biologically characterized. Different 4′-alkyl, 4′-alkenyl, and 4′-alkynyl substituents were introduced in the phenyl ring of the benzyl moiety along with the replacement of the same phenyl ring by the isomeric α- and β-naphthyl groups. Different polar substitutions at the 3′- and 4′-position were also introduced. Novel compounds were tested for their binding affinity at the dopamine, serotonin, and norepinephrine transporter systems in the brain by competing for [^{3}H]WIN 35 428, [^{3}H]citalopram, and [^{3}H]nisoxetine, respectively. Selected compounds were also evaluated for their activity in inhibiting the uptake of [^{3}H]dopamine. Binding results demonstrated that alkenyl and alkynyl substitutions at the 4′-position produced potent compounds in which compound **6** with a vinyl substitution was the most potent. In vivo evaluation of three selected compounds indicated that despite their high potency at the DAT, these compounds stimulated locomotor activity (LMA) less than cocaine when tested across similar dose ranges. In a drug discrimination study procedure, none of these three compounds generalized from cocaine in mice trained to discriminate 10 mg/kg cocaine from vehicle. In a 4 h time course LMA experiment, one of our previous lead piperidine derivatives (**1a**) showed considerable prolonged action. Thus, in this report, we describe a structure−activity relationship study of novel piperidine analogues assessed by both in vitro transporter assays and in vivo behavioral activity measurements.

Introduction

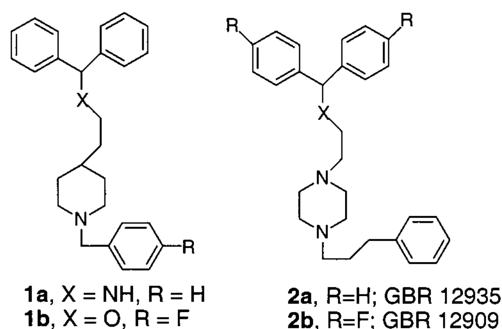
Cocaine is a naturally occurring molecule that is well-known for its strong reinforcing and abuse potential.[1,2] The development of treatments for cocaine abuse is urgently required. A number of approaches have been taken to develop medications for cocaine addiction.[3,4] Cocaine binds to all three monoamine neurotransporter systems in the brain.[5] It is now well-established that binding of cocaine to the dopamine (DA) transporter (DAT), resulting in decreased clearance of neuronally released DA, is responsible for its strong reinforcing effects.[6,7] The DA hypothesis of cocaine addiction was further supported by a series of in vivo experiments and also from molecular biology studies involving a DAT knockout (KO) mice model.[8−10] Thus, binding potencies of DA receptor agonist and DAT specific compounds correlated very well with their relative reinforcing effects in animal experiments.[7,9] In a microdialysis experiment, it was demonstrated that cocaine increases DA preferentially in the nucleus accumbens and this relates to its reinforcing effects.[11] In another recent study, the DA hypothesis for cocaine's reinforcing effects was further strengthened by the demonstration that in DAT KO mice cocaine and amphetamine increase extracellular DA in the nucleus accumbens but not in the caudate putamen.[12] This observation perhaps explains the results of a recent experiment showing self-administration of cocaine by DAT KO mice[13] as cocaine will still block norepinephrine transporter (NET), which is known to accumulate DA efficiently,[14] in DAT KO mice in nucleus accumbens and, thus, will increase the extracellular concentration of DA.[15] These results support a drug development approach targeting the dopaminergic system as a viable avenue to develop medications for cocaine addiction.[16]

Different classes of molecules have been developed for the DAT with an aim to modulate the action of cocaine. These molecules represent a wide variety of structurally diverse ligands with selective affinity for the DAT.[17,18] Our ongoing structure−activity relationship (SAR) studies with piperidine derivatives of modified GBR 12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine, **2b**) developed many potent and selective compounds for the DAT.[19−23] Many of these molecules are more selective than GBR 12909 (**2b**) and 12935 (1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)pipera-

* To whom correspondence should be addressed. Tel: 1-313-577-1064. Fax: 1-313-577-2033. E-mail: adutta@wizard.pharm.wayne.edu.
† Wayne State University.
‡ Current address: Naval Air Warfare Center, Code 4T4200D, China Lake, CA 93555.
§ Virginia Commonwealth University.
|| University of Illinois.

10.1021/jm010316x CCC: $22.00 © 2002 American Chemical Society
Published on Web 12/21/2001

Chart 1



zine, **2a**) (Chart 1).[24] Most importantly, the selectivity of these compounds was exhibited in a pharmacophoric requirement that is different from the conventional GBR molecules.[25] This led us to speculate the involvement of different binding modes for these novel piperidine analogues as compared to the conventional GBR compounds. This notion was further supported by our recent experiment with a novel photoaffinity ligand that was developed based on our piperidine analogue.[26,27] This novel DAT specific photoaffinity ligand was shown to incorporate equally well into the two different domains of the 12-transmembrane domain (Tms) DAT primary sequence, an N-terminal site containing Tms 1–2 and a second site containing Tms 4–6.[27] This result is in sharp contrast to the binding profiles of the other known photoaffinity ligands belonging to either the tropane or the GBR class of compounds, which showed specific labeling preferentially to one of those above two sites.[28,29] The dual hybrid binding profile of the piperidine-based photoaffinity ligand with the DAT indicates the presence of an unique binding profile that is different from the conventional GBR compound.

Our current photoaffinity ligand binding results are also supportive of the ongoing contention that different structural classes of DAT blockers possess nonidentical overlapping binding domains.[30,31] These nonidentical binding interactions are also reflected in their differential in vivo activity as overlapping or different binding domains may lead to changes in transporter function.[32,33] This is evident in the case of some tropane analogues and GBR derivatives that share somewhat identical binding potencies but quite different in vivo activity.[34a–b] Furthermore, cocaine and DA binding sites were shown to involve different amino acid residues on the DAT molecule as demonstrated in mutant DAT molecular experiments.[35,36] This raises the possibility of developing agents selectively targeting the cocaine binding site but sparing the DA reuptake site, which can potentially give rise to a possible cocaine antagonist. In another scenario, compounds that interact with both cocaine and DA binding sites on the DAT, either partially or completely, may potentially give rise to different degrees of indirect partial agonist or full agonist properties.

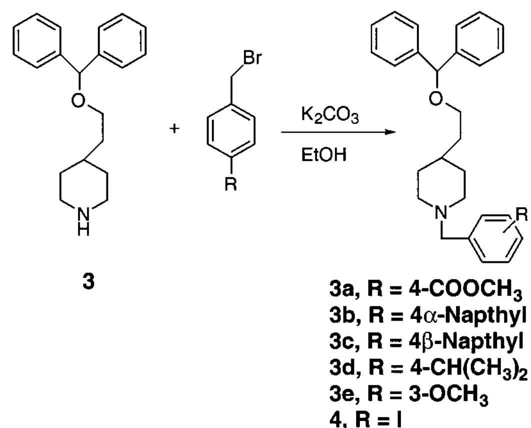
Another important aspect considered in medications development is the duration and onset of action of potential agonists or partial agonists. It is expected that a medicating agent with a long duration of action and slower onset will have less abuse liability as compared to a powerful reinforcer like cocaine, which has a much faster onset and shorter duration of action.[37,38] The validity of this approach was successfully tested in the treatment of heroin addiction by longer-acting methadone.[39] DAT selective GBR derivatives demonstrated a longer duration of action, and recently, a longer-acting decanoate ester of **2b** was shown to decrease cocaine self-administration in a sustained manner without affecting food intake.[40,41] In our own study, some of our previous lead compounds showed considerable prolong duration of action and were much longer than cocaine in this respect. A detailed study on one of our compounds is included here.

In our previous SAR studies, we have shown that the presence of electronegative and electron-withdrawing groups in the aromatic ring of the N-benzyl moiety had a significant influence on binding to the DAT. This indicated an important effect of the nature of N-benzyl moiety substitution in activity and selectivity. In our current study, we wanted to explore the effect of different alkyl, alkene, and alkyne substitutions on the phenyl ring of the N-benzyl group and also the replacement of the phenyl moiety by isomeric naphthyl groups. In addition, polar hydroxy groups and lipophilic iodine substitutions were also introduced. Such structural transformations resulted in the production of compounds with varied lipophilicity, electronic characters, and steric environments. Our objectives were to characterize the effect of these physicochemical parameters on binding activity with selected compounds on in vivo activity.

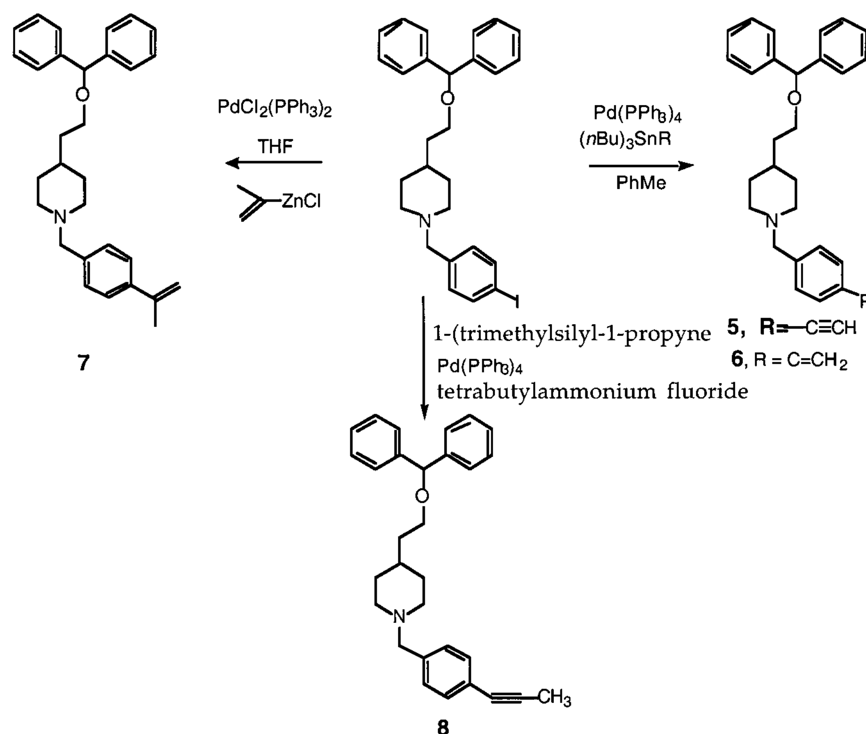
Chemistry. The starting amine (**3**) intermediate was converted into iodo compound **4** (see Scheme 1) by reacting with benzyl iodide in the presence of a base. Similarly, compounds **3a–d** were produced in 80–95% yield by an N-alkylation reaction involving compound **3** with appropriate halides in the presence of a base. This synthetic avenue provided an easy access to our final targets.

Next, compound **4** was used as a starting precursor for the preparation of compounds **5–8** by reacting with appropriate organometallic reagents in the presence of a palladium catalyst as carried out by Blough et al.[42] Thus, Stille-coupling of **4** with tributyl(ethynyl)tin or tributyl(vinyl)tin in the presence of palladium(0) catalyst in refluxing toluene yielded **5** or **6** in 80–90% yield.[43] The in situ prepared (isopropenyl)zinc chloride was reacted with **4** in the presence of dichlorobis(triphenylphosphine) palladium(II) to provide **7** in 40% yield. Last, compound **8** (see Scheme 2) was synthesized

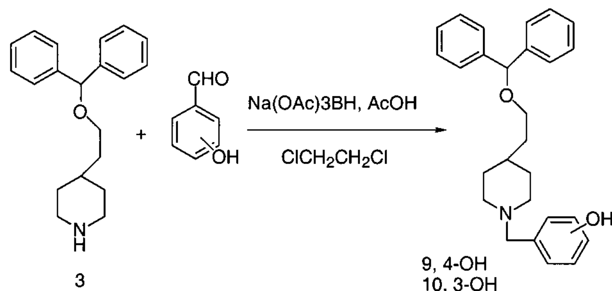
Scheme 1



Scheme 2



Scheme 3



by coupling **4** and 1-(trimethylsilyl)-1-propyne mediated by fluoride ion and palladium catalyst. This was similar to a previous method, although palladium(0) was used.[44] Reductive amination of the intermediate **3** with the appropriate hydroxy-benzaldehyde produced compounds **9** and **10** in good yield (see Scheme 3).

Results and Discussion

In our current SAR studies, we focused on structural changes on the N-substituted benzyl moiety part of our piperidine analogues. In this respect, we concentrated on introducing different alkyl, alkenyl, and alkynyl substitutions at the 4′-position of the phenyl ring along with the introduction of polar substitutions such as methoxy and hydroxy groups thereby altering lipophilic, steric, and electronic environments in these novel analogues. In addition, we also introduced in our series of compounds isomeric naphthyl substitutions by replacing the phenyl ring of the benzyl moiety, which also resulted in considerable changes of steric and lipophilic environments.

The novel iodo analogue **4**, which was used as a precursor for the synthesis of alkene and alkyne derivatives, was tested for its activity. The results demonstrated very high potency and extreme selectivity of this iodo derivative for the DAT (IC_{50}, DAT = 0.96 nM, serotonin (SERT)/DAT = 3041). In fact, compound **4** turned out to be among the few known compounds with a high potency (IC_{50} of <1 nM) that is also highly selective for the DAT (SERT/DAT > 2000).[18] The activity of this compound is interesting considering the fact that the activities of the other corresponding halides from our previous studies, with the exception of a fluoro derivative, were less active. This might be due to the fact that the introduction of a large iodo atom can potentially induce larger changes in steric and lipophilic environments as compared to other halide analogues resulting in favorable interaction.

In our next series of compounds, various 4′-alkyl-, 4′-alkenyl-, and 4′-alkynyl-substituted phenyl derivatives were synthesized. In these derivatives, we wanted to explore the effects of steric, lipophilic, and electronic environments arising from these substituents on activity. In this regard, similar studies were evaluated with the tropane class of compounds, which yielded 3β-phenyl derivatives where some of them exhibited high potencies for both DAT and SERT.[42] We wanted to compare the activities of our derivatives with those of tropane analogues. Our SAR studies demonstrated that moderately bulky isopropyl substitution as in compound **3d** was tolerated well and was quite active. The introduction of unsaturated substituents in the forms of alkenyes and alkynyes derivatives maintained a strong potency and selectivity for the DAT in most part. Compounds **5** and **6** with acetylinic and vinylic groups were the most active for the DAT (IC_{50} 4.10 and 2.96 nM, respectively) whereas isopropenyl analogue **7** was slightly less active as compared to **6** (IC_{50} 11.6 vs 2.96 nM). The activity profile of these derivatives is somewhat different from the corresponding tropane derivatives as some of the tropane derivatives exhibited equipotencies for the DAT and the SERT. In contrast, all of the present substituted

Table 1. Affinity of Drugs at the DAT, SERT, and NET in Rat Striatum and in Inhibition of DA Reuptake

compd	DAT, IC_{50}, nM, [^{3}H]WIN 35, 428[a]	SERT, IC_{50}, nM, [^{3}H]citalopram[a]	NET, IC_{50}, nM, [^{3}H]nisoxetine[a]	[^{3}H]DA uptake inhibition, IC_{50}, nM[a]
cocaine	266 ± 37	737 ± 160	3,530 ± 554	
GBR 12909 (**2b**)	10.6 ± 1.9	132 ± 0	496 ± 22	6.63 ± 0.43
1a	4.50 ± 0.64[b]	1560 ± 210	2620 ± 170	20.6 ± 2.5
3a	8.23 ± 5.49	924 ± 100		1.94 ± 0.31
3b	113 ± 5	4160 ± 900	1490 ± 60	
3c	26.9 ± 3.0	856 ± 210	393 ± 66	
3d	63.1 ± 11.2	1540 ± 240	1060 ± 240	
3e	19.6 ± 2.6	1080 ± 260	472 ± 94	6.68 ± 1.14
4	0.96 ± 0.16	2920 ± 430	1150 ± 120	2.01 ± 0.64
5	4.10 ± 1.30	1040 ± 160	760 ± 212	4.81 ± 1.16
6	2.96 ± 0.61	1470 ± 350	715 ± 37	1.08 ± 0.28
7	11.6 ± 1.8	1030 ± 90	832 ± 28	3.10 ± 1.12
8	65.1 ± 15.0	2410 ± 510	1170 ± 120	
9	240 ± 15	4330 ± 750	3340 ± 760	
10	14.3 ± 3.7	984 ± 94	678 ± 37	10.4 ± 2.2

[a] The DAT was labeled with [^{3}H]WIN 35, 428, the SERT with [^{3}H]citalopram, and the NET with [3H]nisoxetine. Results are the average ± SEM of three independent experiments assayed in triplicates. [b] See ref 23.

Table 2. Selectivity of Various Ligands for Their Activity at Monoamine Transporters

compd	SERT binding/DAT binding	NET binding/DAT binding	[3H]DA uptake/DAT binding
GBR 12909 (**2b**)	12	47	0.62
3a	110		0.23
3b	37	13	
3c	32	15	
3d	24	17	
3e	55	24	0.34
4	3040	1200	2.1
5	250	180	1.2
6	500	240	0.36
7	88	72	0.26
8	37	18	
9	18	14	
10	69	47	0.72

analogues maintained high selectivity for the DAT. Interestingly, it was observed that the terminal H-atom in the acetylinic group of **5** is necessary for potent activity as the replacement of it by a methyl group led to a weaker congener (**8**, IC_{50} 65 vs 4.10 nM). The apparent loss of high potency may be due to the presence of a methyl group imposing steric bulk in the interaction or absence of a relatively acidic acetylinic H-atom. In general, unsaturated alkenyl and alkynyl substitutions were more potent than a simple alkyl substitution.

We observed differential activities in the isomeric (α and *β*) naphthalene derivatives (**3b** and **c**), which might reflect an important contribution of electrostatic interaction differently contributed by these two isomeric compounds. *β*-Naphthalene substitution was found to be more active for the DAT as compared to α-naphthalene (IC_{50} 26.9 vs 113 nM). In our final molecular design, the introduction of polar groups as in compounds **3e**, **9**, and **10** produced a mixture of activities in these molecules. Compound **3e** and its demethylated analogue **10** were active, but interestingly, the 4-hydroxy analogue **9** was not active.

Potent selected compounds were next tested for their activity in inhibiting the DA reuptake process. Overall uptake and binding correlated quite well, with uptake and binding values being less than 4-fold different. The general tendency for higher uptake than binding activity could be a reflection of assay conditions that were not identical in the uptake and binding measurements. Compounds **5**, **7**, and **10** were chosen for further evaluation in in vivo studies. As a part of our goal to develop long-acting molecules for the treatment of cocaine addiction, we additionally evaluated one of our previous lead compounds, **1a**, in extended 4 h locomotor tests and compared it to cocaine.[23]

As shown in Figure 1, cocaine and GBR 12909 (**2b**) produced dose-dependent increases in total distance travelled with significant increases relative to the vehicle control obtained at doses of 10 and 30 mg/kg.[23] These data are presented for comparison purposes and were taken from a previous study.[23] Across the dose range tested, compound **10** failed to alter locomotor activity (LMA) relative to the vehicle controls ($F_{3,28}$ = 1.50, $P > 0.05$). The overall analysis of variance (ANOVA) for compound **5** was significant ($F_{4,35}$ = 3.92, $P < 0.05$); however, Dunnett's post-hoc tests indicated that doses of this compound were not significantly different from the vehicle control. The overall ANOVA for compound **7** was significant ($F_{3,28}$ = 4.00, $P < 0.05$), with doses of 10 and 30 mg/kg significantly increasing total distance traveled relative to the vehicle control. The maximal increase in activity produced by compound **7** was approximately 170% of the vehicle control, whereas cocaine (30 mg/kg) produced maximal increases of approximately 450% of the vehicle control.

Figure 2 shows the 4 h time course LMA experiment. A repeated measures ANOVA indicated a main effect for dose ($F_{2,20}$ = 8.56, $P < 0.05$) and a dose by time interaction ($F_{46,460}$ = 19.23, $P < 0.05$). Examination of the effect of dose within each 10 min interval indicated that cocaine produced stimulant effects greater than the vehicle during intervals 1–7 ($P < 0.05$). Compound **1a** also produced stimulant effects greater than the vehicle during intervals 6 and 7 ($P < 0.05$). Stimulant effects (relative to vehicle) were observed with compound **1a** during intervals 9–24 ($P < 0.05$), whereas cocaine produced effects similar to the vehicle ($P > 0.05$). In our earlier report, this compound was described as exhibiting a locomotor depressant effect when studied within a 1 h time period.[23] The depressant effect was more pronounced at a higher dose (100 mg/kg). Because a longer duration LMA effect for this compound was not evaluated previously, we now wanted to observe this effect at a single dose.

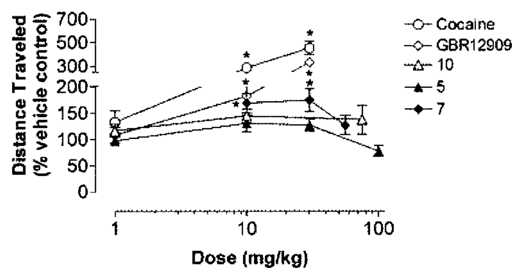


Figure 1. Effects of cocaine, GBR 12909 (**2b**), and compounds **10** ($n = 8$), **5** ($n = 8$), and **7** ($n = 8$) on total distance traveled (cm) in mice during a 60 min test session expressed as a percentage of each individual drug's vehicle control. Brackets indicate SEM; where not indicated, the SEM fell within the data point. * = $P \leq 0.05$ relative to saline control. LMA data for cocaine were taken from Dutta et al. (2001) and are presented for comparison.

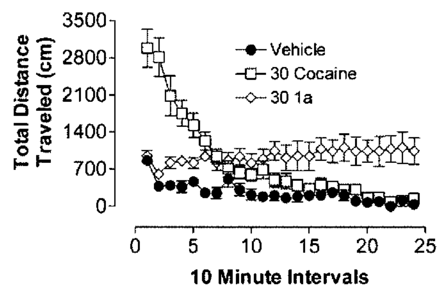


Figure 2. Locomotor time-course effects for vehicle, 30 mg/kg cocaine, and 30 mg/kg compound **1a** over the 240 min (4 h) test session.

Test compounds were administered prior to the start of the discrimination test sessions coinciding with times of obvious activity indicated during LMA tests. Additionally, we tested all compounds with doses in which at least one dose decreased response rates suggesting that the tested dose ranges included behaviorally active doses. As shown in Figure 3, cocaine produced dose-dependent increases in cocaine lever responding. The rate of responding was decreased by approximately 50% relative to saline control levels at the highest dose of cocaine tested (30 mg/kg). Both compounds **10** and **5** maximally produced 30% cocaine lever responding. At a dose of 75 mg/kg of compound **10**, two mice responded 40 and 56% on the cocaine lever, whereas in one mouse 87% cocaine lever responding was obtained. In the remaining three mice, cocaine lever responding was less than 3%. At a dose of 100 mg/kg of compound **5**, two mice responded greater than 90% on the cocaine lever, whereas the remaining four mice responded less than 2% on the cocaine lever. Across the doses tested, **7** produced mainly saline lever responding. With compounds **10**, **5**, and **7**, the rate of responding was slightly depressed at the highest dose tested. Thus, our in vivo results indicated that the relatively nonpolar alkenyl- and alkynyl-substituted compounds **5** and **7** were more active in locomotor stimulation than the relatively polar hydroxy-substituted compound **10**. In this regard, previous studies indicated that certain structurally related benztropine analogues exhibited much less locomotor stimulant properties and did not generalize in a drug discrimination paradigm while exhibiting high potency for the DAT.[45] However, **2a** and **2b** were reported to reproduce a discriminative stimulus effect of cocaine.[46]

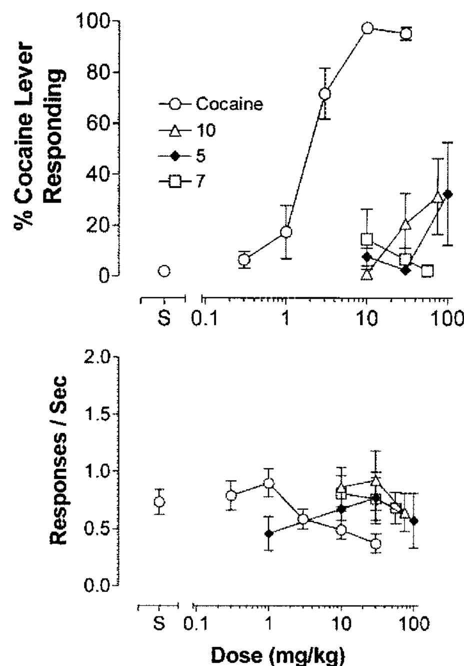


Figure 3. Top frames: effects of cocaine ($n = 12$) and compounds **10** ($n = 6$), **5** ($n = 6$), and **7** ($n = 7$) on the percentage of lever presses emitted upon the cocaine-associated lever and rate of responding in mice trained to discriminate 10 mg/kg cocaine from saline. Brackets indicate SEM; where not indicated, the SEM fell within the data point. Bottom frames: response rate expressed as mean lever presses per second. Each symbol represents the mean number of lever presses emitted during the 15 min test session. Brackets indicate SEM.

Conclusion

In our current study, we have demonstrated that alkyl, alkenyl, and alkynyl substitutions at the 4-position in the phenyl ring of the benzyl group were tolerated well. Among those derivatives, compounds **5** and **6** were the most potent for the DAT. The iodo analogue **4** turned out to be among the few compounds known to date that combine very high potency and selectivity for the DAT, and it will therefore be important to behaviorally characterize it in future experiments. In in vivo studies, compound **7** exhibited more stimulant effects than the other two compounds but was much less stimulating than cocaine at identical doses. In drug discrimination tests, compound **7** did not generalize with the cocaine discriminative stimulus. In an extended 4 h time course LMA study, compound **1a** showed a prolonged stimulant action that was significantly different from cocaine.

Experimental Section

Analytical silica gel-coated thin-layer chromatography (TLC) plates (Si 250F) were purchased from Baker, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid. Flash chromatography was carried out on Baker Silica Gel 40 mM. ^{1}H nuclear magnetic resonance (NMR) spectra were routinely obtained at GE-300 MHz Fourier transform NMR. The NMR solvent used was $CDCl_3$ as indicated. Tetramethylsilane was used as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. and were within ±0.4% of the theoretical value.

[^{3}H]WIN 35 428 (86.0 Ci/mmol), [^{3}H]nisoxetine (80.0 Ci/mmol), and [^{3}H]dopamine ([^{3}H]DA, 48.2 Ci/mmol) were obtained from Dupont-New England Nuclear (Boston, MA). [^{3}H]citalopram (85.0 Ci/mmol) was from Amersham Pharmacia

Biotech Inc. (Piscataway, NJ). Cocaine hydrochloride was purchased from Mallinckrodt Chemical Corp. (St. Louis, MO). WIN 35 428 naphthalene sulfonate was purchased from Research Biochemicals, Inc. (Natick, MA). (−)-Cocaine HCl was obtained from the National Institute on Drug Abuse. GBR 12909 dihydrochloride (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine) was purchased from SIGMA-Aldrich (#D-052; St. Louis, MO).

Procedure A. Synthesis of 4-[2-(Diphenylmethoxy)-ethyl]-1-[(4-methoxycarbonylphenyl)-methyl]piperidine (3a). A mixture of 4-[2-(diphenylmethoxy)ethyl]piperidine (0.23 g, 0.76 mmol), 175 mg of methyl 4-(bromomethyl)benzoate (0.17 g, 0.76 mmol), and powdered K_2CO_3 (0.15 g, 1.1 mmol, 1.5 equiv) in 3 mL of EtOH was refluxed for 1 h. The solvent was evaporated, and the residue was chromatographed (SiO_2; CH_2Cl_2/MeOH) to yield the free base (0.23 g, 80%) as an oil that slowly crystallized (mp 91−3 °C; hexane/Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.2−2.0 (9H, m), 2.83 (2H, bd, J = 11.1 Hz), 3.4−3.6 (4H, m), 3.91 (3H, s), 5.31 (1H, s), 7.07 (2H, d, J = 7.8 Hz), 7.2−7.39 (10H, m), 7.39 (2H, d, J = 7.8 Hz), 7.97 (2H, d, J = 7.8 Hz). The free base was converted into its oxalate salt; mp 129−133 °C; Anal. $C_{31}H_{35}NO_7$.

4-[(2-Diphenylmethoxy)ethyl]-1-(1-naphthylmethyl)piperidine (3b). 4-[(2-Diphenylmethoxy)ethyl]piperidine (0.18 g, 0.61 mmol) was reacted with 2-bromo methylnaphthalene (0.21 g, 0.95 mmol) and K_2CO_3 (0.85 g, 0.61 mmol) in EtOH (10 mL) to give a thick oil, 0.25 g (96%), procedure A. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22−1.33 (2H, m), 1.48−1.53 (1H, m), 1.58−1.66 (4H, m), 1.66−2.02 (2H, dt, J = 1.6, 11.2 Hz, $N(CH)_2$), 2.89−2.92 (2H, bd, J = 11.2 Hz, $N(CH)_2$), 3.47−3.50 (2H, t, J = 6.4 Hz, OCH_2), 3.65 (2H, s, NCH_2Ar), 5.32 (1H, s, Ph_2CHO), 7.22−7.35 (10H, m, ArH), 7.43−7.51 (3H, m, ArH), 7.74 (1H, s, ArH), 7.81 (2H, m, ArH). Free base was converted into its oxalate salt; mp = 194−195 °C; Anal. $C_{31}H_{33}NO$, $(COOH)_2$.

4-[(2-Diphenylmethoxy)ethyl]-1-(2-naphthylmethyl)piperidine (3c). 4-[(2-Diphenylmethoxy)ethyl]piperidine (0.11 g, 0.37 mmol) was reacted with 1-chloro methylnaphthalene (0.12 g, 0.68 mmol) and K_2CO_3 (0.52 g, 3.77 mmol) in EtOH (10 mL) to give a thick oil, 0.14 g (86%), procedure A. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20−1.29 (2H, m), 1.51−1.54 (1H, m), 1.55−1.63 (4H, m), 2.01−2.07 (2H, t, J = 12 Hz, $N(CH)_2$), 2.91−2.95 (2H, bd, J = 12 Hz, $(NCH)_2$), 3.88 (2H, s, NCH_2Ar), 5.33 (1H, s, Ph_2CHO), 7.23−7.55 (13H, m, ArH), 7.76−7.78 (1H, d, J = 8.0 Hz, ArH), 7.84−7.87 (1H, d, J = 9.6 Hz, ArH), 8.31−8.33 (1H, d, J = 8.0 Hz, ArH). Free base was converted into its oxalate salt; mp = 158−160 °C; Anal. $C_{31}H_{33}NO$, $(COOH)_2$.

4-[(2-Diphenylmethoxy)ethyl]-1-[((4-isopropyl)phenyl-)methyl]piperidine (3d). A mixture of 4-[(2-diphenylmethoxy)-ethyl]piperidine (0.18 g, 0.61 mmol), 4-isopropylbenzyl chloride (0.19 g, 1.13 mmol), and K_2CO_3 (0.85 g, 6.16 mmol) in EtOH (10 mL) was stirred at 50 °C for 6 h. The mixture was filtered, and the organic phase was evaporated to give the crude product, which was purified by flash chromatography (EtOAc/Hexane = 1/5) to give a thick oil, 0.21 g (80%), procedure A. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21−1.28 (2H, m), 1.23−1.25 (6H, d, J = 6.4 Hz, $(CH_3)_2CH$), 1.42−1.50 (1H, m), 1.56−1.61 (4H, m), 1.87−1.93 (2H, t, J = 11.2 Hz, $N(CH)_2$), 2.83−2.90 (3H, m, $N(CH)_2$, $PhCH(CH_3)_2$), 3.44 (2H, s, NCH_2Ar), 3.45−3.48 (2H, t, J = 6.4 Hz, $-OCH_2$), 5.31 (1H, s, Ph_2CHO), 7.15−7.34 (14H, m, ArH). Free base was converted into its oxalate salt; mp = 118−120 °C; Anal. $C_{30}H_{37}NO$, $(COOH)_2\cdot0.2H_2O$.

4-[(2-Diphenylmethoxy)ethyl]-1-[((3-methoxyl)phenyl)-methyl]piperidine(3e). 4-[(2-Diphenylmethoxy)ethyl]piperidine (0.15 g, 0.50 mmol) was reacted with 3-methoxy benzyl bromide (0.13 g, 0.65 mol) and K_2CO_3 (0.52 g, 3.77 mmol) in EtOH (10 mL) to give a thick oil, 0.18 g (86%), procedure A. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20−1.30 (2H, m), 1.46−1.50 (1H, m), 1.51−1.64 (4H, m), 1.87−1.93 (2H, t, J = 12 Hz, $N(CH)_2$), 2.84−2.87 (2H, bd, J = 12 Hz, $N(CH)_2$), 3.46−3.50 (4H, m, OCH_2, NCH_2Ph), 3.81 (3H, s, OCH_3), 5.33 (1H, s, Ph_2CHO), 6.79−6.81 (1H, d, J = 8.0 Hz, ArH), 6.90−6.92 (1H, d, J = 8.0 Hz, ArH), 7.21−7.36 (12H, m, ArH). Free base was converted into its oxalate salt; mp = 163−165 °C; Anal. $C_{28}H_{33}NO_2$, $(COOH)_2$.

Synthesis of 4-[2-(Diphenylmethoxy)ethyl]-1-[(4-iodophenyl)methyl]piperidine (4). 4-[(2-Diphenylmethoxy)-ethyl]piperidine (0.23 g, 0.77 mmol) was reacted with 4-iodo benzyl bromide (0.76 g, 2.5 mmol) and K_2CO_3 (1.00 g, 7 mmol) in EtOH (10 mL) to give a thick oil, 0.17 g (46%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21−1.63 (7H, m), 1.87−1.93 (t, J = 11 Hz, 2H, $N(CH_2)-$), 2.79−2.83 (bd, J = 12 Hz, 2H, $N(CH_2)-$), 3.41−3.49 (4H, m), 5.31 (s, 1H, Ph_2CHO-), 7.05−7.63 (14H, m, ArH). Free base was converted into its oxalate salt; mp = 160−161 °C; Anal. $C_{27}H_{30}INO$, $(COOH)_2$.

Synthesis of 4-[2-(Diphenylmethoxy)ethyl]-1-[(4-ethynylphenyl)methyl]piperidine (5). To a solution of 4-[2-(diphenylmethoxy)ethyl]-1-[(4-iodophenyl)methyl]piperidine (0.23 g, 0.496 mmol) in toluene (10 mL) under an N_2 atmosphere were added tetrakis(triphenylphosphine)palladium(0) (30 mg, 0.026 mmol) and tributyl(ethynyl)tin (0.17 g, 0.546 mmol, 1.1 equiv), and the mixture was refluxed. After 3 h, the color was very dark, and the reaction was completed by TLC (4:1; hex:acetone). The solvent was evaporated, and the residue was chromatographed to yield 0.162 g (90%) of the free base as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.2−1.3 (2H, m), 1.4−1.65 (5H, m), 1.9−2.0 (2H, m), 2.84 (2H, bd, J = 11.2 Hz), 3.06 (1H, s), 3.4−3.5 (4H, m), 5.32 (1H, s), 7.2−7.35 (12H, m), 7.44 (2H, d, J = 8 Hz). The free base was converted into its oxalate salt; mp 166−170 °C; Anal. $C_{31}H_{33}NO_5\cdot H_2O$.

Synthesis of 4-[2-(Diphenylmethoxy)ethyl]-1-[(4-vinylphenyl)methyl]piperidine (6). To a solution of 4-[2-(diphenylmethoxy)ethyl]-1-[(4-iodophenyl)methyl]piperidine (0.2 g, 0.384 mmol) in toluene (10 mL) under a nitrogen atmosphere were added tetrakis(triphenylphosphine)palladium(0) (30 mg, 0.026 mmol) and tributyl(vinyl)tin (0.12 g, 0.38 mmol, 1 equiv), and the mixture was refluxed. After 4 h, the color was very dark, and the reaction was completed by TLC (4:1; hexane: acetone). The mixture was filtered through Celite, then the solvent was evaporated, and the residue was chromatographed to yield 0.14 g (86%) of the free base as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.2−2.1 (9H, m), 2.93 (2H, bs), 3.46 (2H, t, J = 6.4 Hz), 3.59 (2H, bs), 5.23 (1H, d, J = 11.6 Hz), 5.29 (1H, s), 5.74 (1H, d, J = 17.6 Hz), 6.7 (1H, dd, J = 11.2 and 16 Hz), 7.19−7.4 (14H, m). The free base was converted into its oxalate salt; mp 136−141 °C; Anal. $C_{31}H_{35}NO_5\cdot3/4H_2O$.

Synthesis of 4-[2-(Diphenylmethoxy)ethyl]-1-[(4-(2-propenyl)phenyl)methyl]piperidine (7). To a stirred solution of 2-bromopropene (0.69 g, 5.76 mmol, 3 equiv) in 4 mL of tetrahydrofuran (THF) at −78 °C was added dropwise 2.88 mL of 2 M n-BuLi in pentane (17.28 mmol, 3 equiv). After the solution was stirred for 15 min, 3.84 mL of 0.5 M ZnCl in THF (17.28 mmol, 3 equiv) was added and the solution remained stirring at −78 °C for a further 30 min. Then, 4-[2-(diphenyl-methoxy)ethyl]-1-[(4-iodophenyl)methyl]piperidine (1 g, 1.92 mmol) dissolved in 2 mL of THF was added dropwise to the solution followed by dichlorobis(triphenylphosphine) palladium(II) (100 mg, 0.14 mmol). The mixture was stirred at −78 °C for 2 h, and then, the cooling bath was removed and allowed to warm and stir at room temperature for 12 h. The solvent was evaporated, and the residue was chromatographed (SiO_2; hexane:acetone) to furnish the free base (0.33 g, 40%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.2−2.0 (9H, m), 2.8−2.9 (2H, bs), 3.46 (2H, t, J = 6.8 Hz), 3.55 (2H, bs), 5.07 (1H, s), 5.30 (1H, s), 5.36 (1H, s), 7.2−7.35 (13H, m), 7.42 (1H, d, J = 8.4 Hz). The free base was converted into oxalate salt; mp 128−132 °C; Anal. $C_{32}H_{35}NO_5\cdot1.5\ H_2O$.

Synthesis of 4-[2-(Diphenylmethoxy)ethyl]-1-[(4-propynylphenyl)methyl]piperidine (8). A mixture of 4-[2-(diphenylmethoxy)ethyl]-1-[(4-iodophenyl)methyl]piperidine (0.5 g, 0.96 mmol), 1-(trimethylsilyl)-1-propyne (0.12 g, 1.15 mmol, 1.2 equiv), tetrakis(triphenylphosphine)palladium(0) (110 mg, 10 mol %), tetrabutylammonium fluoride (0.25 g, 1.2 equiv), and 52 mg of ethanol (1.2 equiv) in 10 mL of THF was heated to 50 °C for 2 h. Saturated K_2CO_3 was added, and the mixture was extracted thrice with CH_2Cl_2. These were collected, dried ($MgSO_4$), and evaporated to a brown oil. The oil was chro-

matographed (SiO_2) eluting with CH_2Cl_2 to remove a dark-colored material and changing (hexane/EtOAc/MeOH) to elute the title compound (140 mg, 41%) as a light brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.2–1.3 (2H, m, H-3ax), 1.4–1.5 (1H, m, H-4), 1.56–1.65 (4H, m, CH_2CH_2O, H-3eq), 1.92 (2H, t, J = 9.6 Hz, H-2ax), 2.05 (3H, s, CH_3), 2.82 (2H, d, J = 11.2 Hz, H-2eq), 3.4–3.5 (4H, m, CH_2O, NCH_2Ar), 5.32 (1H, s, $CH(C_6H_5)_2$), 7.2–7.35 (14H, m, ArH). The compound was precipitated with oxalic acid dihydrate; mp 80–120 °C; Anal. $C_{32}H_{35}NO_5 \cdot 1.75\ H_2O$.

4-[(2-Diphenylmethoxy)ethyl]-1-[((4-hydroxy)phenyl)-methyl]piperidine (9). A mixture of 4-[(2-diphenylmethoxy)-ethyl]piperidine (0.22 g, 0.74 mmol), 4-hydroxybenzaldehyde (0.10 g, 0.82 mmol), and sodium triacetoxyborohydride (0.31 g, 1.47 mmol) in $ClCH_2CH_2Cl$ (10 mL) was stirred at room temperature overnight. After the solvent was removed, the residue was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ and brine. The solution was dried over Na_2SO_4 and evaporated under vacuo to give the crude product, which was purified with flash chromatography (EtOAc/MeOH = 1/1) to give a solid, 0.26 g (86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20–1.28 (m, 2H), 1.43–1.66 (m, 5H), 1.93–2.00 (t, J = 11 Hz, 2H, $N(CH)_2$), 2.90–2.94 (d, J = 11 Hz, 2H, $N(CH)_2$), 3.41 (s, 2H, NCH_2Ph), 3.44–3.48 (t, J = 6.6 Hz, 2H, OCH_2), 5.30 (s, 1H, Ph_2CHO), 6.63–6.65 (d, J = 7.8 Hz, 2H, Ar–H), 7.08–7.11 (d, J = 7.8 Hz, 2H, Ar–H), 7.23–7.34 (m, 10H, Ar–H). Free base was converted into its oxalate salt; mp 220–222 °C; Anal. [$C_{27}H_{31}NO_2 \cdot 0.5(CO_2H)_2 \cdot 0.31H_2O$].

4-[(2-Diphenylmethoxy)ethyl]-1-[((3-hydroxy)phenyl)-methyl]piperidine (10). 4-[(2-Diphenylmethoxy)ethyl]piperidine was reacted with 3-hydroxybenzaldehyde and sodium-triacetoxyborohydride in $ClCH_2CH_2Cl$ to give 0.18 g (83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.28 (m, 2H), 1.43–1.65 (m, 5H), 1.99–2.11 (t, J = 11 Hz, 2H, $N(CH)_2$), 2.95–2.98 (d, J = 11 Hz, $N(CH)_2$), 3.42–3.46 (t, J = 5.7 Hz, 2H, OCH_2), 3.48 (s, 2H, NCH_2Ph), 5.29 (s, 1H, Ph_2CHO), 6.68–6.76 (m, 2H, Ar–H), 7.07–7.12 (d, J = 7.5 Hz, 1H, AR–H), 7.22–7.31 (m, 10H, ArH). Free base was converted into its oxalate salt; mp 161–161 °C; Anal.$C_{27}H_{31}NO_2 \cdot (CO_2H)_2$.

Transporter Binding Assays. The affinity of test compounds for the rat DAT, SERT, and NET was assessed by the inhibition of binding of [^{3}H]WIN 35 428, [^{3}H]citalopram, and [^{3}H]nisoxetine, respectively, exactly as described by us previously.[23] Briefly, rat striatum was the source for DAT, and cerebral cortex was the source for SERT and NET (see Table 1). The final [Na^+] was 30 mM for DAT and SERT assays and was 225 mM for NET assays. All binding assays were conducted at 0–4 °C, for a period of 2 h for [^{3}H]WIN 35 428 and [^{3}H]citalopram binding and 3 h for [^{3}H]nisoxetine binding. Nonspecific binding of [^{3}H]WIN 35 428 and [^{3}H]citalopram binding was defined with 100 μM cocaine and that of [^{3}H]-nisoxetine binding with 1 μM desipramine. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted out in 10% (v/v) DMSO. Additions from the latter stocks resulted in a final concentration of DMSO of 0.5%, which by itself did not interfere with radioligand binding. At least five triplicate concentrations of each test compound were studied, spaced evenly around the IC_{50} value.

DAT Uptake Assays. Uptake of [^{3}H]DA into rat striatal synaptosomes was measured exactly as described by us previously.[23] Briefly, rat striatal P_2 membrane fractions were incubated with the test drug for 8 min in uptake buffer (for composition see ref 47) followed by the additional presence of [^{3}H]DA for 4 min at 25 °C. Nonspecific uptake was defined with 100 μM cocaine. Construction of inhibition curves and dissolvement of test compounds were as described above.

LMA. Subjects. Adult male Swiss Webster mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 30–35 g were used. Mice were housed five per cage, had continuous access to food and water, and were allowed to acclimate to the vivarium environment 1 week prior to the start of any experiment. The mice were housed in an AALAC-accredited animal facility with a controlled temperature (22–24 °C) on a 12 h light–dark cycle, and all testing occurred during the light component.

Apparatus and Procedure. The LMA chambers and procedure have been described in detail elsewhere.[23] Briefly, four automated activity monitoring chambers were used that permitted the simultaneous recording of two mice. Mice were injected with the vehicle or test compound and placed immediately into the test chambers where their activity was recorded for 60 or 240 min. The total distance traveled (cm) during the experiment session was recorded for each mouse. The raw data at each test dose were converted to a percentage of the vehicle control total distance traveled (60 min session only). A one-factor (dose) ANOVA was conducted on each drug, and its respective vehicle was followed by Dunnett's post-hoc tests when the overall ANOVA was significant (60 min session). A repeated measures ANOVA was conducted on a drug dose (between subjects factor) and 10 min interval (within subjects factor) followed by Dunnett's post-hoc tests when the overall ANOVA was significant (240 min session). Next, simple effects analyses were conducted at each 10 min interval to examine the effect of drug dose. The α-level for all comparisons was set at 0.05.

Drugs. Cocaine (National Institute of Drug Abuse) was dissolved in 0.05% sterile saline. Compounds **5**, **7**, and **10** were dissolved in Alkamuls EL-620:ethanol:water in a 1:1:18 ratio. All drugs were administered by the intraperitoneal (ip) route in a volume of 10 mL/kg.

Drug Discrimination. Subjects. Adult male Swiss Webster mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used. Mice were individually housed, maintained on a 12 h light–dark cycle, and provided with continuous access to water. Training and testing occurred during the light component. Mice were maintained at 35 ± 5 g by supplemental postsession feedings.

Apparatus and Procedure. The drug discrimination chambers and procedure have been described in detail elsewhere.[23] Briefly, experimental sessions were conducted in light- and sound-attenuated chambers equipped with two response levers separated by a trough into which a 0.01 mL dipper cup could be presented. Each chamber was equipped with a house light.

Mice were initially trained to press one of the two levers under a fixed ratio 1 FR1 schedule of sweetened condensed milk. The response requirement was gradually increased to FR20. Subsequently, the mice were reinforced for pressing the opposite lever until reliable responding was obtained under FR20 conditions. Discrimination training occurred daily (Monday–Friday) in 15 min experimental sessions. Mice were injected with cocaine (10 mg/kg) or saline ip 10 min prior to the start of the session. The cocaine- and saline-associated levers were counterbalanced across the mice. Responses on the injection appropriate lever resulted in delivery of the sweetened milk solution. Responses on the injection inappropriate lever resulted in resetting the response requirement on the correct lever. A pseudo-random schedule was used to determine which injection was administered, with the restriction that the same injection was not given on more than two consecutive sessions and over 30 training sessions the number of saline and cocaine injections were approximately equal.

Testing commenced when (i) a mouse completed the first fixed ratio (FFR) on the correct lever on at least 8 or 10 consecutive days and (ii) at least 80% of the total responses were made on the correct lever during those eight sessions. Tests were conducted on Tuesdays and Fridays, provided that the mouse completed the FFR on the correct lever during the most recent cocaine and saline training sessions; otherwise, a training session was administered. During test days, responding on either lever was reinforced with milk.

Analyses. The percentage of responses on the cocaine lever was calculated for each mouse during training and test sessions by dividing the number of lever presses emitted upon the cocaine lever by the total number of lever presses emitted on both levers, and then, this quotient was multiplied by 100. Additionally, the rate of responding was calculated for each

mouse by dividing the total number of responses emitted on both levers by 900 s. Individual cocaine lever responding percentages and responses per second were then averaged (± the standard error of the mean (SEM)). If a mouse failed to complete a FFR, then its data was excluded from calculations of mean cocaine lever responding but were included for mean response rate calculations.

Drugs. Cocaine HCl (National Institute of Drug Abuse) was dissolved in 0.05% sterile saline. Compounds **5**, **7**, and **10** were dissolved in Alkamuls EL-620:ethanol:water in a 1:1:18 ratio. All drugs were administered by the ip route in a volume of 10 mL/kg at a time prior to the start of the test sessions coinciding with a time of obvious activity indicated during LMA tests. Both **5** and **7** were administered 20 min prior to the session start, and **10** was administered 30 min prior to the session start.

Acknowledgment. This work was supported by the National Institute on Drug Abuse, Grant No. DA 12449 (A.K.D.).

References

(1) Substance Abuse and Mental Health Services Administration National Household Survey on Drug Abuse: Main Findings 1997; Department of Health and Human Services: Washington, D.C., 1998.

(2) Johanson, C. E.; Schuster, R. S. Cocaine. In *Psychopharmacology: the Fourth Generation of Progress*; Bloom, F. E., Kupfer, D. J., Eds.; Raven Press: New York, 1995; pp 1685–1697.

(3) McCance, E. F. Overview of potential treatment medications for cocaine dependence. *NIDA Res. Monogr.* **1997**, *175*, 36–72.

(4) Carroll, F. I.; Howell, L. L.; Kuhar, M. J. Pharmacotherapies for Treatment of Cocaine Abuse: Preclinical Aspects. *J. Med. Chem.* **1999**, *42*, 2721–2736.

(5) Ritz, M. C.; Cone, E. J.; Kuhar, M. J. Cocaine inhibition of ligand binding at dopamine, norpinephrine and serotonin transporters: A structure–activity study. *Life Sci.* **1990**, *46*, 635–645.

(6) Ritz, M. C.; Lamb, R. J.; Goldberg, R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **1987**, *237*, 1219–1223.

(7) Spealman, R. D.; Madras, B. K.; Bergman, J. Effects of Cocaine and Related Drugs in Nonhuman Primates. II. Stimulant Effects on Schedule-Controlled Behavior. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 142–149.

(8) (a) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* **1991**, *14*, 299–302. (b) Koob, G. F.; Bloom, F. E. Cellular and molecular mechanisms of drug dependence. *Science* **1988**, *242*, 715–723.

(9) Witkins, J. M.; Nichols, D. E.; Terry, P.; Katz, J. L. Behavioral effects of selective dopaminergic compounds in rats discriminating cocaine injections. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 706–713.

(10) Giros, B.; Jaber, M.; Jones, S. R.; Wightman, R. M.; Caron, M. G. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **1996**, *379*, 606–612.

(11) Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5274–5278.

(12) Carboni, E.; Spielewoy, C.; Vacca, C.; Nosten-Bertrand, M.; Giros, B.; Di Chiara, G. Cocaine and Amphetamine Increase Extracellular Dopamine in the Nucleus Accumbens of Mice Lacking the Dopamine Transporter Gene. *J. Neurosci.* **2001**, *21*, RC141.

(13) Rocha, B. A.; Fumagalli, F.; Gainetdinov, R. R.; Jones, S.; Ator, R.; Giros, B.; Miller G. W.; Caron, M. Cocaine self-administration in dopamine transporter knockout mice. *Nat. Neurosci.* **1998**, *1*, 132–137.

(14) Yamamoto, B. K.; Novotney, S. Regulation of Extracellular Dopamine by the Norepinephrine Transporter. *J. Neurochem.* **1998**, *71*, 274–280.

(15) Eshleman, A. J.; Carmolli, M.; Cumbay, M.; Martens, C. R.; Neve, K. A.; Janowsky, A. Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 877–885.

(16) Smith, M. P.; Hoepping, A.; Johnson, K. M.; Trzcinska, M.; Kozikowski, A. P. Dopaminergic agents for the treatment of cocaine abuse. *Drug Discovery Today* **1999**, *4*, 322–332.

(17) Carrol, F. I.; Lewin, A. H.; Kuhar, M. J. Dopamine transporter uptake blockers: Structure activity relationships. In *Neurotransmitter Transporters: Structure and Function*; Reith, M. E. A., Eds.; Human Press: Totowa, NJ, 1997; pp 263–295.

(18) Singh, S. Chemistry, design, and structure–activity relationship of cocaine antagonists. *Chem. Rev.* **2000**, *100*, 925–1024.

(19) Dutta, A. K.; Xu, C.; Reith, M. E. A. Structure–Activity Relationship Studies of Novel 4-[2-[Bis(4-fluorophenyl)methoxy]-ethyl]-1-(3-phenylpropyl)piperidine Analogues: Synthesis and Biological Evaluation at the Dopamine and Serotonin Transporter Sites. *J. Med. Chem.* **1996**, *39*, 749–756.

(20) Dutta, A. K.; Coffey, L. L.; Reith, M. E. A. Highly selective, novel analogues of 4-[2-(diphenylmethoxy)ethyl]-1-benzylpiperidine for the dopamine transporter: Effect of different aromatic substitutions on their affinity and selectivity. *J. Med. Chem.* **1997**, *40*, 35–43.

(21) Dutta, A. K.; Coffey, L. L.; Reith, M. E. A. Potent and selective ligands for the dopamine transporter (DAT): Structure–activity relationship studies of novel 4-[2-(diphenylmethoxy)ethyl]-1-(3-phenylpropyl)piperidine analogues. *J. Med. Chem.* **1998**, *41*, 699–705.

(22) Dutta, A. K.; Xu, C.; Reith, M. E. A. Tolerance in the Replacement of the benzhydrylic O-atom in 4-[2-(Diphenyl-methoxy)-ethyl]-1-benzylpiperidine derivatives by an N-atom: Development of new-generation potent and selective N-analogue molecules for the dopamine transporter. *J. Med. Chem.* **1998**, *41*, 3293–3297.

(23) Dutta, A. K.; Fei, X.-S.; Beardsley, P.; Newman, J.; Reith, M. E. A. Structure–Activity Relationship Studies of 4-[2-(Diphenylmethoxy)-ethyl]-1-benzylpiperidine Derivatives and their N-analogues: Evaluation of Behavioral Activity of O- and N-Analogues and their Binding to Monoamine transporters. *J. Med. Chem.* **2001**, *44*, 937–948.

(24) Andersen, P. H. Biochemical and Pharmacological Characterization of [^{3}H] GBR 12935 Binding in Vitro to Rat Striatal Membranes: Labeling of the Dopamine Uptake Complex. *J. Neurochem.* **1987**, *48*, 1887–1896.

(25) Van der Zee, P.; Koger, H. S.; Gootjes, J.; Hespe, W. Aryl 1,4-dialk(en)ylpiperazines as selective and very potent inhibitors of dopamine uptake. *Eur. J. Med. Chem.* **1980**, *15*, 363–370.

(26) Dutta, A. K.; Fei, X.-S.; Vaughan, R. A.; Gaffaney, J. D.; Wang, N.; Lever, J. R.; Reith, M. E. A. Design, Synthesis, and Characterization of a Novel, 4-[2-(Diphenylmethoxy)ethyl]-1-Benzyl Piperidine-Based, Dopamine Transporter Photoaffinity Label. *Life Sci.* **2001**, *68* (16), 1839–1849.

(27) Vaughan, R. A.; Gaffaney, J. D.; Lever, J. R.; Reith, M. E. A.; Dutta, A. K. Dual Incorporation of Photoaffinity Ligands on Dopamine Transporters Implicates Proximity of Labeled Domains. *Mol. Pharmacol.* **2001**, *59*, 1157–1164.

(28) Vaughan, R. A.; Kuhar, M. J. Dopamine Transporter Ligand Binding Domains: Structural and Functional Properties Revealed by Limited Proteolysis. *J. Biol. Chem.* **1996**, *271*, 21672–21680.

(29) Vaughan, R. A.; Agoston, G. E.; Lever, J. R.; Newman, A. H. Differential binding of tropane-based photoaffinity ligands on the dopamine transporter. *J. Neurosci.* **1999**, *19*, 630–636.

(30) Berger, P.; Elsworth, J. D.; Reith, M. E. A.; Tanen, D.; Roth, R. H. Complex interaction of cocaine with the dopamine uptake carrier. *Eur. J. Pharmacol.* **1990**, *176*, 251–252.

(31) Saadouni, S.; Lyamani-Refahi, F.; Costentin, J.; Bonnet, J.-J. Cocaine and GBR 12783 recognize nonidentical, overlapping binding domains on the dopamine neuronal carrier. *Eur. J. Pharmacol.* **1994**, *268*, 187–197.

(32) Rothman, R. B.; Grieg, N.; Kim, A.; de Costa, B. R.; Rice, K. C.; Carroll, F. I.; Pert, A. Cocaine and GBR 12909 produce equivalent motoric reponses at different occupancy of the dopamine transporter. *Pharmacol. Biochem. Behav.* **1992**, *43*, 1135–1142.

(33) Tella, S. R.; Ladenheim, B.; Andrews, A. M.; Goldberg, S. R.; Cadet, J. L. Differential reinforcing effects of cocaine and GBR 12909: Biochemical evidence for divergent neuroadaptive changes in the mesolimbic dopaminergic system. *J. Neurosci.* **1996**, *16*, 7416–7427.

(34) (a) Izenwasser, S.; Terry, P.; Heller, B.; Witkin, J. M.; Katz, J. L. Differential relationships among dopamine transporter affinities and stimulant potencies of various uptake inhibitors. *Eur. J. Pharmacol.* **1994**, *263*, 277–283. (b) Katz, J. L.; Izenwasser, S.; Kline, R. H.; Allen, A. C.; Newman, A. H. Novel 3a-diphenylmethoxytropane analogues: selective dopamine uptake inhibitors with behavioral effects distinct from those of cocaine. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 302–315.

(35) Kitayama, S.; Shimada, S.; Xu, H.; Markham, L.; Donovan, D.; Uhl, G. Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7782–7785.

(36) Giros, B.; Wang, Y.-M.; Suter, S.; McLeskey, S. B.; Pifl, C.; Caron, M. G. Delineation of discrete domains for substrate, cocaine and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J. Biol. Chem.* **1994**, *269*, 15985–15988.

(37) Negus, S. S.; Brandt, M. R.; Mello, N. K. Effects of the long-acting monoamine reuptake inhibitor indatraline on cocaine self-administration in rhesus monkeys. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 60−69.
(38) Gorelick, D. A.; The rate hypothesis and agonist substitution approaches to cocaine abuse treatment. *Adv. Pharmacol.* **1998**, *42*, 995−997.
(39) Ball, J. C., Ross, A., Eds. *The Effectiveness of Methadone Maintenance Treatment*; Springer-Verlag: New York, 1991.
(40) Rothman, R. B.; Glowa, J. R. A review of the effects of dopaminergic agents on humans, animals, and drug-seeking behavior, and its implications for medication development. Focus on GBR 12909. *Mol. Neurobiol.* **1995**, *11*, 1−19.
(41) Glowa, J. R.; Fantegrossi, W. E.; Lewis, D. B.; Matecka, D.; Rice, K. C.; Rothman, R. B. Sustained decrease in cocaine-maintained responding in rhesus monkeys with 1-[2-[bis(4-fluorophenyl)-methoxy]ethyl]4-(3-hydroxy-3-phenylpropyl)piperazinyl decanoate, a long-acting ester derivative of GBR 12909. *J. Med. Chem.* **1996**, *39*, 4689−4691.
(42) Blough, B. E.; Abraham, P.; Lewin, A. H.; Kuhar, M. J.; Boja, J. W.; Carroll, F. I. Synthesis and transporter binding properties of 3β-(4′-Alkyl-, 4′-alkenyl-, and 4′-alkynylphenyl)nortropane-2β-carboxylic acid methyl esters: Serotonin transporter selective analogues. *J. Med. Chem.* **1996**, *39*, 4027−4035.
(43) McKean, D. R.; Parrinello, G.; Renaldo, A. F.; Stille, J. K. Synthesis of functional styrenes via palladium-catalyzed coupling of aryl bromides with vinyl tin reagents. *J. Org. Chem.* **1987**, *52*, 422−424.
(44) Krause, M.; Ligneau, X.; Stark, H.; Garbarg, M.; Schwartz, J.-C.; Schunack, W. 4-Alkynylphenyl imidazoylpropyl as selective histamine H_3-receptor antagonists with high oral central nervous system activity. *J. Med. Chem.* **1998**, *41*, 4171−4176.
(45) Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J.; Kline, R. H.; Newman, A. H. Novel *N*-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane analogues: selective ligands for the dopamine transporter. *J. Med. Chem.* **1997**, *40*, 4329−4339.
(46) Melia, K. F.; Spealman, R. D. Pharmacological characterization of the discriminative-stimulus effects of GBR 12909. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 626−632.
(47) Xu, C.; Coffey, L. L.; Reith, M. E. A. Translocation of dopamine and binding of 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (WIN 35, 428) measured under identical conditions in rat striatal synaptosomal preparations. Inhibition by various blockers. *Biochem. Pharmacol.* **1995**, *49*, 339−350.

JM010316X