Structure−Activity Relationship Studies on a Novel Series of (*S*)-2*β*-Substituted 3α-[Bis(4-fluoro- or 4-chlorophenyl)methoxy]tropane Analogues for in Vivo Investigation

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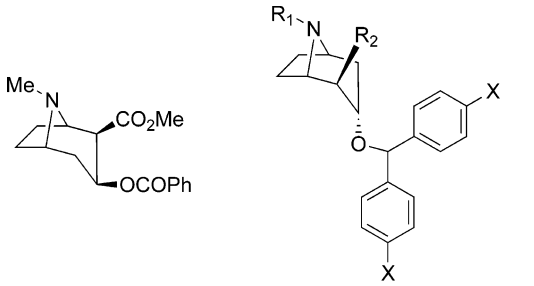
In general, 3α-(diphenylmethoxy)tropane (benztropine)-based dopamine uptake inhibitors do not demonstrate cocaine-like pharmacological activity in models of psychostimulant abuse and have been proposed as potential medications for the treatment of cocaine addiction. However, several (S)-2-carboalkoxy-substituted-3α-[bis(4-fluorophenyl)methoxy]tropane analogues were discovered to stimulate locomotor activity and substitute in subjects trained to discriminate cocaine, suggesting a role of the 2-position substituent in mediating these cocaine-like actions. Herein, we describe the synthesis of a series of novel N- and 2-substituted-3α-[bis-(4-fluoro- or 4-chlorophenyl)methoxy]tropane analogues. Most of these analogues demonstrated high affinity binding to the dopamine transporter (DAT; K_i = 1.8−40 nM), and selectivity over the other monoamine transporters and muscarinic M_1 receptors. When the (*S*)-2-carboalkoxy substituent was replaced with (*S*)-2-ethenyl, the resulting analogue **11** demonstrated the highest DAT binding affinity in the series (K_i = 1.81 nM) with DAT selectivity over serotonin transporters (SERT; 989-fold), norepinephrine transporters (NET; 261-fold) and muscarinic receptors (90-fold). When the 4′-F groups of compounds **5** (K_i = 2.94 nM) and **8** (K_i = 6.87 nM) were replaced with 4′-Cl in the (*S*)-2-carboalkoxy series, DAT binding affinities were slightly reduced (K_i = 12.6 and 14.6 nM for **6** and **7**, respectively), yet inhibition of dopamine uptake potency remained comparably high (IC_{50} range = 1.5−2.5 nM). Interestingly, the 4′-Cl analogue (±)-**6** substituted less in rats trained to discriminate cocaine than the 4′-F analogue (±)-**5**. These studies demonstrate that manipulation of the 2-, N-, and 3-position substituents in the 3α-(diphenylmethoxy)tropane class of dopamine uptake inhibitors can result in ligands with high affinity and selectivity for the DAT, and distinctive in vivo pharmacological profiles that cannot be predicted by their effects in vitro.

Introduction

Inhibition of dopamine uptake via the dopamine transporter (DAT) has been characterized as being the primary mechanism underlying the psychomotor stimulant and reinforcing actions of cocaine and has been targeted in the discovery of potential medications for the treatment of cocaine abuse (for recent reviews, see refs 1−3). Many studies have been focused on the elucidation of molecular structure and function of the DAT, as well as characterizing the binding sites of cocaine and other dopamine uptake inhibitors. Cocaine and its 3-aryl analogues have been used as molecular probes to explore the topology of the cocaine binding site(s) on the DAT.[4−12] Structure−activity relationship (SAR) studies have provided insight into the possible mode of cocaine binding to the DAT and how this may differ from other structurally diverse dopamine uptake inhibitors.[1,13,14] Exploiting these differences and further examining SAR in vivo has been a primary focus of our drug discovery efforts toward a medication to treat cocaine abuse and addiction.

Investigation of SAR within the 3α-(diphenylmethoxy)tropane class of dopamine uptake inhibitors has led to the discovery that unlike 3-aryl cocaine analogues, benztropine derivatives do not require a substituent at the 2-position of the tropane ring for binding with high affinity to the DAT.[15−19] Recent comparisons of the SAR of compounds from these two classes of dopamine uptake inhibitors further support different binding modes or domains on the DAT protein.[14,20] Further studies have revealed that the benztropine-based dopamine uptake inhibitors not only have distinct structural requirements from cocaine and its 3-aryl analogues for binding to the DAT, but also have different behavioral profiles in animal models of cocaine abuse.[21−24]

Meltzer et al. synthesized all eight stereoisomers of 2-carbomethoxy-3-[bis(4-fluorophenyl)methoxy]tropane and discovered that the *S*-(+)-enantiomer (*S*-(+)-difluoropine) was the most



1; cocaine

2; R_1 =Me, R_2 =H, X =F
3; R_1=butyl, R_2=H, X=F
4; R_1 =allyl, R_2 =H, X =F
5; R_1 =Me, R_2 =CO_2Me, X=F
6; R_1=Me, R_2 =CO_2Me, X=Cl
7; R_1=Me, R_2=CO_2Et, X=Cl
8; R_1=Me, R_2=CO_2Et, X=F

Figure 1. Chemical structures of cocaine (**1**) and 3α-[bis(4-fluorophenyl)methoxy]tropane analogues (**2**−**8**).

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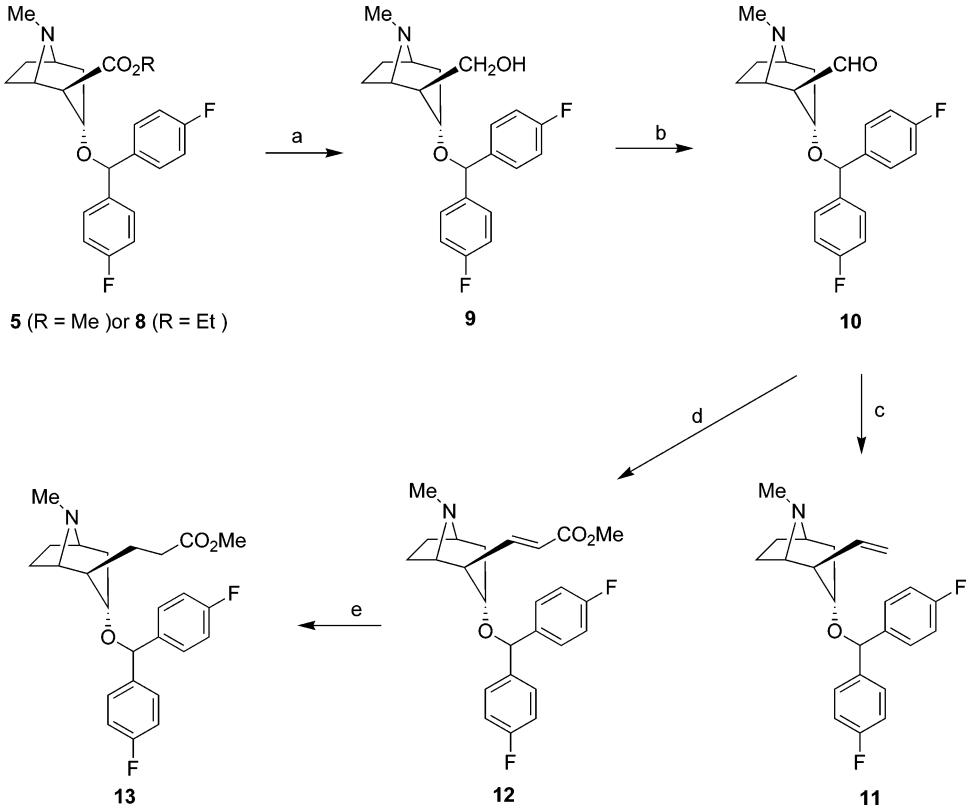
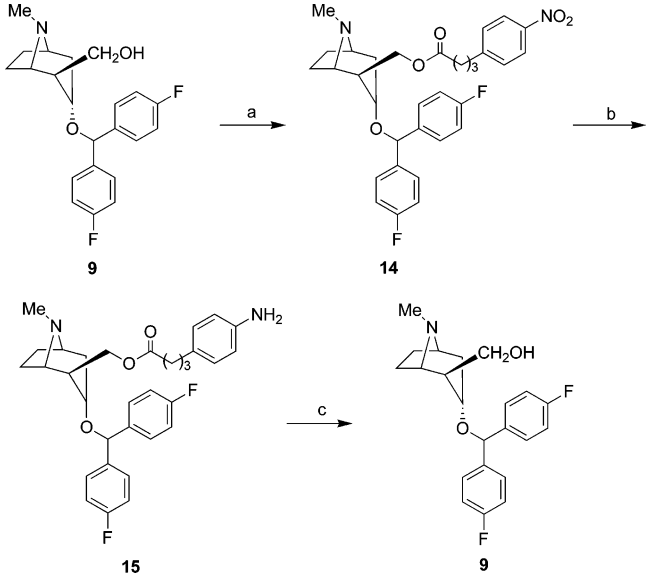
Scheme 1[a]



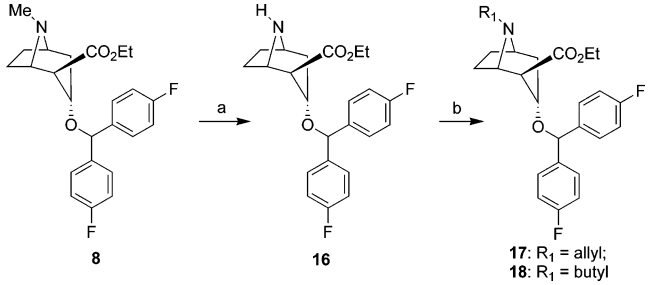
[a] Reagents and conditions: (a) $LiAlH_4$, anhyd EtO_2, 0 °C to r.t, 3 h; (b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (c) $Ph_3P^+CH_3\ Br^-$, BuLi, THF; (d) $(CH_3O)_2P(O)CH_2CO_2Me$, $^iPr_2NEt$, CH_3CN; (e) H_2, Raney Ni, MeOH.

Scheme 2[a]



[a] Reagents and conditions: (a) 4-(4-nitropheyl)butyryl chloride, Et_3N, CH_2Cl_2 0 °C to r.t; (b) H_2, Raney Ni, MeOH; (C) $LiAlH_4$, Et_2O.

Scheme 3[a]



[a] Reagents and conditions: (a) (i) ACE-Cl, Na_2CO_3, 1,2-dichloroethane, reflux, (ii) MeOH, r.t; (b) allyl bromide or butyl bromide, K_2CO_3, DMF, 50 °C.

active[19] (Figure 1, **5**). Subsequently we reported an enantioselective synthesis of a series of *S*-(+)-2*β*-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropanes and showed that these compounds were also potent dopamine uptake inhibitors.[25,26] An SAR comparison of these DAT ligands with their structurally identical *R*-(−)-2*β*-carboalkoxy-3*β*-(3,4-dichlorophenyl)tropanes demonstrated that although the *S*-(+)-2*β*-carboalkoxybenztropines showed consistently lower affinities than the identically 2-substituted *R*-(−)-3-aryltropanes in binding to the DAT, they inhibited dopamine uptake with high and equivalent potencies.[26]

To further investigate the SAR at the 2-position, a novel series of (S)-2*β*-substituted 3α-[bis(4-fluoro- or 4-chlorophenyl)-methoxy]tropanes was prepared and evaluated for binding at the DAT, SERT, NET, and muscarinic M_1 receptors. In addition, we have recently discovered that the N-substituted-3α-[bis(4-fluorophenyl)methoxy]tropanes can antagonize the effects of cocaine in rodents.[27,28] Therefore, several N-substituted 2*β*-carboethoxy analogues were prepared and evaluated for their potential use as tools for in vivo investigation.

Chemistry

S-2*β*-Carbomethoxy- and 2*β*-carboethoxy-3α-[bis(4-chlorophenyl)methoxy] tropanes (**6** and **7**) and their racemates were prepared according to our previously reported procedure.[25,26]

In Scheme 1, *S*-(+)-2*β*-carboalkoxy-3α-[bis(4-fluorophenyl)-methoxy]tropane (**5** or **8**[26]) was reduced with $LiAlH_4$ to give the corresponding alcohol **9**. Swern oxidation of the alcohol provided aldehyde **10** in nearly quantitative yield. Wittig reaction

Table 1. Binding Data for (*S*) and (±)-2-Substituted 3α-[Bis(4-fluoro- and chlorophenyl)methoxy]tropanes

compd	DAT binding K_i [a,b] (nM)	DA uptake IC_{50} [a,b] (nM)	SERT K_i [a,b] (nM)	NET K_i [a,b] (nM)	M_1 K_i [a,b] (nM)
S-**5**	2.94 ± 0.36	1.50 ± 0.2[c]	690 ± 58[c]	269 ± 39[c]	133 ± 4.2[c]
(±)-**5**[c]	21.3 ± 3.6[f]	2.94 ± 0.4	1750 ± 240	474 ± 65	302 ± 43
S-**6**	12.6 ± 0.40	2.46 ± 0.2	528 ± 39	2150 ± 325	382 ± 37
(±)-**6**	23.4 ± 1.5	NT	NT	NT	NT
S-**7**	14.6 ± 0.39	1.52 ± 0.2	1560 ± 91	3350 ± 154	3060 ± 150
(±)-**7**	22.0 ± 0.84	NT	NT	NT	NT
S-**8**	6.87 ± 0.33	1.8 ± 0.2[c]	1850 ± 270[c]	629 ± 31[c]	1890 ± 130[c]
(±)-**8**[c]	26.2 ± 3.4[f]	3.6 ± 0.4	3740 ± 490	1020 ± 120	1860 ± 190
4,4-diCl[e]	17.5 ± 0.88[f]	23.4 ± 3.0[d]	1640 ± 236[d]	2980 ± 182[d]	40.6 ± 8.0[d]
cocaine	71.8 ± 4.6	236 ± 21[g]	286 ± 38[g]	3300 ± 170[g]	61400 ± 11000[g]

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. K_i values were analyzed by PRISM. [b] Binding methods were conducted as previously reported[29,30] except that the DAT assay was run in sucrose buffer. [c] Data from ref 25 and included for reference. [d] Data from ref 29 and included for reference. [e] 3α-(bis-Cl-phenylmethoxy)tropane. [f] The K_i values for these compounds at DAT were assessed using different methods[29,30] than those used for the other compounds. In our experience the values obtained using those methods give K_i values that are approximately 3-fold higher than those obtained with the methods used for the other compounds. [g] Data from ref 30 and included for reference. NT = not tested.

of **10** with methyltriphenylphosphonium bromide gave alkene **11**, while Horner−Wadsworth−Emmons reaction of the aldehyde **10** with trimethyl phosphonoacetate afforded the α,β-unsaturated ester **12**, as a single crystalline isomer, which was reduced by catalytic hydrogenation to give compound **13**.

In Scheme 2, compound **9** was converted to the ester **14** by reaction with 4-(*p*-nitrophenyl)butyric acid chloride, which was prepared from the carboxylic acid in refluxing $SOCl_2$. Catalytic reduction of the nitro group of **14** with 10% Pd/C was unsuccessful. However, reduction with Raney Ni successfully produced the aniline **15** in good yield. The optical rotation for **14** was $[\alpha]_{25}^{D}$ +3.5° (c = 1.0, $CHCl_3$); however, when it was converted to **15**, the rotation changed from "(+)" to "(−)" (**15**: $[\alpha]_{25}^{D}$ −5.4° (c = 1.0, $CHCl_3$). To ensure that the *S*-stereochemistry remained unchanged, compound **15** was reduced to the alcohol **9** with $LiAlH_4$. This product was analytically identical to the original **9**, and measurement of the optical rotation showed it changed back to (+) from (−). This experiment confirmed that no enantiomeric conversion of **15** had taken place during hydrogenation and that the optical rotation conversion was independent of the stereochemistry at the asymmetric C-2.

In Scheme 3, N-demethylation of the ethyl ester **8**[25,26] using the Oloffson procedure with ACE-Cl, resulted in the formation of the N-nor product **16**, which was easily converted to **17** and **18** by treatment with the corresponding alkyl bromides in DMF under mildly basic conditions.

Results and Discussion

Binding affinities of all novel compounds were evaluated in radiolabeled ligand displacement assays for DAT, SERT, NET, and muscarinic M_1 receptors in rat brain. The methods for these in vitro assays are briefly described in the Experimental Methods Section, and results are shown in Table 1. The methods for the SERT, NET, and M_1 binding assays have been previously described.[29,30] Pirenzepine, though not exclusively labeling M_1 receptors, has been shown to be a useful ligand for M_1 receptor identification.[31] Inhibition of [^{3}H]-dopamine uptake in rat synaptosomes was also evaluated using a previously reported procedure[29,30] for compounds (*S*)-**6** and (S)-**7** for comparison with the 4-F analogues (*S*)-**5** and (*S*)-**8**, and their racemates.

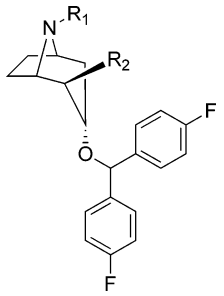
We found that replacement of the 4-F groups in compounds (*S*)-**5** and (*S*)-**8** with a Cl group, (*S*)-**6** and (*S*)-**7**, modestly reduced DAT binding affinities by 4- and 2-fold, respectively. However, the IC_{50} values for inhibiting dopamine uptake changed minimally, and remained relatively high (IC_{50} = 2.5 and 1.5 nM for (*S*)-**6** and (*S*)-7, respectively).

Compounds (±)-**5** and (±)-**6** were selected to compare their cocaine-like subjective effects in rats trained to discriminate cocaine from saline injections. The racemates were used for behavioral testing because multigram quantities needed for behavioral evaluation were more conveniently synthesized as racemates. Further, these compounds (Table 1) and previously prepared racemates[25] demonstrate high DAT affinity (though approximately one-half as potent as the *S*-enantiomers) and selectivity for the DAT over other monoamine transporters. Compound (±)-**5** fully substituted for the cocaine discriminative stimulus (Figure 2, top left panel), whereas its parent compound (**2**), which lacks a substitution in the 2 position, as previously reported,[21] produced approximately 65% cocaine-appropriate responding. Extending the pretreatment time for administration of **2** was previously reported to result in full substitution for cocaine.[21] In contrast to (±)-**5** and despite having high binding affinity at the DAT, the 4′4″-diCl analogue, (±)-**6,** did not produce cocaine-like behavioral effects (Figure 2 top right). The lack of substitution for cocaine occurred despite the study of a range of doses from those having no effects to those that virtually eliminated responding (Figure 2, bottom right panel). A study of the time course of effects of (±)-**6** (Desai et al., submitted) indicated that time only diminished its cocaine-like behavioral activity.

To further explore SAR at the N-position of the F-analogues, a series of N-substituted (S)-2-substituted-3α-[bis(4-fluorophenyl)methoxy]tropane analogues were synthesized and evaluated for binding affinities at the DAT, NET, SERT, and muscarinic M_1 receptors (Table 2). Most of the compounds in this series demonstrated high affinity for the DAT. Increasing the carbon chain length at the 2-position of the tropane ring did not significantly affect that affinity (e.g. **12**, DAT K_i = 4.69 nM, and **13**, DAT K_i = 3.74 nM, compared to **5,** DAT K_i = 2.94 nM). However, increasing the steric bulk of this substituent did result in an approximate 10-fold reduction in DAT binding affinity (e.g. **14** and **15**). DAT binding affinities were retained by conversion of the ester to alcohol or alkene (**9** and **11**), indicating that the ester function is not required for this class of ligands to bind to the DAT with high affinity. In fact, compound **11** (K_i = 1.81 nM) showed the highest DAT binding affinity in this series. This observation is consistent with previously described results with the 3-aryltropanes, wherein at the 2-position H-bonding interactions with the DAT protein are not required for high affinity binding and may indicate that lipophilicity is more important than H-bonding interactions.[10,32]

N-Demethylation (**16**) and substitution of the *N*-methyl of compound **8** with other alkyl groups (**17**, **18**) resulted in comparable DAT binding affinities. These compounds also had affinities at the DAT that were comparable to the analogues **3** and **4** that had no substitution in the 2-position (Table 2). However, the 2-substituted compounds demonstrated much

Table 2. Binding Affinities of Novel S-2-Substituted 3α-[Bis(4-fluorophenyl)methoxy]tropanes at the DAT, SERT, NET, and Muscaranic M_1 Receptors



compound	R_1	R_2	DAT $K_i \pm$ SEM (nM)[a]	SERT $K_i \pm$ SEM (nM)[a]	NET $K_i \pm$ SEM (nM)[a]	M_1 $K_i \pm$ SEM (nM)[a]
2, (AHN 1-055)	Me	H	4.11 ± 0.50[b]	3260 ± 110[b]	844 ± 57	11.6 ± 0.93[b]
3 (JHW 007)	butyl	H	9.70 ± 0.92[c]	1350 ± 150[c]	1490 ± 190[c]	399 ± 27[c]
4, (AHN 2-005)	allyl	H	8.79 ± 0.52[c]	2850 ± 63[c]	1570 ± 160[c]	177 ± 21[c]
9	Me	CH_2OH	3.40 ± 0.32	910 ± 64.5	983 ± 97	109 ± 15
11	Me	$CH{=}CH_2$	1.81 ± 0.21	1790 ± 110	473 ± 64	163 ± 23
12	Me	$CH{=}CHCO_2Me$	4.69 ± 0.60	572 ± 51	269 ± 35	1380 ± 81
13	Me	$CH_2CH_2CO_2Me$	3.74 ± 0.07	1070 ± 120	454 ± 43	3110 ± 440
14	Me	$CH_2OCO(CH_2)_3C_6H_4NO_2$-p	39.9 ± 4.3	3780 ± 390	2130 ± 160	708 ± 100
15	Me	$CH_2OCO(CH_2)_3C_6H_4NH_2$-p	27.6 ± 0.85	1390 ± 150	440 ± 18	342 ± 34
16	H	CO_2Et	8.87 ± 1.0	2150 ± 220	563 ± 74	17100 ± 2500
17	allyl	CO_2Et	11.0 ± 0.94	2280 ± 130	935 ± 110	11400 ± 1700
18	butyl	CO_2Et	8.18 ± 0.17	2500 ± 330	580 ± 16	3200 ± 300

[a] Binding methods were conducted as previously reported[29,30] except that the DAT assay was run in sucrose buffer. [b] Included for reference (ref 34). [c] Included for reference (ref 16).

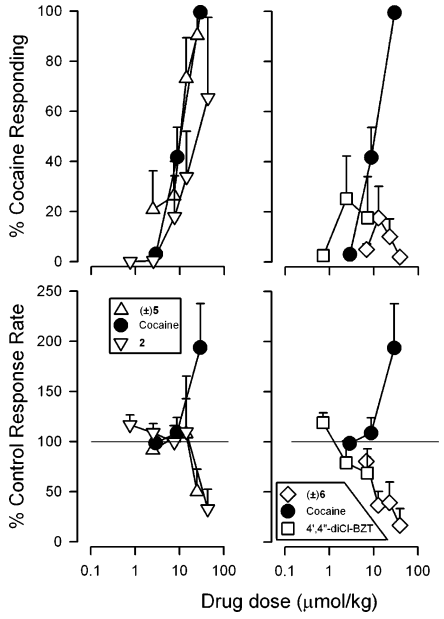


Figure 2. Effects of cocaine (**1**), **2**, (±)-**5,** (±)-**6**, and 4″,4″-diCl-BZT (3α-(bis-Cl-phenylmethoxy)tropane**)** in rats trained to discriminate injections of cocaine (10 mg/kg; 29.4 μmol/kg) from saline. Ordinates: percentage of responses on the cocaine-appropriate lever (top panels) or rates at which responses were emitted as a percentage of responses after saline administration (bottom panels). Abscissae: drug dose in μmol/kg (log scale). Each point represents the effect in six rats. The percentage of responses emitted on the cocaine-appropriate lever was considered unreliable and not plotted, if fewer than half of the subjects responded at that dose. Note that **2** failed to substitute for cocaine with a pretreatment time of 5 min, whereas (±)-**5** substituted for the discriminative stimulus effects of cocaine. In contrast, neither (±)-**6** nor 4″,4″-diCl-BZT fully substituted for cocaine. Effects of **2** and 4″,4″-diCl-BZT were previously published.[21]

higher selectivity for the DAT over muscarinic receptors than did the 2-unsubstituted compounds (Table 3). These data suggest that the 2-carboalkoxy group may not be playing a significant role in binding to the DAT, but clearly and adversely affects binding at muscarinic receptors.

Table 3. Binding Affinity Selectivity at DAT over SERT, NET, and Muscarinic M_1 Receptors

compound	SERT/DAT	NET/DAT	M_1/DAT
2	793	148	3
3	139	154	41
4	324	179	20
5	234	92	45
6	42	171	30
8	269	92	275
9	259	279	31
11	989	261	90
12	122	57	294
13	286	121	832
14	95	53	18
15	50	16	12
16	242	63	1928
17	207	85	1036
18	306	71	391
4,4-diCl	94	170	2
cocaine	4	46	853

In general, none of these ligands demonstrated high binding affinities to the norepinephrine (NET) and serotonin (SERT) transporters. Therefore, with the exception of **14** and **15**, which showed relatively reduced DAT/M_1 selectivity, all new compounds showed high binding selectivity to the DAT over SERT, NET, and muscarinic M_1 receptors. Notably, compound **16** is the most DAT/M_1 selective benztropine reported to date (Table 3).

In summary, a series of novel *S*-2*β*-substituted-3α-[bis(4-fluorophenyl)methoxy]tropanes and several *N*- or bis(4-chlorophenyl)methoxy-substituted-(*S*)-2*β*-carboethoxy analogues were synthesized. Binding evaluation revealed that all of the compounds showed high affinity for the DAT and demonstrated high DAT selectivity over the NET, SERT, and muscarinic M_1 receptor. Several structurally variant substitutions at the 2-position of the tropane ring were well tolerated, although groups with steric bulk caused a slight decrease in DAT binding affinity, suggesting some limitations in tolerance to steric bulk

at this position. Substitution at the tropane nitrogen, in the 2-carboethoxy series, resulted in retention of high DAT binding affinity and selectivity. Indeed, these analogues (**16**, **17**, and **18**) demonstrated the highest DAT/ M_1 selectivity reported to date for the benztropine class of dopamine uptake inhibitors. Behavioral studies demonstrated that the F-analogue (±)-**5** produced cocaine-like discriminative stimulus effects in rats. However, when the 4-Fs on the 3α-diphenylmethoxy substituent were replaced with Cl groups, the resulting (±)-**6** was unlike cocaine. A comparison of binding profiles across the monoamine transporters and muscarinic receptors between **5** and **8** and all the other benztropine analogues reported to date,[1,33] including **6** and **7** herein, does not explain the different behavioral profiles of these compounds. Hence, further investigation into the role of the 2-substituent and its affect on the rate of DAT occupancy is underway. In addition, compounds **7**, **11**, **13**, **17**, and **18** are also currently being evaluated in animal models of cocaine abuse in order to further characterize SAR in vivo and define the role of various substituents on the tropane ring, in this intriguing class of dopamine uptake inhibitors.

Experimental Section

All chemicals and reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis, Inc., and used without further purification. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 or a Varian Mercury Plus 400 instruments. Proton chemical shifts are reported as parts per million (δ , ppm) relative to tetramethylsilane (0.00 ppm) as an internal standard. Coupling constants are measured in hertz. Chemical shifts for ^{13}C NMR spectra are reported as δ relative to deuterated chloroform ($CDCl_3$, 77.5 ppm, CD_3OD 49.3). Infrared spectra were recorded as a neat film on NaCl plates with a Perkin-Elmer Spectrum RX I FT-IR system. 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 an HP-1 (cross-linked methylsilicone 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 min, programmed to 295 °C at 15 °C/min, and maintained at 295 °C for 10 or 23 min. Microanalyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and agree with ± 0.4% of calculated values). All column chromatography was performed using silica gel (Merck, 230−400 mesh, 60 Å).

***S*-(+)-2β-Carbomethoxy-3α-[bis(4-chlorophenyl)methoxy]tropane (6).** *S*-(+)-alloecgonine methyl ester[26] (309 mg, 1.55 mmol), 4,4′-dichlorobenzhydrol (786 mg, 3.10 mmol), *p*-toluenesulfonic acid monohydrate (443 mg, 2.33 mmol), and benzene (15 mL) were placed in a 50 mL round-bottom flask fitted with a Dean−Stark trap and condenser. The reaction mixture was heated to reflux for 24 h. The solvent was then removed, and the residue was diluted with water (20 mL), basified with NH_4OH to pH 9, and extracted with $CHCl_3$ (3 × 20 mL). The combined organic layer was dried (K_2CO_3) and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH/NH_4OH, 97:3:1) to afford *S*-**6** (472 mg, 70%) as an oil, which solidified slowly to a white solid after standing at room temperature. Mp: 109−110 °C (lit.[33] mp: 110−112 °C). $[\alpha]_{25}^{D}$ +17.3° (c = 1.0, $CHCl_3$); IR: 1732, 1069, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.17−1.68 (m, 6H), 2.18 (s, 3H), 2.71 (m, 1H), 3.09 (m, 1H), 3.59 (m, 1H), 3.68(s, 3H), 3.95 (d, J = 4.8 Hz, 1H), 5.32 (s, 1H), 7.30−7.12 (m, 8H), ppm; GC-MS (*m*/*z*) 433 (M+); Anal. ($C_{23}H_{25}NCl_2O_3$) for C, H, N.

(±)-6 was prepared by the same procedure in 75% yield. Mp: 108−110 °C. The IR, ^{1}H NMR, and GC-MS spectra for **(±)-6** were identical to *S*-(+)-**6**. Anal. ($C_{23}H_{25}NCl_2O_3$) for C, H, N.

***S*-(+)-2β-Carboethoxy-3α-[bis(4-chlorophenyl)methoxy]tropane (7). S−(+)-**7** was prepared in 78% yield according to the above procedure. Mp: 88−89.5 °C. $[\alpha]_{24}^{D}$ +17.0° (c = 1.0, $CHCl_3$); IR: 1733, 1222, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7.0 Hz, 3H), 2.16−1.65 (m, 6H), 2.18 (s, 3H), 2.68 (m, 1H), 3.08 (m, 1H), 3.58 (m, 1H), 3.97 (d, J = 4.8 Hz, 1H), 4.22−4.00 (m, 2H), 5.30 (s, 1H), 7.30−7.16 (m, 8H), ppm; GC-MS (*m*/*z*) 447 (M+); anal. ($C_{24}H_{27}NCl_2O_3$) for C, H, N.

(±)-7 was prepared in 74% yield by the same procedure. Mp: 87.5−89.5 °C. The IR, ^{1}H NMR, and GC-MS spectra for **(±)-7** were identical to *S*-(+)-**7**. Anal. ($C_{24}H_{27}NCl_2O_3$) for C, H, N.

***S*-(+)-2β-Hydroxymethyl-3α-[bis(4-fluorophenyl)methoxy]-tropane (9).** A solution of **5** or **8**[26] (3.03 mmol) in 8 mL of anhydrous diethyl ether was added dropwise to a suspension of $LiAlH_4$ (115 mg, 3.03 mmol) in the same solvent (8 mL) at 0 °C. After the addition, the ice−H_2O bath was removed and the reaction mixture was allowed to warm to room temperature for 3 h. H_2O (0.3 mL) was added carefully at 0 °C, followed by the addition of 0.5 mL of aqueous NaOH (2 N). The resulting mixture was filtered, the filtrate was dried (K_2CO_3) and filtered, and the ether was removed in vacuo. The residue was purified by flash column chromatography (eluting with 5−10% ($CHCl_3$/MeOH/NH_4OH; CMA) to give the product (1.02 g, 90%) as a colorless oil. $[\alpha]_{25}^{D}$ +37.5° (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.14−1.79 (m, 7H), 2.22 (s, 3H), 3.12 (m, 1H), 3.24 (m, 1H), 3.46 (dd, J = 2.8, 10.4 Hz, 1H), 3.56 (d, J = 6.0 Hz, 1H), 3.93 (dd, J = 2.4, 10.0 Hz, 1H), 5.38 (s, 1H), 6.98 (m, 4H), 7.22 (m, 4H), ppm; Anal. ($C_{22}H_{25}NF_2O_2$) for C, H, N.

***S*-(+)-2β-Formyl-3α-[bis(4-fluorophenyl)methoxy]tropane (10).** To a solution of oxalyl chloride (1.64 mL, 2.0 M solution in CH_2Cl_2, 3.28 mmol) under argon at −78 °C was added a solution of DMSO (469 mg, 6.02 mmol) in dry CH_2Cl_2 (1 mL). After 0.5 h, a solution of the alcohol **9** (1.02 g, 2.73 mmol) in dry CH_2Cl_2 (3 mL) was added and the reaction mixture was stirred for 1 h at −78 °C, followed by addition of triethylamine (1.71 mL, 12.3 mmol) at the same temperature. The reaction mixture was then allowed to warm to room temperature and diluted with H_2O (20 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried (K_2CO_3) and concentrated to yield the aldehyde (994 mg, 98%). IR: 1723, 1222, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.21−1.58(m, 7H), 2.23(s, 3H), 3.06(m, 1H), 3.57(m, 1H), 3.99(m, 1H), 5.37(s, 1H) 6.98(m, 4H), 7.24(m, 4H), 9.59(s, 1H), ppm. The aldehyde was used in the following steps without further purification.

***S*-(+)-2β-Ethenyl-3α-[bis(4-fluorophenyl)methoxy]tropane (11).** Butyllithium (0.22 mL, 2.5 M solution in hexane, 0.55 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (195 mg, 0.55 mmol) in dry THF (3 mL) at 0 °C, under argon. The resulting yellow-orange solution was stirred for 30 min, and the ice−H_2O bath was then removed. The crude aldehyde **10** (169 mg, 0.46 mmol) in 2 mL of THF was added, and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with H_2O (20 mL), and the two layers were separated. The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried (K_2CO_3) and concentrated. The residue was purified by column chromatography to afford the product (105 mg, 62%) as an oil. $[\alpha]_{25}^{D}$ +5.6° (c = 1.0, $CHCl_3$); IR: 1222 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08−1.75 (m. 6H), 2.21 (s, 3H), 2.47 (m, 1H), 2.99 (m, 1H), 3.07 (m, 1H), 3.32 (d, J = 5.2 Hz, 1H), 4.88 (d, J = 17.2 Hz, 1H), 4.96 (d, J = 10.4 Hz, 1H), 5.37 (s, 1H), 5.96 (m, 1H), 6.98 (m, 4H), 7.30 (m, 4H), ppm; ^{13}C NMR δ 24.9, 25.4, 36.1, 42.0, 50.1, 60.6, 65.9, 74.0, 79.8, 114.1, 115.1, 115.3, 128.3, 128.5, 138.5, 140.9, 160.8, 163.3, ppm; GC-MS (*m*/*z*) 369 (M+), 368 (M − 1); Anal. ($C_{23}H_{25}NF_2O$) for C, H, N.

***S*-(+)-2β-(2′-(methoxycarbonyl)eth-1′-enyl)-3α-[bis(4-fluorophenyl)methoxy] tropane (12).** To a suspension of LiCl (80 mg, 1.89 mmol) in dry acetonitrile (8 mL) at room temperature under an argon atmosphere were added trimethyl phosphonoacetate (343 mg, 1.89 mmol), *N*,*N*-diisopropylethylamine (203 mg, 1.57 mmol), and the aldehyde (**10**, 583 mg, 1.57 mmol). The reaction mixture was allowed to stir for 24 h, acetonitrile was then removed under reduced pressure. The residue was diluted with H_2O (20 mL)

and extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried (K_2CO_3) and concentrated. The crude product was purified by column chromatography (3% CMA) to afford the product (580 mg, 86%) as a colorless oil, which solidified slowly to a colorless solid Mp 80−82 °C. $[\alpha]_{25}^{D}$ +26.5° (*c* = 1.0, $CHCl_3$); IR: 1718, 1222, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.16−1.78 (m, 6H), 2.20 (s, 3H), 2.58 (d, *J* = 8.0 Hz, 1H), 3.12−3.00 (m, 2H), 3.34 (d, *J* = 6.0 Hz, 1H), 3.70 (s, 3H), 5.35 (s, 1H), 5.68 (dd, *J* = 1.2, 16 Hz, 1H), 7.08−6.97 (m, 5H), 7.24 (m, 4H), ppm; ^{13}C NMR δ 25.4, 25.9, 36.4, 42.3, 49.4, 51.8, 60.9, 65.4, 73.6, 80.4, 115.5, 115.7, 120.8, 128.5, 128.6, 138.3, 151.0, 161.0, 163.4, 167.2, ppm; GC-MS(*m*/*z*) 427 (M+); Anal. ($C_{25}H_{27}NF_2O_3$) for C, H, N.

***S*-(+)-2β-(2′-(Methoxycarbonyl)ethyl)-3α-[bis(4-fluorophenyl)-methoxy] tropane (13).** A large spatula tip of Raney Ni (in H_2O) was placed in a Parr bottle and washed (×3) with MeOH. The unsaturated compound **12** (580 mg, 1.36 mmol) in MeOH (40 mL) was added, and the mixture was shaken under an atmosphere of H_2 (40 psi) for 1.5 h. GC-MS showed the complete absence of starting material. The reaction mixture was then filtered, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (Et_2O/ Et_3N = 95/5) to give (510 mg. 88%) as an oil. $[\alpha]_{25}^{D}$ +24.8° (*c* = 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.06−1.48(m, 9H), 2.17(t, *J* = Hz, 2H), 2.21(s, 3H), 2.94(m, 1H), 3.03 (m, 1H), 3.16 (d, *J* = 5.2 Hz, 1H), 3.66 (s, 3H), 5.34 (s, 1H), 6.99 (m, 4H), 7.25 (m, 4H), ppm; ^{13}C NMR δ 24.8, 25.9, 28.3, 32.6, 35.9, 42.4, 45.6, 51.9, 61.3, 65.3, 73.1, 79.7, 115.4, 115.6, 128.5, 128.6, 138.6, 138.7, 160.9, 163.3, 174.1, ppm; GC-MS(*m*/*z*) 429 (M+); Anal. ($C_{25}H_{29}NF_2O_3$) for C, H, N.

***S*-(+)-2β-{[4-(4′-Nitrophenyl)butryl]oxymethyl}-3α-[bis(4-fluorophenyl) methoxy]tropane (14).** 4-(4′-Nitrophenyl)butyric acid (414 mg, 1.98 mmol) in $SOCl_2$ (8 mL) was stirred at reflux for 3 h. Excess of $SOCl_2$ was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (20 mL). To the solution was added alcohol **9** (492 mg, 1.32 mmol), followed by the slow addition of triethylamine (1.1 mL, 7.9 mmol) at 0 °C. The reaction mixture was then allowed to stir at room temperature for 3 h. The mixture was diluted with H_2O (30 mL), the two layers were separated, and the aqueous layer was further extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (K_2CO_3) and concentrated. The residue was purified by column chromatography (eluting with Et_2O/Et_3N = 97/3) to give the product (644 mg, 87%) as an oil. $[\alpha]_{25}^{D}$ +3.5° (*c* = 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.08−1.62 (m, 11H), 2.15 (s, 3H, N−CH_3), 2.72 (t, *J* = 7.6 Hz, 2H, $COCH_2$), 3.07−2.98 (m, 2H), 3.30 (d, *J* = 5.2 Hz, 1H), 4.00 (dd, *J* = 9.2, 11.2 Hz, 1H, CH_2O), 4.09 (dd, *J* = 6.0, 11.2 Hz, 1H, CH_2O), 5.34 (s, 1H, $OCHAr_2$), 6.96 (m, 4H, Ar−H), 7.24 (m, 4H, Ar−H), 7.32 (d, *J* = 8.8 Hz, 2H, Ar−H), 8.15 (d, *J* = 8.8 Hz, 2H, Ar−H), ppm; ^{13}C NMR δ 24.2, 25.5, 25.9, 33.3, 34.9, 35.4, 45.8, 60.8, 62.8, 66.2, 70.1, 79.4, 115.1, 115.3, 123.7, 128.3, 128.4, 129.2, 138.4, 146.5, 149.2, 160.8, 163.2, 172.8, ppm; Anal. ($C_{32}H_{34}N_2F_2O_5$) for C, H, N.

***S*-(−)-2β-{[4-(4′-Aminophenyl)butyryl]oxymethyl}-3α-[bis(4-fluorophenyl) methoxy]tropane (15).** A spatula tip of Raney Ni (in H_2O) was placed in a Parr bottle and washed (×3) with MeOH. A solution of the nitro compound **14** (523 mg, 0.98 mmol) and MeOH/EtOAc (mL/mL) was shaken under an atmosphere of H_2 (30 psi) overnight. TLC showed complete absence of starting material. The mixture was then filtered, and the filtrate was concentrated. The residue was purified by column chromatography (5% CMA) to give the product as an oil. $[\alpha]_{25}^{D}$ −5.4° (*c*=1.0, $CHCl_3$); IR: 1727, 1603, 1221, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.18−1.63 (m, 11H), 2.15 (s, 3H), 2.51 (t, *J* = 7.6 Hz, 2H), 3.07−2.98 (m, 2H), 3.30 (d, *J* = 5.2 Hz, 1H), 3.70−3.40 (brs, 2H, NH_2), 3.95 (dd, *J* = 9.2, 11.0 Hz, 1H), 4.10 (dd, *J* = 6.0, 11.0 Hz, 1H), 5.32 (s, 1H), 6.63 (d, *J* = 8.8 Hz, 2H), 6.96 (m, 6H), 7.22 (m, 4H), ppm; ^{13}C NMR δ 24.6, 25.7, 27.0, 33.8, 34.5, 35.9, 42.1, 45.8, 61.1,63.0, 66.2, 70.4, 79.6, 115.3, 115.5, 128.6, 128.7, 129.5, 131.6, 138.6, 144.7, 161.0, 163.4, 173.7, ppm; Anal. ($C_{32}H_{36}N_2F_2O_3$) for C, H, N.

***S*-(+)-*N*-Nor-2β-Carboethoxy-3α-[bis(4-fluorophenyl)methoxy]tropane (16).** Compound **8** (652 mg, 1.57 mmol) was dissolved in anhydrous 1,2-dichloroethane (10 mL). To the solution were added 1-chloroethyl chloroformate (ACE-Cl, 0.68 mL, 6.28 mmol) and Na_2CO_3 (833 mg, 7.85 mmol), and the mixture was warmed to reflux for 3 h. TLC showed complete absence of starting material. After cooling to room temperature, the reaction mixture was filtered. The solvent in the filtrate was removed in vacuo, and the residue was dissolved in MeOH (20 mL). The reaction mixture was then stirred at reflux for 1 h. MeOH was removed in vacuo. The residue was diluted with H_2O (50 mL), basified to pH 9 with $NaHCO_3$, and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was dried (K_2CO_3) and concentrated. The residue was purified by column chromatography (eluting with 5% CMA) to give the product (610 mg, 97%) as an oil. $[\alpha]_{25}^{D}$ +33.8° (*c* = 1.0, $CHCl_3$); IR: 3326, 1728, 1212, cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.97−1.65 (m, 5H), 2.22−2.06 (m, 2H), 2.65 (s, 1H), 3.48 (m, 1H), 3.79 (m, 1H), 3.83 (d, (*J* = Hz, 1H), 4.17−4.04 (m, 2H), 5.37 (s, 1H), 7.00 (dd, *J* = Hz, 4H), 7.25 (m, 4H), ppm; ^{13}C NMR δ 14.6, 28.9, 29.2, 36.0, 50.6, 53.5, 55.8, 51, 71.2, 80.6, 115.4, 115.6, 128.5, 128.7, 138.3, 161.0, 163.4, 173.2, ppm; GC-MS (*m*/*z*) 401 (M+); Anal. ($C_{23}H_{25}NF_2O_3$) for C, H, N.

***S*-(+)-*N*-Allyl-2β-carboethoxy- 3α-[bis(4-fluorophenyl)methoxy]-tropane (17).** Compound **16** (85 mg, 0.21 mmol) and allyl bromide (51 mg, 0.42 mmol) were combined in DMF (3 mL). To the solution was added K_2CO_3 (58 mg, 0.42 mmol), and the mixture was heated to 50 °C overnight. The reaction was quenched with H_2O (20 mL), and the mixture was extracted with $CHCl_3$ (3 × 30 mL). The combined organic layer was dried (K_2CO_3) and concentrated. The residue was purified by column chromatography (eluting with 3% CMA) to give the product (86 mg, 92%) as an oil. $[\alpha]_{25}^{D}$ +23.2° (*c* = 1.0, $CHCl_3$); IR: 1730, 1221, cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (t, *J* = 7.2 Hz, 3H), 2.18−1.58 (m, 6H), 2.69 (m, 1H), 2.81 (dd, *J* = 7.2, 13.6 Hz, 1H), 2.94 (dd, *J* = 5.6, 13.6 Hz, 1H), 3.15 (m, 1H), 3.68 (m, 1H), 4.00 (d, *J* = 2.0 Hz, 1H), 4.18−4.02 (m, 2H), 5.12−5.02 (m, 2H), 5.34 (s, 1H), 5.78 (m, 1H), 6.98 (m, 4H), 7.25 (m, 4H), ppm; ^{13}C NMR δ 14.2, 24.6, 25.9, 36.3, 51.8, 56.7, 59.8, 60.2, 60.4, 70.7, 80.3, 115.2, 115.4116.3, 128.3, 128.4, 136.8, 138.3, 138.4, 160.8, 163.3, 172.5, ppm; GC-MS (*m*/*z*) 441 (M+); Anal. ($C_{26}H_{29}NF_2O_3$) for C, H, N.

***S*-(+)-*N*-Butyl-2β-carboethoxy-3α-[bis(4-fluorophenyl)methoxy]-tropane (18).** Compound **18** was obtained in 87% yield by the procedure described above for **17** using *n*-butyl bromide as the alkylating agent. $[\alpha]_{25}^{D}$ +20.4° (*c* = 1.0, $CHCl_3$); IR: 1729, 1603, 1223, cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.40−1.20 (m. 4H), 2.21−1.74 (m, 8H), 2.68 (m, 1H), 3.12 (m, 1H), 3.69 (m, 1H), 3.99 (d, *J* = 4.8 Hz, 1H), 4.09 (m, 2H), 5.34 (s, 1H), 6.98 (m, 4H), 7.23 (m, 4H), ppm; ^{13}C NMR δ 14.3, 14.4, 20.7, 24.9, 26.2, 31.5, 36.5, 52.1, 53.5, 60.6, 60.7, 61.0, 71.1, 80.5, 110.0, 115.4, 115.6, 128.5, 128.6, 138.8, 161.0, 163.5, 172.7, ppm; GC-MS (*m*/*z*) 457 (M+); Anal. ($C_{27}H_{33}NF_2O_3$) for C, H, N.

Biological Assays. Dopamine Transporter Binding Assay. Brains from male Sprague−Dawley rats weighing 200−225 g (Taconic Labs) were removed, and striatum was dissected and quickly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of ice cold modified sucrose phosphate buffer (0.32 M sucrose, 7.74 mM Na_2HPO_4, 2.26 mM NaH_2PO_4, pH adjusted to 7.4) using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 20000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 10 mg/mL. Ligand binding experiments were conducted in assay tubes containing 0.5 mL sucrose phosphate buffer for 120 min on ice. Each tube contained 0.5 nM ^3H WIN 35428 (specific activity 84 Ci/mmol) and 1.0 mg striatal tissue (original wet weight). Nonspecific binding was determined using 0.1 mM cocaine HCl. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% PEI (polyethyleneimine), using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, MD). The filters were washed twice with 5 mL cold buffer and transferred to scintillation vials. Beckman

Ready Safe (3.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Data were analyzed by using GraphPad Prism software (San Diego, CA).

Serotonin Transporter Binding Assay. Brains from male Sprague−Dawley rats weighing 200−225 g (Taconic Labs, Germantown, NY) were removed, and midbrain was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25 °C), using a Brinkman Polytron and centrifuged at 50000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 15 mg/mL. Ligand binding experiments were conducted in assay tubes containing 0.5 mL of buffer for 60 min at room temperature. Each tube contained 1.4 nM [^{3}H]citalopram (Amersham Biosciences, Piscataway, NJ) and 1.5 mg of midbrain tissue (original wet weight). Nonspecific binding was determined using 10 mM fluoxetine. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.3% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, MD). The filters were washed twice with 3 mL of cold buffer and transferred to scintillation vials. Beckman Ready Value (3.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Each compound was tested with concentrations ranging from 0.01 nM to 100 mM for competition against binding of [^{3}H]Citalopram, in at least three independent experiments, each performed in triplicate. Data were analyzed with GraphPad Prism software (San Diego, CA).

Norepinephrine Transporter Binding Assay. Brains from male Sprague−Dawley rats weighing 200−225 g (Taconic Labs, Germantown, NY) were removed, and frontal cortex was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25 °C), using a Brinkman Polytron and centrifuged at 50000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 80 mg/mL. Ligand binding experiments were conducted in assay tubes containing 0.5 mL of buffer for 60 min at 0−4 °C. Each tube contained 0.5 nM [^{3}H]nisoxetine (PerkinElmer Life Sciences, Boston, MA) and 8 mg of frontal cortex tissue (original wet weight). Nonspecific binding was determined using 1 mM desipramine. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, MD). The filters were washed twice with 3 mL of cold buffer and transferred to scintillation vials. Beckman Ready Value (3.0 mL) was added, and the vials were counted using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Each compound was tested with concentrations ranging from 0.01 nM to 100 mM for competition against binding of [^{3}H]nisoxetine, in at least three independent experiments, each performed in triplicate. Data were analyzed by using GraphPad Prism software (San Diego, CA).

Muscarinic M_1 Binding Assay. Whole frozen rat brains excluding cerebellum (Taconic, Germantown, NY) were thawed in ice-cold buffer (10 mM Tris-HCl, 320 mM sucrose, pH 7.4) and homogenized with a Brinkman polytron in a volume of 10 mL/g of tissue. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The resulting supernatant was then centrifuged at 10000*g* for 20 min at 4 °C. The resulting pellet was resuspended in a volume of 1 g/5 mL in 10 mM Tris buffer (pH 7.4). Assays were conducted in binding buffer (10 mM Tris-HCl, 5 mM $MgCl_2$). The total volume in each tube was 0.5 mL, and the final concentration of membrane after all additions was approximately 20 mg (original wet weight). [^{3}H]Pirenzepine (PerkinElmer Life Sciences, Boston, MA) final concentration 3 nM was added, and the incubation was continued for 1 h at 37 °C. The incubation was terminated by the addition of 3 mL of ice-cold buffer (10 mM Tris-HCl, pH 7.4) and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.5% polyethylenimine in water) using a Brandel Cell Harvester (Brandel Instruments, Gaithersburg, MD). The filters were washed with two additional 5 mL washes and transferred to scintillation vials. Beckman Ready Value Scintillation Cocktail (3 mL) was added to the vials, which were counted the next day using a Beckman liquid scintillation counter (Beckman Coulter, Fullerton, CA). Nonspecific binding was defined as binding in the presence of 10 μM QNB (quinuclidinyl benzilate). Each compound was tested with concentrations ranging from 0.01 nM to 100 μM for competition against binding of [^{3}H]pirenzepine, in at least three independent experiments, each performed in triplicate. Displacement data were analyzed with GraphPad Prism software (San Diego, CA).

Dopamine Uptake Assay. The tissue was homogenized in ice cold buffer (5 mM HEPES, 0.32 M sucrose) using 10 strokes with a Teflon glass homogenizer followed by centrifugation at 1000*g* for 10 min at 4 °C. The supernatant was saved and recentrifuged at 10000*g* for 20 min at 4 °C. The supernatant was then discarded, and the pellet was gently resuspended in ice cold incubation buffer (127 mM NaCl, 5 mM KCl, 1.3 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.498 mM HEPES acid, 10 mM d-glucose, 1.14 mM L-ascorbic acid, pH 7.4) and placed on ice for 15 min. The synaptosomal tissue preparation was incubated in buffer in glass test tubes at 37 °C to which 10 μM pargyline and either the drug being tested or no drug was added, as appropriate. After a 10 min preincubation in the presence of drug, [^{3}H] dopamine (final concentration, 0.5 nM) (Amersham Biosciences, Piscataway, NJ) was added to each tube and the incubation was carried on for 5 min. The reaction was terminated by the addition of 3 mL of ice cold buffer to each tube and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.1% polyethylenimine in water) using a Brandel cell harvester (Brandel Instruments, Gaithersburg, MD). After filtration, the filters were washed with two additional 5 mL washes and transferred into scintillation vials. Beckman Ready Value (Beckman-Coulter Instruments, Fullerton, CA) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). The reported values represent specific uptake from which nonspecific uptake was subtracted (defined as uptake in the presence of 100 μM (−)cocaine HCl). Data were analyzed using the nonlinear regression analysis of GraphPad Prism Software, (San Diego CA).

Drug Discrimination Procedure. Cocaine Discrimination. Male Sprague−Dawley rats (Charles River, Wilmington, MA) weighing approximately 350 g were individually housed with free access to water under a 12 h light/dark cycle (lights on 07.00 h). All testing was between 09:00 and 15:00 h. Rats were fed approximately 15 g standard lab chow daily at least 30 min after testing.

Rats were tested in two-lever operant-conditioning chambers (modified Med Associates, model ENV 007, St. Albans, VT; inside 29.2 × 24.2 × 21 cm)) housed within light- and sound-attenuating enclosures with white noise present throughout testing. Ambient illumination of the chamber was by a lamp in the top center of the front panel. Levers were set 17 cm apart, with a pair of green light emitting diodes (LEDs) above one lever and a pair of yellow LEDs above the other. A force on the lever of 0.4 N through 1 mm was required to register a response and produced a click; reinforced responses dispensed one 45 mg pellet (BioServe, Frenchtown, NJ) into the centrally located food tray.

Rats were initially trained to press both levers under a fixed-ratio 20-response schedule of food reinforcement. Following i.p. cocaine injection, responses on only one lever were reinforced; following saline, responses on the other lever were reinforced. The assignment of cocaine- and saline-appropriate levers was counterbalanced across rats. Immediately after injection, rats were placed inside the experimental chambers and a 5 min time-out period was initiated, during which all lamps were extinguished and responding had no scheduled consequences. All lamps were then illuminated and responses on the appropriate lever were reinforced. Responses on the inappropriate lever reset the FR 20 response requirement on the appropriate lever. Each food presentation was followed by a 20 s time-out period. Sessions ended after 20 food presentations or 15 min, whichever occurred first.

Training sessions for which cocaine (C) and saline (S) injections were administered were ordered in an SCCS sequence, with test sessions conducted after consecutive SC or CS training sessions. Test sessions were conducted if the subject attained criterion performance on both of the immediately preceding saline and cocaine training sessions. The criteria were at least 85% appropriate responding overall and during the first FR of the session. Test sessions were identical with training sessions, with the exception that 20 consecutive responses on either lever were reinforced. Before test sessions, different doses of cocaine or doses of test compounds were administered.

For each rat, the overall response rate and the percentage of responses occurring on the cocaine-appropriate lever were calculated. The mean values were calculated for each measure at each drug dose tested. Data from any rat which failed to respond at a rate exceeding 0.02 responses per second was not included in the calculation of mean cocaine-appropriate responding at that dose. If less than three rats met the response rate requirement, no mean value was calculated for percentage of cocaine-appropriate responding at that dose.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Newman, A. H.; Kulkarni, S. Probes for the Dopamine Transporter: New Leads toward a Cocaine-Abuse Therapeutic − A Focus on Analogues of Benztropine and Rimcazole. *Med. Res. Rev.* **2002**, *22*(5), 429−464.

(2) Carroll, F. I. 2002 Medicinal Chemistry Division Award Address: Monoamine Transporters and Opioid Receptors. Targets for Addiction Therapy. *J. Med. Chem.* **2003**, *46*(10), 1175−1194.

(3) Dutta, A. K.; Zhang, S.; Kolhatkar, R.; Reith, M. E. A. Dopamine Transporter as Target for Drug Development of Cocaine Dependence Medications. *Eur. J. Pharmacol.* **2003**, *479*, 93−106.

(4) 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.

(5) Lewin, A. H.; Gao, Y.; Abraham, P.; Boja, J. W.; Kukar, M. J.; Carroll, F. I. 2β-Substitued Analogues of Cocaine. Synthesis and Inhibition of Binding to the Cocaine Receptor. *J. Med. Chem.* **1992**, *35*, 135−140.

(6) 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.

(7) Davies, H. M. L.; Saikali, E.; Sexton, T. Childers, S. R. Novel 2-substituted Cocaine Analogs: Binding Properties at Dopamine Transporter Sites in Rat Striatum. *Eur. J. Pharmacol.-Mol. Pharmcol. Sect.* **1993**, *244*, 93−97.

(8) Carroll, F. I.; Gray, J. L.; Abraham, P.; Kuzemko, M. A.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. 3-Aryl-2-(3′-substituted-1′,2′,4-oxadiazol-5′-yl)tropane analogues of Cocaine: Affinities at the Cocaine binding Site at the Dopamine, Serotonin and Norepinephrine Transporter. *J. Med. Chem.* **1993**, *36*, 2886−2890.

(9) Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; 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.

(10) Kozikowski, A. P.; Saiah, M. K. E.; Johnson, K. M.; Bergmann, J. S. Chemistry an Biology of the 2β-alkyl-3β-phenyl Analogues of Cocaine: Subnanomolar Affinity Ligands That Suggest New Pharmacophore Model at the C-2 Position. *J. Med. Chem.* **1995**, *38*, 3086−3093.

(11) Kotian, P.; Muscarella, S. W.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. Synthesis, Ligand Binding and Quantitative Structure−Activity Relationship Study of 3β-(4′-substituted phenyl)-2β-heterocyclic Tropanes: Evidance for an Electrostatic Interaction at the 2-Position. *J. Med. Chem.* **1996**, *39*, 2753−2763.

(12) Xu, L.; Kelkar, S. V.; Lomenzo, S. A.; Izenwasser, S.; Katz, J. L.; Kline, R. H.; Trudell, M. L. Synthesis, Dopamine Transporter Affinity, Dopamine Uptake Inhibition, and Locomotor Stimulant Activity of 2-Substituted 3β-phenyltropane Derivatives. *J. Med. Chem.* **1997**, *40*, 858−863.

(13) Reith, M. E. A.; Berfield, J. L.; Wang, L. C.; Ferrer, J. V.; Javitch, J. A. The Uptake Inhibitors Cocaine and Benztropine Differentially Alter the Conformation of the Human Dopamine Transporter. *J. Biol. Chem.* **2001**, *276*, 29012−29018.

(14) Ukairo, O. T.; Bondi, C. D.; Newman, A. H.; Kulkarni, S. S.; Kozikowski, A. P., Pan, S.; Surratt, C. K. Recognition of benztropine by the dopamine transporter (DAT) differs from that of the classical dopamine uptake inhibitors cocaine, methylphenidate and mazindol as a function of a DAT transmembrane 1 aspartic acid residue. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 575−583.

(15) Newman, A. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L. Novel 3α-Diphenylmethoxytropane Analogues are Potent Dopamine Uptake Inhibitors without Cocaine-like Behavioral Profiles. *J. Med. Chem.* 1**994**, *37*, 2258−2261.

(16) Newman, A. H.; Kline, R. H.; Allen, A. C.; George, C.; Izenwasser, S.; Katz, J. L. Novel 4′- and 4′,4″-Substituted-3α-(diphenylmethoxy)-tropane Analogues are Potent and Selective Dopamine Uptake Inhibitors. *J. Med. Chem.* **1995**, *38*, 3933−3940.

(17) Kline, R. H.; Izenwasser, S.; Katz, J. L.; Newman, A. H. 3′-Chloro-3α-(diphenylmethoxy)tropane But Not 4′-Chloro-3α-(diphenylmethoxy)tropane Produces a Cocaine-like Behavioral Profile. *J. Med. Chem.* **1997**, *40*, 851−857.

(18) Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J. L.; Kline, R. H.; Newman, A. H. Novel N-Substituted 4′,4"-Difluoro-3α-(diphenylmethoxy)-tropane Analogues: Selective Ligands for the Dopamine Transporter. *J. Med. Chem.* **1997**, *40*, 4329−4339.

(19) 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.

(20) Loland, C. J.; Desai, R. I.; Gerstbrein, K.; Sitte, H. H.; Newman, A. H.; Katz, J. L.; Gether, U. Relationship Between Conformational Changes in the Dopamine Transporter and Cocaine-like Subjective Effects of Uptake Inhibitors, *J. Biol. Chem.* **2006**, submitted.

(21) Katz, J. K.; Izenwasser, S., Kline, R. H., Allen, A. C.; Newman, A. H. Novel 3α-Diphenylmethoxytropane Analogs: Selective Dopamine Uptake Inhibitors with Behavioral Effects Distinct from Those of Cocaine. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 302−315.

(22) Katz, J. L.; Kopajtic, T.; Agoston, G. E.; Newman, A. H. Effects of N-Substituted Analogues of Benztropine: Diminished Cocaine-like Effects in Dopamine Transporter Ligands. *J. Pharmacol. Exp. Ther.* **2004**, *288*, 302−315.

(23) Woolverton, W. L.; Hecht, G. S.; Agoston, G. E.; Katz, J. L.; Newman, A. H. Further Studies of the Reinforcing Effects of Benztropine Analogs in Rhesus Monkeys. *Psychopharmacology* **2001**, *154*, 375−382.

(24) Li, S.-M.; Newman, A. H.; Katz, J. L. Place Conditioning and Locomotor Effects of N-Substituted, 4′, 4″-Difluorobenztropine Analogues in Rats. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1223−1230.

(25) Zou, M.-F.; Agoston, G. E.; Belov, Y.; Kopajtic, T.; Katz, J. L.; Newman, A. H. Enantioselective Synthesis of S-(+)-2β-Carboalkoxy-3α-[bis(4-fluorophenyl)-methoxy]tropanes as Novel Probes for the Dopamine Transporter. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1249−1252.

(26) Zou, M.-F.; Kopajtic, T.; Katz, J. L.; Newman, A. H. Structure−Activity Relationship Comparison of (*S*)-2β-Substituted 3α-[bis(4-fluorophenyl)-methoxy]tropanes and (*R*)-2β-Substituted 3β-(3,4-Dichlorophenyl)tropanes at the Dopamine Transporter. *J. Med. Chem.* **2003**, *46*, 2908−2916.

(27) Desai, R. I.; Kopajtic, T.; Koffarnus, M.; Newman, A. H.; Katz, J. L. Identification of a Dopamine Transporter Ligand that Blocks the Stimulant Effects of Cocaine. *J. Neurosci.* **2005**, *25*(8), 1889−1893.

(28) Desai, R.; Kopajtic, T.; French, D.; Newman, A. H.; Katz, J. L. Relationship Between In Vivo Occupancy at the Dopamine Transporter and Behavioral Effects of Cocaine, GBR 12909 and Benztropine Analogues. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 397−404.

(29) Katz, J. L.; Agoston, G. E.; Alling, K. L.; Kline, R. H.; Forster, M. J.; Woolverton, W. L.; Kopajtic, T. A.; Newman, A. H. Dopamine Transporter Binding Without Cocaine-like Behavioral Effects: Synthesis and Evaluation of Benztropine Analogues Alone and in Combination with Cocaine in Rodents. *Psychopharmacology* **2001**, *154*, 362−374.
(30) Kulkarni, S.; Grundt, P.; Kopajtic, T.; Katz, J. L.; Newman, A. H. Structure−Activity Relationship at Monoamine Transporters for a series of N-Substituted-3α-(bis[4-fluorophenyl]methoxy)tropanes: Comparative Molecular Field Analysis, Synthesis, and Pharmacological Evaluation. *J. Med. Chem*. **2004**, *47*, 3388−3398.
(31) Watson, M.; Yamamura, H. I.; Roeske, W. R. A unique regulatory profile and regional distribution of [^{3}H]pirenzepine binding in the rat provide evidence for distinct M1 and M2 muscarinic receptor subtypes. *Life Sci*. **1983**, *32*, 3001−3011.
(32) Xu, L.; Kelkar, S. V.; Izenwasser, S.; Kopajtic, T.; Katz, J. L.; Klein-Stevens, C.; Zhu, N.; Lomenzo, S. A.; Winfield, L.; Trudell, M. L. Synthesis and Biological Evaluation of 2-Substituted 3β-Tolyltropane Derivatives at Dopamine, Serotonin, and Norepinephrine Transporters *J. Med. Chem*. **2002**, *45*, 1203−1210.
(33) Meltzer, P. C.; Liang, A. Y.; Madras, B. K. 2-Carbomethoxy-3-(diarylmethoxy)-1αH,5αH-tropane Analogues: Synthesis and Inhibition of Binding at the Dopamine Transporter and Comparison with Piperazines of the GBR Series. *J. Med. Chem*. **1996**, *39*, 371−379.
(34) Grundt, P.; Kopajtic, T. A.; Katz, J. L.; Newman, A. H. The Effect of 6-substituted-4′, 4″-Difluorobenztropines on Monoamine Transporters and the Muscarinic M1 Receptor. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 3295−3298.

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