Novel 4′-Substituted and 4′,4″-Disubstituted 3α-(Diphenylmethoxy)tropane Analogs as Potent and Selective Dopamine Uptake Inhibitors†

Amy Hauck Newman,* Richard H. Kline, Andrew C. Allen, Sari Izenwasser, Clifford George,‡ and Jonathan L. Katz

Psychobiology Section, National Institutes of Health, National Institute on Drug Abuse - Division of Intramural Research, Box 5180, Baltimore, Maryland 21224, and Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375-5000

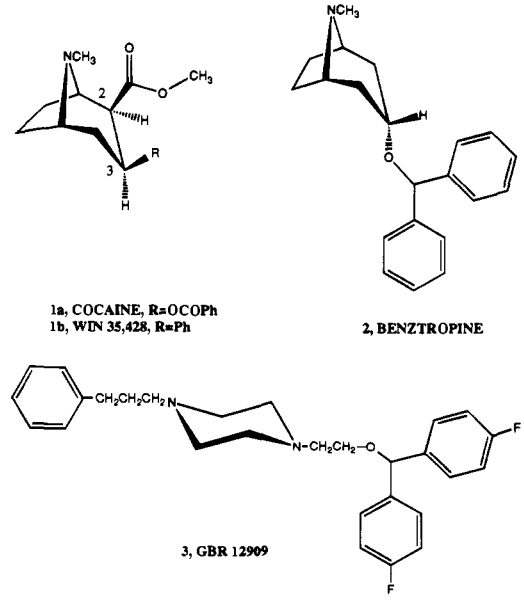
Received May 15, 1995®

A series of 4′-substituted and 4′,4″-disubstituted 3α-(diphenylmethoxy)tropane analogs were prepared as novel probes for the dopamine transporter. These compounds were evaluated in radiolabeled binding assays for the dopamine, norepinephrine, and serotonin transporters. All of these compounds monophasically displaced [^{3}H]WIN 35,428 binding in rat caudate putamen with K_i values ranging from 11.8 to 2000 nM. The most potent compound in this series was 4′,4″-difluoro 3α-(diphenylmethoxy)tropane **7c** with a K_i = 11.8 nM. All of the compounds inhibited dopamine uptake in rat caudate putamen (IC_{50} = 24–4456 nM) which correlated significantly (r = 0.907; p > 0.0001) with binding affinities at the dopamine transporter. None of the compounds demonstrated high-affinity binding at the norepinephrine (K_i > 4800 nM) or serotonin (K_i > 690 nM) transporters. Therefore, the most potent dopamine uptake inhibitors in this series were highly selective for the dopamine transporter. Preliminary behavioral studies of several of these analogs (**7a–e**) suggested that the compounds did not display a cocaine-like behavioral profile, despite their ability to inhibit dopamine uptake. The present data coupled with the observed differences from cocaine in structure–activity relationships suggested that the 3α-(diphenylmethoxy)tropane analogs may be interacting at a different active site than cocaine on the dopamine transporter and that an additional binding domain might be exploited for the identification of potential therapeutics for the treatment of cocaine abuse.

Introduction

The significant public health and social problems resulting from cocaine (**1a**) abuse has stimulated research efforts directed toward elucidating the central mechanisms by which cocaine exerts its behavioral effects. The data from these studies suggest that the primary mechanism of the behavioral effects of cocaine appears to be related to the inhibition of dopamine uptake[1,2] that results in an elevated concentration of dopamine in the synapse. As a consequence, considerable emphasis has been directed toward the dopamine transporter as a target for pharmacological tools for research and potential therapeutics for the treatment of cocaine abuse.

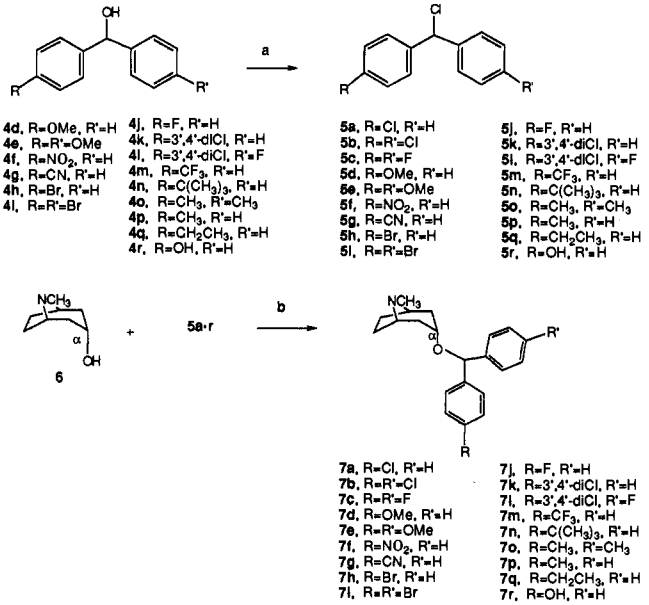
The cocaine recognition site on the dopamine transporter has been characterized with a large number of cocaine analogs,[3–12] and structure–activity relationships within this series continue to evolve with the synthesis of novel compounds. In a preliminary report, we recently described a series of tropane analogs that bind to the dopamine transporter and block dopamine uptake.[13] Several of these 4′-substituted and 4′,4″-disubstituted analogs of benztropine (**2**, 3α-(diphenylmethoxy)-1αH,5αH-tropane) bind with high affinity to the dopamine transporter and inhibit dopamine uptake.[13] However, the lead compound, 4′-chloro 3α-(diphenylmethoxy)tropane, did not produce stimulation of locomotor activity or cocaine-like subjective effects in a drug discrimination model, in rodents.[13] These results were intriguing from a number of different standpoints. First, to our knowledge, all of the potent dopamine uptake inhibitors that have been evaluated behaviorally demonstrate cocaine-like actions.[14,15] Thus, the distinction between the benztropine analogs and other dopamine uptake inhibitors is certainly worth investigating. Second, the structure–activity relationships for the benztropine analogs differ considerably from those of



cocaine and another structural analog, GBR 12909 (**3**). For example, none of the benztropine analogs have a substituent in the 2-position, which has been deemed necessary for high-affinity binding of the cocaine analogs

† This paper was presented in part at the 24th Annual Meeting of the Society for Neuroscience, Miami, FL, November 1994.
‡ Naval Research Laboratory.
® Abstract published in *Advance ACS Abstracts*, September 1, 1995.

Scheme 1[a]



[a] (a) $SOCl_2$; (b) 160 °C.

to the dopamine transporter.[3,4,16] In fact, Meltzer et al. recently demonstrated that placing a β-2-methyl ester substituent on 4′,4″-difluoro 3α-(diphenylmethoxy)tropane actually results in an inactive compound.[17] Third, when the 3-position aryl ether system of benztropine is in the α- or axial stereochemistry, higher affinity binding results, as compared to its β-conformer.[13] In contrast, high-affinity binding of cocaine and the WIN series of cocaine analogs (i.e., WIN 35,428, **1b**) is only achieved when the 3-aryl system is in the β- or equatorial conformation.[3] Finally, from the small series of compounds reported previously,[13] substitution in the *para*-position of one or both phenyl rings significantly affected binding affinity at the dopamine transporter. In contrast, generally, *para*-substitution in the phenyl ring of either the cocaine or WIN series of analogs does not change binding affinity as dramatically.[3] The apparent distinct structure–activity relationships and behavioral profiles of this initial series of compounds encouraged us to continue preparing benztropine analogs with substitution in the *para*-position of one or both phenyl rings. It was anticipated that the synthesis and evaluation of these tropane analogs would result in the expansion of structure–activity relationships at the dopamine transporter, the determination of binding selectivities at the other monoamine transporters, and, further, the characterization of their behavioral pharmacology as compared to that of cocaine.

Chemistry

The synthesis of compounds **7a–r** is depicted in Scheme 1. The 4′-substituted or 4′,4″-disubstituted benzhydrols **4d–r** were converted to the benzhydryl chlorides **5d–r** (**5a–c** were purchased from Lancaster Chemical Co.) in refluxing thionyl chloride. The benzhydryl chlorides were then added, neat or in a minimal volume of anhydrous diethyl ether, to tropine (**6**) at 160 °C. The synthesis of compound **7r** was accomplished via the O-debenzylation of 4′-benzyloxy 3α-(diphenylmethoxy)tropane, which was prepared using the conditions described above. The desired products were isolated and purified as the hydrochloride salts, and in most cases the yields were good. Notable exceptions were the compounds with strong electron-withdrawing substituents, particularly the 4′-NO_2 group (**7f**), which were obtained in lower yields than the other substituted analogs. This appeared to be due to the relative instability of the intermediate benzhydryl chloride **5f** at the melt temperature which resulted in decomposition before reaction with tropine, as observed by TLC. Physical properties of compounds **7f–r** are in Table 1. The physical properties of compounds **7a–e** and **10** were previously reported.[13] To prepare the β-stereoisomer of 4′-chlorobenztropine (**10**), pseudotropine (**9**) was prepared stereoselectively from tropinone with lithium borohydride. The melt reaction, as seen in Scheme 2, was then used to prepare the desired product **10**. The yield (31%) of this reaction was slightly lower than in the 3-α-series (74%)[13] which may be due to the increased time that was necessary for the reaction to be completed (60 min vs 2–10 min).

The configuration of **7c** from the X-ray results is that shown in Figure 1. The two phenyl groups were near normal to one another with a dihedral angle between least squares planes through the rings of 90.6°. This configuration differed only slightly from that of the X-ray structure of benztropine[18] where the largest differences were rotations of the phenyl groups as measured by C–O–C–C torsion angles of 5.8° and 7.1°. The nitrogen to phenyl ring centroid distances remained unchanged with distances of 7.05 and 6.94, and 7.07 and 6.91 Å for **7c** and benztropine, respectively.

Results and Discussion

All of the 4′-substituted and 4′,4″-disubstituted 3α-(diphenylmethoxy)tropane analogs **7a–r** and **10** were evaluated for displacement of radiolabeled ligand binding at the dopamine (DAT), serotonin (5HTT), and norepinephrine (NET) transporters as well as muscarinic m_1 and m_2 receptors, Table 2. All of the compounds monophasically displaced [^{3}H]WIN 35,428 binding at the dopamine transporter with a wide range of affinities (K_i = 11.8–2000 nM). The most potent compound in this series was the 4′,4″-difluoro analog **7c** (K_i = 11.8 nM). All of the 4′-halogen- and 4′,4″-dihalogen-substituted compounds bound with higher affinity at the dopamine transporter than the unsubstituted parent compound, benztropine, with F > Cl > Br.

In general, increasing steric bulk on one and especially on both phenyl rings significantly decreased binding affinity at the dopamine transporter, i.e., **7n** (K_i = 1918 nM) and **7e** (K_i = 2000 nM) vs **7d** (K_i = 78.4 nM). Further, both electronic and lipophilic properties of these substituents appear to be playing a role since the halogenated compounds were more potent than the parent drug and the compounds with more strongly electron-withdrawing substituents, such as a 4′-CF_3 substituent in **7m** (K_i = 635 nM), were less potent than the comparably sized 4′-CH_3-substituted analog **7p** (K_i = 187 nM).

As noted previously, in the benztropine series, the 3α-diphenyl ether was favored over the 3β-configuration, i.e., **7a** (K_i = 30 nM) vs **10** (K_i = 854 nM).[13] This configuration (α- or axial aryl) is opposite to the favored β- or equatorial configuration of the aryl substituents for the cocaine and WIN analogs.[3]

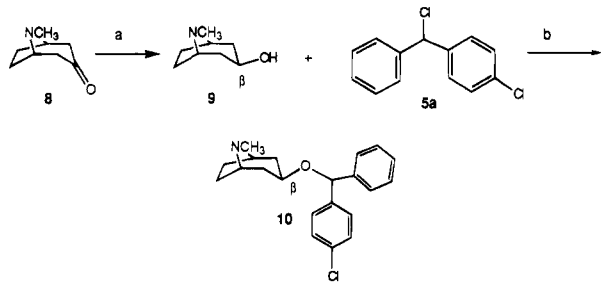
None of the 4′-substituted or 4′,4″-disubstituted 3α-(diphenylmethoxy)tropane analogs bound with high

Table 1. Physical Properties of Compounds **7f–r**

compd	recryst solv	mp, °C	MS *m/z*	formula	yield, %
7f	acetone	145–151	M + 352	$C_{21}H_{25}N_2O_3Cl·0.75H_2O$	21
7g	2-PrOH	106–108	M + 332	$C_{22}H_{25}N_2OCl·0.75H_2O$	40
7h	acetone	210–211	M + 386 M + 2 388	$C_{21}H_{25}NOBrCl$	70
7i	acetone	199–200	NA[a]	$C_{21}H_{24}NOBr_2Cl$	65
7j	acetone	175	M + 325	$C_{21}H_{25}NOFCl·0.25H_2O$	62
7k	2-PrOH/ether	219–221	M + 375	$C_{21}H_{24}NOCl_3$	58
7l	EtOAc	205–206	M + 393	$C_{21}H_{23}NOCl_3F$	63
7m	EtOAc	180–182	M + 375	$C_{22}H_{25}NOF_3Cl·0.5H_2O$	50
7n	acetone	243–245	M + 363	$C_{25}H_{34}NOCl$	52
7o	acetone	220–222	M + 335	$C_{23}H_{30}NOCl$	63
7p	EtOAc	197–199	M + 321	$C_{22}H_{28}NOCl$	60
7q	EtOAc	150–153	M + 335	$C_{23}H_{30}NOCl$	45
7r	MeOH	240–241	NA[a]	$C_{21}H_{26}NO_2Cl$	38

[a] Molecular ion peak not observable.

Scheme 2[a]



[a] (a) $LiBH_4$, THF, −78 °C; (b) 160 °C.

affinity to either norepinephrine or serotonin transporters. At the norepinephrine transporter, none of the compounds displaced >60% of [^{3}H]desmethylimipramine binding at a concentration of 10 μM. For example, compound **7c** was >700-fold more potent at the dopamine transporter as compared to the norepinephrine transporter. Further this compound was >200-fold selective for the dopamine vs serotonin transporter. Hence, these are some of the most selective compounds for the dopamine transporter over the other monoamine transporters reported to date.

All of these analogs bound with high affinity (K_i = 0.95–70.8 nM) to muscarinic m_1 receptors. In all cases, substitution at the 4′- and/or 4″-position(s) served to decrease affinity at this site, with the 4′,4″-dibromo analog **7i** having >70-fold lower affinity for these receptors than the parent benztropine. Interestingly, though affinities at m_1 and m_2 sites were correlated (r = 0.954; p < 0.0011), some of these compounds were remarkably selective for the m_1 over m_2 sites, such as compound **7m** which was nearly 300-fold selective for m_1 sites.

The parent drug benztropine demonstrated a >100-fold higher affinity for m_1 receptors compared to the dopamine transporter. In contrast, the compounds that were most potent at the dopamine transporter, **7c**,**l**,**b**,**k**, had similar binding affinities at muscarinic m_1 receptors, suggesting that chemical modification that increased binding affinities at the dopamine transporter did not concomitantly increase binding affinities at the muscarinic receptors, and therefore, a separation of these actions appears to be achievable. In fact, affinity at the dopamine transporter was correlated with affinity at neither m_1 (r = 0.323; p = 0.1653) nor m_2 (r = 0.353; p = 0.1267) sites, likewise suggesting that dopamine uptake inhibition and antimuscarinic actions will be separable within the 3α-(diphenylmethoxy)tropane series of compounds.

All of the 4′-substituted or 4′,4″-disubstituted 3α-(diphenylmethoxy)tropane analogs were evaluated for inhibition of [^{3}H]dopamine uptake in rat caudate putamen, Table 3. Compounds **7a–c** have been previously reported as dopamine uptake inhibitors.[19,20] All of the compounds that bound with high affinity to the dopamine transporter were potent inhibitors of dopamine uptake. This was evidenced by the high, significant correlation (r = 0.907; p < 0.0001) between log K_i values for binding and log IC_{50} values for inhibiting dopamine uptake, Figure 2. These results correspond with the recently reported data of Carroll et al.[4] and Bennett et al.[21] who have demonstrated that for hundreds of cocaine analogs, with a variety of chemical modifications, dopamine uptake inhibition was well correlated with binding affinity at the dopamine transporter.

However, in contrast to the cocaine analogs,[22] behavioral studies on compounds **7a–e** demonstrated that several of the present dopamine uptake inhibitors were not efficacious locomotor stimulants.[13,23] Further, also in contrast to the cocaine analogs,[14] the present compounds did not produce a cocaine-like discriminative stimulus as demonstrated by their lack of generalization to the cocaine cue in rats trained to discriminate 10 mg/kg cocaine from saline.[13,23] The observation that these tropane analogs are neurochemically similar and yet behaviorally distinct from cocaine is very intriguing, and at this time, the reasons for this dichotomy are unclear. There are several potential explanations which warrant further investigation. For example, all of the benztropine analogs bind with high affinity to muscarinic sites, and those actions may have interfered with the expression of a cocaine-like psychomotor stimulant effect. In the series that has been behaviorally tested, to date, there is not a large difference in binding affinities at the muscarinic m_1 sites (K_i range = 3.6–39.6), and the separation of binding affinities for the dopamine transporter and muscarinic m_1 sites is negligible. Therefore, at this time, we cannot fully assess the role of the muscarinic antagonist properties of these compounds in their behavioral effects. Compounds that retain high affinity at the dopamine transporter but have significantly lower affinities for the muscarinic m_1 sites are necessary to fully determine whether this receptor system is playing a role in the pharmacology of these compounds.

Alternatively, it has been reported previously that [^{3}H]WIN 35,428 exhibits two binding components on the dopamine transporter.[24,25] Further, a two-site model for

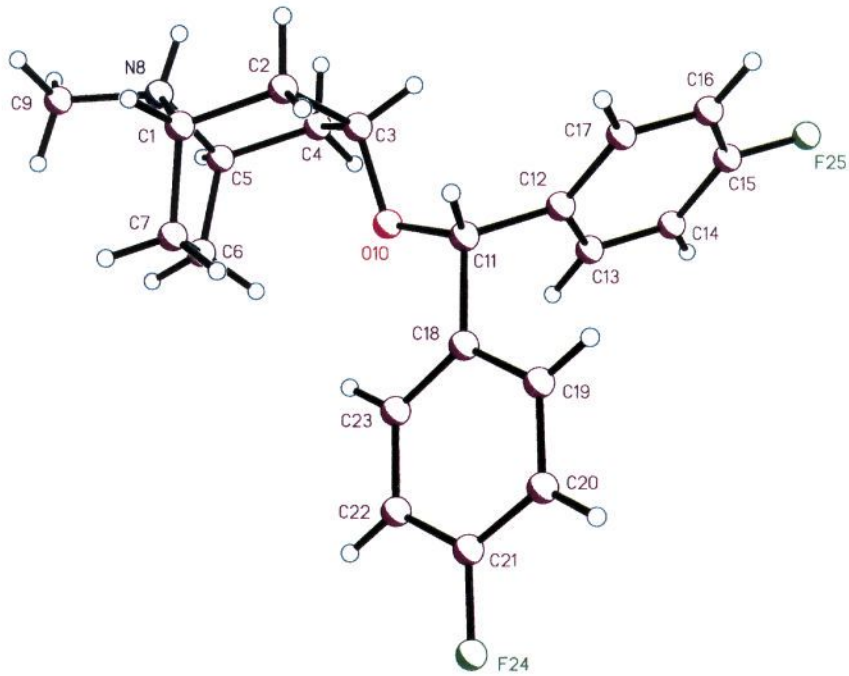


Figure 1. Thermal ellipsoid plot drawn from experimental coordinates of **7c**.

Table 2. Results of Radiolabeled Binding Experiments on 4′-Substituted and 4′,4″-Disubstituted 3α-(Diphenylmethoxy)tropane Analogs

compd	substitution	DAT K_i, nM (% error)[a]	% I at 10 μM[b,c]		K_i, nM[c]	
			NET	5HTT	m_1	m_2
7c	4′,4″-diF	11.8 (11)[e]	30 (>8500)[d]	62 (2440)[d]	6.1	48.8
7l	3′,4′-diCl, 4″-F	18.9 (14)	57.8 (>9000)[d]	80.3 (691)[d]	33	369
7b	4′,4″-diCl	20.0 (14)[e]	43 (>6000)[d]	54 (2960)[d]	35	1490
7k	3′,4′-diCl	21.1 (19)	56.5 (4870)[d]	66.2 (1790)[d]	12.5	141
7a	4′-Cl	30.0 (12)[e]	32	34	3.6	36.6
7j	4′-F	32.2 (10)	37.9	35.2	1.1	15.0
7h	4′-Br	37.9 (7)	37	39	3.7	39.1
7d	4′-OMe	78.4 (8)[e]	30.9	30.5	4.2	18.9
7i	4′,4″-diBr	91.6 (13)	41.1	41	70.8	66%[b]
2		118 (9)[e]	18.3	17.2	0.95	2.6
7p	4′-CH_3	187 (5)	31.8	35.4	2.7	37.5
7g	4′-CN	196 (9)	16	40	7.1	74.0
7f	4′-NO_2	197 (8)	29	54 (3570)[d]	7.1	41.9
7r	4′-OH	297 (13)	27.3	23.3	2.1	17.7
7o	4′,4″-diCH_3	420 (7)	35.1	35.5	47.2	716
7q	4′-CH_2CH_3	520 (8)	32.7	42.7	12.6	703
7m	4′-CF_3	635 (10)	34.6	49.1	5.4	1510
10	3β-4′-Cl	854 (7)[e]	27.9	37.2	4.1	87.4
7n	4′-C(CH_3)$_3$	1918 (7)	37.6	24.7	148	54%[b]
7e	4′,4″-diOMe	2000 (7)[e]	34	31	39.6	1000
cocaine		32 (16)/388 (57)[f]				
GBR 12909		11.6 (31)[f]				

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. [b] Percent inhibition of radiolabeled ligand binding at a concentration of 10 μM. [c] Data provided by NOVASCREEN. [d] K_i value in nanomolar. [e] Data from ref 13. [f] Data from ref 24.

the binding of cocaine and several other dopamine uptake inhibitors is preferred over a one-site model when assays are conducted with either [^{3}H]cocaine[26] or [^{3}H]WIN 35,428.[24] In addition, locomotor activity is well correlated with the binding affinities of the compounds at the high-affinity site, whereas no correlation exists between this behavior and binding at the low affinity component.[24] Since benztropine and the analogs reported herein monophasically displace [^{3}H]WIN 35,428 and do not produce locomotor stimulant activity, it may be speculated that these compounds are interacting at a low-affinity component on the dopamine transporter, which may not be associated with psychomotor stimulant actions. Of course, further characterization of compounds that interact exclusively at one component or the other is necessary before any conclusions can be drawn.

Summary

A series of 4′-substituted and 4′,4″-disubstituted 3α-(diphenylmethoxy)tropane analogs have been prepared that bind with high affinity to the dopamine transporter and inhibit dopamine uptake. None of these compounds bind with high affinity to the other monoamine transporters and therefore are selective dopamine uptake inhibitors.

Structure–activity relationships have been derived and demonstrate that *para*-substitution at one or both

Table 3. [^{3}H]Dopamine Uptake Inhibition by 4′-Substituted and 4′,4″-Disubstituted 3α-(Diphenylmethoxy)tropane Analogs[a]

compd	substitution	[^{3}H]DA uptake inhibition IC_{50}, nM
7l	3′,4′-diCl, 4″-F	24
7h	4′-Br	29
7i	4′,4″-diBr	34
7k	3′,4′-diCl	47
7j	4′-F	48
7c	4′,4″-diF	71
7b	4′,4″-diCl	75
7a	4′-Cl	115
7f	4′-NO_2	219
7g	4′-CN	222
2		403
7d	4′-OMe	468
7p	4′-CH_3	512
7r	4′-OH	677
7q	4′-CH_2CH_3	984
7m	4′-CF_3	2155
7o	4′,4″-diCH_3	2536
7e	4′,4″-diOMe	2876
10	3β-4′-Cl	3519
7n	4′-$C(CH_3)_3$	4456

[a] Each value represents data from at least three independent experiments, each performed in triplicate.

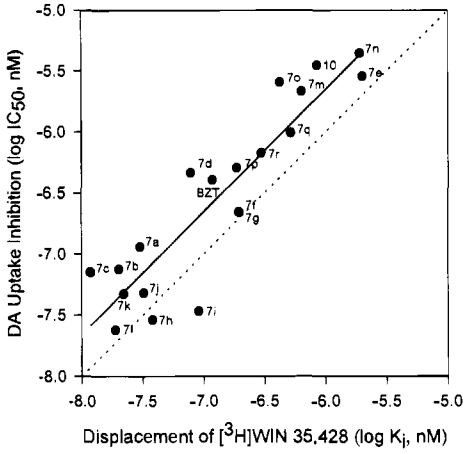


Figure 2. Correlation of K_i values for displacement of [^{3}H]-WIN 35,428 and IC_{50} values for inhibition of dopamine uptake in tissue from rat caudate putamen.

of the phenyl rings of benztropine significantly changes the binding characteristics of these compounds as compared to the unsubstituted parent drug. Further, these modifications serve to decrease binding affinities at muscarinic receptors and do not significantly increase the binding affinities for the other monoamine transporters. Comparable *para*-substitutions in the analogous phenyl rings of the structurally similar WIN series of cocaine analogs do not similarly affect binding affinities at the dopamine transporter suggesting that there may be a difference in the way that these compounds interact at the active site. The novel aspects of this series of 4′-substituted or 4′,4″-disubstituted 3α-(diphenylmethoxy)tropane analogs are further supported by findings that they did not demonstrate cocaine-like behavioral effects.

On the basis of the neurochemical and behavioral results, these novel benztropine analogs may have potential as therapeutics for the treatment of cocaine abuse. These compounds inhibit dopamine uptake and thus would provide elevated levels of extracellular dopamine that may alleviate some of the symptoms of cocaine abstinence,[27] in a manner similar to the way in which the nicotine patch or nicotine chewing gum protects against withdrawal symptoms. Further, due to their lack of cocaine-like behavioral effects, these compounds may not be subject to abuse themselves. Thus a compound from or related to this series could serve to keep drug abusers from seeking cocaine but not become a substitute addictive drug.

Future studies are being directed toward exploiting the differences in the structure–activity relationships at the dopamine transporter and the muscarinic sites. Highly selective and potent dopamine uptake inhibitors will undoubtedly be very useful in further defining the role of the dopamine transporter in the pharmacology of cocaine.

Experimental Section

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR data were recorded on a Bruker (Billerica, Mass) AC-300 instrument. Samples were dissolved in an appropriate deuterated solvent. Proton chemical shifts (δ) are reported as parts per million (ppm) relative to tetramethylsilane (Me_4Si; 0.00 ppm) which was used as an internal standard. Carbon chemical shift values (δ) are reported in parts per million relative to deuterated chloroform ($CDCl_3$; 77.0 ppm). Mass spectra were recorded on a Hewlett Packard (Palo Alto, CA) 5971A mass selective ion detector in the electron-impact mode with sample introduction via a HP-5890 series II gas chromatograph fitted with an HP-1 column (cross-linked methyl silicone gum), 25 m × 0.2 mm i.d., 50 μm film thickness. Ultrapure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and transfer line temperatures were 250 and 280 °C, respectively. The initial oven temperature was 100 °C, held for 3.0 min, programmed to 295 °C at 15.0 °C/min, and maintained at 295 °C for 10.0 min. Infrared spectra were recorded in KBr with a Perkin-Elmer 1600 Series FTIR. Microanalyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and agree within 0.4% of calculated values. TLC solvent used was $CHCl_3$/MeOH/NH_4OH, 90:10:1, unless otherwise indicated. All chemicals and reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis, Inc.

Synthesis of 3α-(Diphenylmethoxy)tropanes 7a–q. General Method. The benzhydrol **4d–q** (11 mmol) was dissolved in 10 mL of $SOCl_2$, at 0 °C, under an atmosphere of argon. The reaction mixture was warmed to reflux and allowed to stir at this temperature for 2–18 h. The reaction flask was cooled in an ice bath, and the volatiles were removed *in vacuo*. Addition of dry toluene (2 × 5 mL) and removal *in vacuo* ensured the complete removal of $SOCl_2$. The resulting viscous oil was determined spectroscopically to be the desired alkyl chloride. The benzhydryl chloride (crude **5d–q** or commercially available **5a–c**) was directly added dropwise to a tropine (1.41 g, 10 mmol) melt (160 °C oil bath) over 2 min. Evolution of HCl gas over 3–30 min resulted in a bronze oil which solidified to a glass upon cooling. The crude product was dissolved in 25 mL of $CHCl_3$ and transferred to a separatory funnel. Ion pairing of the desired product was achieved by extraction with 2.8 N HCl (2 × 25 mL, to remove residual tropine) followed by washing the aqueous fraction with $CHCl_3$ (2 × 10 mL). The combined organic fractions were evaporated to an off-white foam which was recrystallized to give the pure product as the HCl salt (21–83% yield).

3α-[(4′-Hydroxyphenyl)phenylmethoxy]tropane (7r). 3α-[(4′-Benzyloxy)(diphenylmethoxy)]tropane hydrochloride (synthesized via the General Method reported above in 41% yield) (1.0 g, 2.22 mmol) was dissolved in absolute methanol (100 mL) and reduced (H_2, 30 psi) over 10% palladium on activated carbon for 3.5 h. After removal of the catalyst via filtration through Celite, the solvent was evaporated *in vacuo* leaving 0.74 g (95% yield) of a white solid residue which was recrystallized from methanol. Anal. ($C_{21}H_{25}NO_2$·HCl) C, H, N.

Pseudotropine (9). Tropinone (3.0 g, 21.6 mmol) was placed in a dry 250 mL round bottom flask, purged with argon.

Anhydrous THF (75 mL, without stabilizer) was added, and the solution was cooled in a dry ice/acetone bath to −78 °C. In another dry, argon-purged flask, $LiBH_4$ (1.41 g, 64.6 mmol) was dissolved in anhydrous THF (50 mL) and cooled to −78 °C. The contents of this flask were transferred to the first flask via cannula, under argon. This flask was rinsed once with additional THF (25 mL). The reaction was allowed to proceed for 6 h at −78 °C and then the mixture warmed gradually to room temperature overnight. The reaction mixture was cooled in an ice water bath, and the reaction was carefully quenched with H_2O (10 mL) followed by neutralization with 2.8 N HCl. The volatiles were removed *in vacuo*, and the residue was taken up in 1 N HCl (100 mL) and heated to reflux for 60 min to dissociate the pseudotropine−boron complex formed. The acidic solution was adjusted to pH = 10−11 with 15% NaOH and extracted with $CHCl_3$/2-propanol (3:1, 10 × 50 mL). The organic fractions were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* resulting in 2.58 g (85% yield) of a white solid product. Recrystallization from toluene/petroleum ether gave small white needles, mp 106−109 °C (lit.[28] mp 109 °C).

3β-[(4′-Chlorophenyl)phenylmethoxy]tropane (10). 2-Chlorobenzhydryl chloride (8.5 mmol) was added to a pseudotropine (8.5 mmol, free base) melt as described for compounds **7a−q** (160 °C oil bath) and allowed to stir at this temperature for 1 h. The crude product was worked up in the same way as described above resulting in an off-white foam which was recrystallized from acetone to give pure **10** as the HCl salt (31% yield), mp 124−126 °C. Anal. ($C_{21}H_{25}NOCl_2 \cdot 0.25H_2O$) C, H, N.

Representative spectral data of 7a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82−1.89 (m, 4H, H-2,4$_{ax,eq}$), 1.94−1.97 (m, 2H, H-6,7$_{exo}$), 2.08−2.12 (m, 2H, H-6,7$_{endo}$), 2.24 (s, 3H, N-CH_3), 3.07 (br t, 2H, H-1,5), 3.54 (t, J = 4.6 Hz, 1H, H-3$_{eq}$), 5.37 (s, 1H, Ph-CH-Ph), 7.20−7.31 (m, 9H, H-aromatics); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 25.79 (t, C-6,7), 36.17 (t, C-2,4), 40.89 (q, CH_3-N), 60.13 (d, C-1,5), 69.25 (d, C-3), 79.89 (d, C-9), 127.42, 128.16, 128.43, 128.48, 128.80 (d, C-protonated aromatic), 132.74, 141.58, 142.43 (s, C-nonprotonated aromatic); IR (KBr) 697, 735 (aro-H out of plane), 826, 1043, 1079 (R-O-R), 1320, 1401, 1449, 1484, 1590 (aro-db stretch) 2525 (tertiary amine HCl) cm^{-1}; EIMS 341 m/z (parent ion, 1, with isotopic abs for Cl at 343), 235 (10), 201 (15), 165 (20), 140 (100, base), 124 (40), 83 (82), 82 (50).

10: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31−1.38 (m, 2H, H-6,7$_{endo}$), 1.72−1.80 (m, 4H, H-2,4$_{ax,eq}$), 1.87−1.94 (m, 2H, H-6,7$_{exo}$), 2.23 (s, 3H, N-CH_3), 3.15 (brs, 2H, H-1,5), 3.62 (pentet, J = 7 Hz, 1H, H-3$_{ax}$), 5.44 (s, 1H, Ph-C*H*-Ph), 7.20−7.33 (m, 9H, H-aromatics); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 26.70 (t, C-6,7), 36.18 (t, C-2,4), 38.17 (q, CH_3-N), 59.94 (d, C-1,5), 69.25 (d, C-3), 79.40 (d, C-9), 126.80, 127.87, 128.23, 128.57, 128.77 (d, C-protonated aromatic), 133.26, 140.77, 141.60 (s, C-nonprotonated aromatic); IR (KBr) 697, 735 (aro-H out of plane), 1008, 1055, 1079, 1085 (R-O-R), 1402, 1448, 1484, 1590 (aro-db stretch) 2543 (tertiary amine HCl) cm^{-1}; EIMS 341 m/z (parent ion, 1, with isotopic abs for Cl at 343), 235 (5), 201 (15), 165 (20), 140 (9), 125 (100, base), 96 (20), 83 (90), 82 (50).

Single-Crystal X-ray Analysis of 7c. A clear plate 0.15 × 0.50 × 0.60 mm^3 crystal was selected for data collection, $C_{21}H_{24}O\ N^+Cl^-H_2O$, fw = 397.88. Data were collected on a computer-controlled diffractometer with an incident beam graphite monochromator (Nicolet R3m/V with Mo Kα radiation, λ = 0.71073 Å, T = 293 K). A least-squares refinement using 25 centered reflections within 21 < 2θ < 30° gave the monoclinic $P2_1/c$ cell, a = 22.062(4), b = 9.712(2), and c = 9.493(2) Å, β = 91.93(2)°, V = 2032.9(7) Å^3, Z = 4, and d_{calcd} = 1.300 gm/cm^3. A total of 2864 reflections were measured in the θ/2θ mode to $2\theta_{max}$ = 45°, of which there were 2664 independent reflections. Corrections were applied for Lorentz and polarization effects. A face-indexed numerical absorption correction was applied, μ = 0.22 mm^{-1}, and maximum and minimum transmission were 0.97 and 0.89, respectively. The structure was solved by direct methods and refined on F^2 with a full matrix least-squares SHELXL93 program.[29] The 250 parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Carbon hydrogens used a riding model in which the coordinate shifts of the carbons were applied to the attached hydrogens, C−H = 0.96 Å, H angles idealized, and $U_{iso}(H) = 1.1 \cdot U_{eq}(C)$. The final R values for the 2122 observed reflections with $F_o > 4\sigma(|F_o|)$ were R = 0.041, and R_w^2 = 0.128 for all data. The goodness of fit parameter was 0.88, and final difference Fourier excursions were 0.15 and −0.16 eÅ^{-3}. Tables of coordinates, bond distances, and bond angles have been deposited with the Crystallographic Data Centre, Cambridge CB2 1EW, England.

Pharmacology. Dopamine Transporter 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 vol 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, and 10 mM D-glucose, pH adjusted to 7.4) using a Brinkman polytron and centrifuged at 20000g for 10 min at 4 °C. The resulting pellet was then washed two more times by resuspension in ice-cold buffer and centrifugation at 20000g for 10 min at 4 °C. Fresh homogenates were used in all experiments.

Binding assays were conducted in modified Krebs-HEPES buffer on ice. The total volume in each tube was 0.5 mL, and the final concentration of membrane after all additions was 0.5% (w/v) corresponding to 200−300 mg of protein/sample. Triplicate samples of membrane suspension were preincubated for 5 min in the presence or absence of the compound being tested. [^{3}H]WIN 35,428 (2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalene disulfonate; specific activity 82.4 Ci/mmol, final concentration 1.5 nM; New England Nuclear, Boston, MA) 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). 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 ca. 36%. Under these assay conditions, an average experiment yielded approximately 6000 dpm total binding/sample and ca. 250 dpm nonspecific binding, defined as binding in the presence of 100 μM cocaine. Each compound was tested with concentrations ranging from 0.01 nM to 100 μM for competition against binding of [^{3}H]WIN 35,428 in three independent experiments, each performed in triplicate.

[^{3}H]Dopamine Uptake Assay. 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.[30] 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, specific activity 50 Ci/mmol; Amersham Corp., Arlington Heights, IL) 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% poly(ethylenimine) in water). The filters were rinsed and placed in scintillation vials to which 1 mL of methanol and 2 mL of 0.2 M HCl were added to extract the accumulated [^{3}H]-dopamine. Radioactivity was determined by liquid scintillation spectrometry at an efficiency of ca. 30%. The reported values represent specific uptake from which nonspecific binding to filters was subtracted.

Analysis of Data. Saturation and displacement data were analyzed by the use of the nonlinear least-squares curve-fitting

computer program LIGAND.[31] Data from replicate experiments were modeled together to produce a set of parameter estimates and the associated standard errors of these estimates. In each case, the model reported fit significantly better than all others according to the F test at $p < 0.05$. The K_i values reported are the dissociation constants derived for the unlabeled ligands. Uptake data were analyzed using standard analysis of variance and linear regression techniques.[32] IC_{50} values were calculated using the linear portion of the concentration–response curve (linear regression $p < 0.05$).

In both saturation and competition experiments, two components of [^{3}H]WIN 35,428 binding were apparent. Analysis of the data utilizing the LIGAND program revealed a high-affinity component with a K_D of 7 ± 5 nM and a B_{max} of 445 ± 338 fmol/mg of protein and a low-affinity component with a K_D of 126 ± 115 nM and a B_{max} of 1995 ± 559 fmol/mg of protein. Competition of [^{3}H]WIN 35,428 binding by cocaine also revealed two binding sites (Table 2).

Serotonin (5HTT) and norepinephrine transporter (NET) and muscarinic m_1 and m_2 receptor binding data were provided by NOVASCREEN. The radiolabeled ligands used and the methods were from the following published procedures: 5HTT, [^{3}H]citalopram (specific activity 70–87 Ci/mmol, final ligand concentration 0.7 nM);[33] NET, [^{3}H]desmethylimipramine (specific activity 40–70 Ci/mmol, final ligand concentration 3.0 nM);[34] muscarinic m_1 receptors, [^{3}H]pirenzepine (specific activity 70–87 Ci/mmol, final ligand concentration 1.0 nM);[35] and muscarinic m_2 receptors, [^{3}H]AF DX 384 (specific activity 70–120 Ci/mmol, final ligand concentration 5.0 nM).[36]

Acknowledgment. A.C.A. and R.H.K. were supported by an NIDA-sponsored National Research Council Fellowship. We thank Brett Heller for expert technical assistance and Dr. Jeff Witkin for valuable editorial assistance. Animals used in this study, at NIDA, were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and all experimentation was conducted according to the guidelines of the Institutional Care and Use Committee of the Division of Intramural Research, National Institute on Drug Abuse, NIH, and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication (NIH) 85-23, revised 1985. Radiolabeled ligand binding experiments for the norepinephrine and serotonin transporters and the muscarinic m_1 and m_2 receptors were provided by NOVASCREEN under the NIMH contract.

References

(1) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine Receptors on Dopamine Transporters are Related to Self-Administration of Cocaine. *Science* **1987**, *237*, 1219–1223.
(2) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The Dopamine Hypothesis of the Reinforcing Properties of Cocaine. *Trends Neurosci.* **1991**, *14*, 299–301.
(3) Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Cocaine Receptor: Biochemical Characterization and Structure-Activity Relationships of Cocaine Analogues at the Dopamine Transporter. *J. Med. Chem.* **1992**, *35*, 969–981.
(4) Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A.; Boja, J. W.; Kuhar, M. J. Cocaine and 3β-(4′-substituted-phenyl)tropane-2β-carboxylic acid ester and amide Analogues. New High-Affinity and Selective Compounds for the Dopamine Transporter. *J. Med. Chem.* **1995**, *38*, 379–388.
(5) Davies, H. M. L.; Saikali, E.; Sexton, T.; Childers, S. R. Novel 2-Substituted Cocaine Analogs: Binding Properties at Dopamine Transport Sites in Rat Striatum. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1993**, *244*, 93–97.
(6) Kline, R. H., Jr.; Wright, J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-Arylecgonine Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake. *J. Med. Chem.* **1990**, *33*, 2024–2027.
(7) Kline, R. H., Jr.; Wright, J.; Eshleman, A. J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-Carbamoylecgonine Methyl Ester Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake. *J. Med. Chem.* **1991**, *34*, 702–705.
(8) Kline, R. H., Jr.; Eshleman, A. J.; Wright, J.; Eldefrawi, M. E. Synthesis of Substituted 3-Carbamoylecgonine Methyl Ester Analogues: Irreversible and Photoaffinity Ligands for the Cocaine Receptor/Dopamine Transporter. *J. Med. Chem.* **1994**, *37*, 2249–2252.
(9) Kozikowski, A. P.; Roberti, M.; Xiang, L.; Bergmann, J. S.; Callahan, P. M.; Cunningham, K. A.; Johnson, K. M. Structure-Activity Relationship Studies of Cocaine: Replacement of the C-2 Ester Group by Vinyl Argues Against H-Bonding and Provides an Esterase-resistant, High Affinity Cocaine Analogue. *J. Med. Chem.* **1992**, *35*, 4764–4766.
(10) Simoni, D.; Stoelwinder, J.; Kozikowski, A. P.; Johnson, K. M.; Bergmann, J. S.; Ball, R. G. Methoxylation of Cocaine Reduces Binding Affinity and Produces Compounds of Differential Binding and Dopamine Uptake Inhibitory Activity: Discovery of a Weak Cocaine "Antagonist". *J. Med. Chem.* **1993**, *36*, 3975–3977.
(11) Milius, R. A.; Saha, J. K.; Madras, B. K.; Neumeyer, J. L. Synthesis and Receptor Binding of N-Substituted Tropane Derivatives. High-affinity Ligands for the Cocaine Receptor. *J. Med. Chem.* **1991**, *34*, 1728–1731.
(12) Kelkar, S. V.; Izenwasser, S.; Katz, J. L.; Klein, C. L.; Zhu, N.; Trudell, M. L. Synthesis, Cocaine Receptor Affinity, and Dopamine Uptake Inhibition of Several New 2β-Substituted 3β-Phenyltropanes. *J. Med. Chem.* **1994**, *37*, 3875–3877.
(13) Newman, A. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L. Novel 3α-(Diphenylmethoxy)tropane Analogs: Potent Dopamine Uptake Inhibitors without Cocaine-like Behavioral Profiles. *J. Med. Chem.* **1994**, *37*, 2258–2261.
(14) Cline, E. J.; Terry, P.; Carroll, F. I.; Kuhar, M. J.; Katz, J. L. Stimulus Generalization from Cocaine to Analogs with High *in vitro* Affinity for Dopamine Uptake Sites. *Behav. Pharmacol.* **1992**, *3*, 113–116.
(15) Woolverton, W. L. Discriminitive Stimulus Effects of Cocaine. In *Drug Discrimination: Applications to Drug Abuse Research*; Glennon, R. A., Jarbe, T. U. C., Frankenheim, J., Eds.; NIDA Research Monograph 116; NIDA: Washington, DC, 1991; pp 61–74.
(16) Lewin, A. H.; Gao, Y.; Abraham, P.; Boja, J.; Kuhar, M. J.; Carroll, F. I. 2β-Substituted analogues of cocaine. Synthesis and Inhibition of Binding to the Cocaine Receptor. *J. Med. Chem.* **1992**, *35*, 135–140.
(17) Meltzer, P. C.; Liang, A. Y.; Madras, B. K. The Discovery of an Unusually Selective and Novel Cocaine Analog: Difluoropine. Synthesis and Inhibition of Binding at Cocaine Recognition Sites. *J. Med. Chem.* **1994**, *37*, 2001–2010.
(18) Jones, P. G.; Kennard, O.; Horn, A. S. 3-(Diphenylmethoxy)-8-methyl-8-azoniabicyclo[3.2.1]octane methanesulphonate monohydrate (Benztropine methanesulphonate monohydrate). *Acta Crystallogr.* **1978**, *34B*, 3125–3127.
(19) 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.
(20) van der Zee, P.; Hespe, W. A. Comparison of the Inhibitory Effects of Aromatic Substituted Benzhydryl Ethers on the Uptake of Catecholamines and Serotonin into Synaptosomal Preparations of the Rat Brain. *Neuropharmacology* **1978**, *17*, 483–490.
(21) Bennett, B. A.; Wichems, C. H.; Hollingsworth, C. K.; Davies, H. M. L.; Thornley, C.; Sexton, T.; Childers, S. R. Novel 2-Substituted Cocaine Analogs: Uptake and Ligand Binding Studies at Dopamine, Serotonin and Norepinephrine Transport Sites in the Rat Brain. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1176–1186.
(22) Cline, E. J.; Scheffel, U.; Boja, J. W.; Carroll, F. I.; Katz, J. L.; Kuhar, M. J. Behavioral Effects of Novel Cocaine Analogs: A Comparison with *in vivo* Receptor Binding Potency. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 1174–1179.
(23) Katz, J. L.; Izenwasser, S.; Allen, A. C.; Newman, A. H. Behavioral Effects of Novel 4′- and 4′,4″-substituted-3α-diphenylmethoxytropane Analogs. *Soc. Neurosci. Abstr.* **1994**, *20*, 1628 (no. 664.19). Newman, A. H.; Kline, R. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L. Novel 4′- and 4′,4″-substituted-3α-diphenylmethoxytropane Analogs are Potent and Selective Dopamine Uptake Inhibitors. *Soc. Neurosci. Abstr.* **1994**, *20*, 1628 (no. 664.20).
(24) 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.
(25) Madras, B. K.; Spealman, R. D.; Fahey, M. A.; Neumeyer, J. L.; Saha, J. K.; Milius, R. A. Cocaine Receptors Labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane. *Mol. Pharmacol.* **1989**, *36*, 518–524.

(26) Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D. Effects of Cocaine and Related Drugs in Nonhuman Primates. I. [^{3}H]Cocaine Binding Sites in Caudate Putamen. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 131–141.
(27) Rothman, R. B. High Affinity Dopamine Reuptake Inhibitors as Potential Cocaine Antagonists: A Strategy for Drug Development. *Life Sci.-Pharmacol. Lett.* **1990**, *46*, PL-17–PL-21.
(28) *The Merck Index*, 11th ed.; Merck and Co.: Rohway, NJ, 1989; No. 7937, p 1259.
(29) Sheldrick, G. M. *SHELXL93. Program for Crystal Refinement*; University of Gottingen: Germany, 1993.
(30) Izenwasser, S.; Werling, L. L.; Cox, B. M. Comparison of the Effects of Cocaine and Other Inhibitors of Dopamine Uptake in Rat Striatum, Nucleus Accumbens, Olfactory Tubercle, and Medial Prefrontal Cortex. *Brain Res.* **1990**, *520*, 303–309.
(31) Munson, P. J.; Rodbard, D. Ligand: A Versatile Approach for Characterization of Ligand-Binding Systems. *Anal. Biochem.* **1980**, *107*, 220–239.
(32) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 6th ed.; Iowa State University Press: Ames, IA, 1967; pp 135–171.
(33) D'Amato, R. J.; Largent, B. L.; Snowman, A. M.; Snyder, S. H. Selective Labeling of Serotonin Uptake Sites in Rat Brain by [^{3}H]Citalopram Contrasted to Labeling of Multiple Sites by [^{3}H]-Imipramine. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 364–371.
(34) Raisman, R.; Sette, M.; Pimoule, M.; Briley, M.; Langer, S. Z. High Affinity [^{3}H]Desipramine Binding in the Peripheral and Central Nervous System: A Specific Site Associated with the Neuronal Uptake of Noradrenaline. *Eur. J. Pharmacol.* **1982**, *78*, 345–351.
(35) Watson, M.; Roeske, W. R.; Yamamura, H. I. [^{3}H]Pirenzepine and [^{3}H]Quinuclidinyl Benzilate Binding to Rat Cerebral Cortical and Cardiac Muscarinic Cholinergic Sites: Characterization and Regulation of Antagonist Binding to Putative Muscarinic Subtypes. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 419–427.
(36) Hammer, R.; Giraldo, E.; Schiavi, G. B.; Monferini, G.; Ledinsky, H. Binding Profile of a Novel Cardioselective Muscarine Receptor Antagonist AF-DX 116 to Membranes of Peripheral Tissues and Brain in the Rat. *Life Sci.* **1986**, *38*, 1653–1662.

JM9503512