Monoamine Transporter Binding, Locomotor Activity, and Drug Discrimination Properties of 3-(4-Substituted-phenyl)tropane-2-carboxylic Acid Methyl Ester Isomers

F. Ivy Carroll,*,† Scott P. Runyon,† Philip Abraham,† Hernan Navarro,† Michael J. Kuhar,‡ Gerald T. Pollard,§ and James L. Howard§

Chemistry and Life Sciences, and Howard Associates, MCB/HLB Complex, Research Triangle Institute, Research Triangle Park, North Carolina 27709, Yerkes National Primate Center of Emory University, 954 Gatewood Road NE, Atlanta, Georgia 30329

Received July 9, 2004

The monoamine transporter binding properties, gross behavior, and locomotor activity effects in mice and drug discrimination results in cocaine-trained rats of the 2β,3β-, 2β,3α-, 2α,3β-, and 2α,3α-isomers of several 3-(4-substituted-phenyl)tropane carboxylic acid methyl esters were compared (**2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c**). The 2β,3β-isomer showed the highest affinity for the dopamine transporter (DAT), and the 2β,3α-isomer showed the next highest affinity. The order of potency for the 2β,3β-isomer is 4′-chloro (**2c**) = 4′-iodo (**2e**) > 4′-bromo (**2d**) = 4′-methyl (**2f**) > 4′-fluoro (**2b**) > 4′-hydrogen (**2a**). In the case of the 2β,3α-isomer, the order of affinity was 4′-bromo (**3d**) > 4′-iodo (**3e**) = 4′- chloro (**3c**) > 4′-methyl (**3f**) > 4′-fluoro (**3b**) > 4′-hydrogen (**3a**). The 4′-hydrogen, 4′-fluoro, and 4′-methyl 2α,3β-isomers, **4a**, **4b**, and 4**f**, had the lowest affinity for the DAT. While most of the compounds showed their highest affinity at the DAT, none were selective relative to the other two monoamine transporters. In general, the 2α,3α- and 2α,3β-isomers were more toxic (death and convulsions) than the 2β,3β- and 2β,3α-isomers. With the exception of the 2α,3α-isomers, all compounds produced the locomotor activity stimulation typical of dopaminergic drugs. The ED_{50} ranges for the 2β,3β- (**2a**−**f**), 2β,3α- (**3a**−**f**), and 2α,3β-isomers (**4a**−**f**) in the locomotor assay were 0.1−1.2, 6.6−21.8, and 2.4−11.7 mg/kg, respectively. With the exception of the 2α,3α-isomer, all compounds generalized to cocaine. The 2β,3β-isomers were at least 10-fold more potent than cocaine and the other three sets of isomers in this test.

Introduction

Cocaine (**1**) is a potent stimulant with strong reinforcing effects. Cocaine blocks the reuptake of dopamine (DA), serotonin (5-HT), and norepinephrine (NE). It also exerts effects on the cholinergic, muscarinic, and σ receptors and sodium channels.[1,2] It was hypothesized that the dopamine transporter (DAT) was the key target in cocaine's reinforcing effects.[3] Over the last several years, substantial animal data have accumulated that support this hypothesis.[1,4,5] Significant effort was also directed toward defining structure−activity relationship (SAR) studies to characterize the cocaine binding site on the DAT and potentially lead to compounds useful for the various animal behavioral studies.[4−8] Since cocaine (**1**) was the drug of interest, we started our SAR efforts by studying cocaine analogues. The cocaine structure has four sites of asymmetry, although there are only eight possible isomers. We found that only natural cocaine has appreciable affinity for the DAT; the other seven isomers were 60−600 times weaker.[9] After completing these studies, we turned our attention to the 3-phenyltropane class of monoamine uptake inhibitors.[10,11] This class was both more potent in in vitro and in vivo studies and more chemically and metabolically stable.[12] Our laboratory, as well as others, have reported the syntheses and biological evaluations of a number of analogues from this class (for recent reviews see refs 6, 7, and 12).

Like cocaine, the 3β-phenyltropane-2β-carboxylic acid methyl ester (WIN 35,065−2, **2a**) has four sites of asymmetry but only eight possible isomers. The other diastereoisomers are the 2β,3α-, 2α,3β-, and 2α,3α-isomers (**3a**, **4a**, and **5a**, respectively). By far the most studied isomers have been the 2β,3β-compounds.[5,6,12] Somewhat surprisingly, the only compound reported to receive clinical evaluation from the 3-phenyltropane class was the 2α,3β-compound brasofensine (**6**).[13,14] This compound was also reported to be less potent than its 2β,3β-isomer but was chosen for development due to its better toxicological profile.[15] Since much effort from our laboratory as well as others has been devoted to the development of 2β,3β-phenyltropane analogues as potential treatment pharmacotherapies for cocaine abuse, it is important to gain information about the relative potencies and animal behavioral profiles of the 3-phenyltropane class. To do this, the monoamine transporter binding properties, gross effects, and behavioral pharmacological profiles of WIN 35,065−2 and its 2α,3β-, 2β,3α-, and 2α,3α-isomers as well as the similar isomers of several 4-substituted analogues (see structures **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c**) were compared.

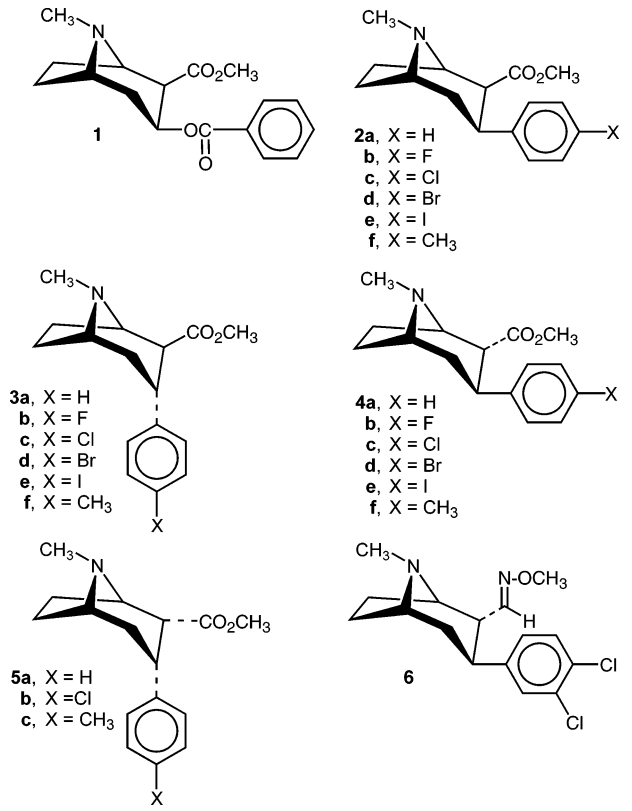
* Corresponding author. Address: Research Triangle Institute, Post Office Box 12194, Research Triangle Park, NC 27709-2194. Phone: 919-541-6679. Fax: 919-541-8868. E-mail: fic@rti.org.

† Chemistry and Life Sciences, Research Triangle Institute.

‡ Yerkes National Primate Center of Emory University.

§ Howard Associates, MCB/HLB Complex, Research Triangle Institute.

10.1021/jm0401311 CCC: $27.50
Published on Web 11/05/2004



Chemistry

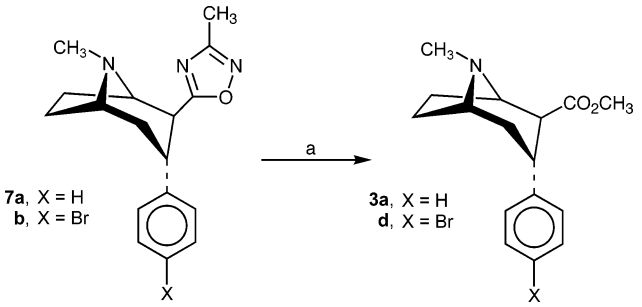
The synthesis of several compounds listed in Table 1 has been previously described. Compounds **2a**,[11,16] **2b**,[11] **2c**,[16] **2d**,[16] **2e**,[16] **2f**,[16] **3b**,[17] **3c**,[17] **3e**,[17] **3f**,[17] **4a**,[11] **4b**,[11] **4c**,[18] **4e**,[19] and **4f**[18] were prepared according to literature methods. Conversion of (1*R*)-3α-(4-substituted phenyl)-2*β*-(3-methyl-[1,2,4]oxadiazole-5-yl)tropanes **7a** and **7b**[20] to the desired tropanes **3a** and **3d** was accomplished using nickel boride in methanol (Scheme 1). Isomerization of (1*R*)-3*β*-(4-bromophenyl)tropane-2*β*-carboxylic acid methyl ester (**2d**) with sodium methoxide in methanol at reflux provided **4d**. The 2α,3α-tropanes **5b** and **5c** were prepared by catalytic hydrogenation of (1*R*)-3-(4-chlorophenyl)trop-2-ene-2-carboxylic acid methyl ester (**8a**) and (1*R*)-3-(4-methylphenyl)trop-2-ene-2-carboxylic acid methyl ester (**8b**)[21] over Raney nickel in methanol, respectively (Scheme 2).

Biology

The binding affinities of the target compounds **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c** along with reference compound cocaine, at the DAT, serotonin transporter (5-HTT), and norepinephrine transporter (NET) were determined via competition binding assays using the previously reported procedures.[20] The results are listed in Table 1. Some of the data was taken from previous reports.[22] The final concentration of radioligands in the assays was 0.5 nM [^{3}H]WIN 35,428 for the DAT, 0.2 nM [^{3}H]paroxetine for the 5-HTT, and 1.0 nM [^{3}H]nisoxetine for the NET.

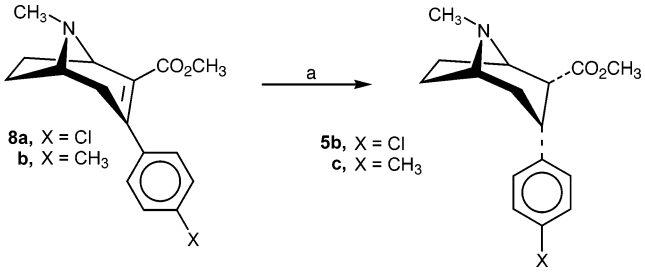
The behavioral pharmacology was done with the previously reported procedures.[8] Behaviorally relevant doses for **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c** were determined by observation of gross behavior in mice given 1, 10, and 100 mg/kg (Table 2). Stimulant and/or depressant effects on locomotor activity were determined in mice (Table 3). Generalization with the cocaine cue was determined by lever choice in a drug discrimination task in rats. Table 4 shows the percent of rats choosing the cocaine lever at various doses of compounds, along with ED_{50} values.

Scheme 1[a]



[a] Reagents and Conditions: (a) Nickel boride, MeOH.

Scheme 2[a]

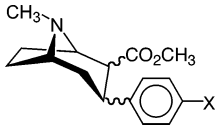


[a] Reagents and Conditions: (a) Raney nickel, H_2, 50 psi, MeOH.

Results and Discussion

With the goal of separating the stimulant and local anesthetic effects of cocaine from its toxicity and dependence liability, Clarke and co-workers[10,11] synthesized and determined the biological properties of a few 3-(substituted-phenyl)tropane-2-carboxylic acid methyl esters. The 2*β*,3*β*-compounds WIN 35,065−2 (**2a**) and WIN 35,428 (**2b**) were reported to be 5−60-fold more potent than cocaine in behavioral studies, including locomotor activity, and in preventing or reversing reserpine-induced eyelid ptosis in mice. The analogous 2α,3*β*-isomer, **4b**, was reported to be inactive in the locomotor screen. Numerous subsequent studies from our laboratory, as well as others, obtained similar results for many other 3*β*-(4-substituted-phenyl)tropane-2*β*-carboxylic acid methyl esters (see refs 6, 7, and 12 for reviews). In contrast, only a few studies on the in vitro and in vivo activity of other isomers have been reported. Reith et al.[23] reported that the 2α,3*β*-isomer, **4a**, was much less potent than the 2*β*,3*β*-isomer, **2a**, in DA and 5-HT uptake using striatum or rat cortex. Ritz et al.[24] reported that **4a** had lower affinity than **2a** at the rat brain DAT, 5-HTT, and NET, and Madras et al.[25] found that **4a** was much less potent than **2a** in DAT binding using cynomologus monkey caudate putamem. The 2*β*,3*β*-isomer, **2e**, was found to be more potent than the 2α,3*β*-isomer, **4e**, in binding to the DAT, 5-HTT, and NET.[19,26] In preliminary studies, we found that the 2*β*,3α-isomer of 3-(substituted-phenyl)tropane-2*β*-carboxylic acid methyl esters in some cases was more selective for binding to the DAT relative to the 5-HTT and NET with only slightly lower DAT affinity than the 2*β*,3*β*-isomer.[17,27] Others have reported similar findings.[28]

Table 1. Monoamine Transporter Binding Properties of 3-(4-Substituted-phenyl)tropane-2-carboxylic Acid Methyl Esters



			IC_{50}, nM (K_i, nM)		
compd	X	isomer	DAT[c]	NET[c]	5-HTT[c]
cocaine			89.1	3300 (1990)	1050 (45)
2a[a]	H	2β,3β	23 ± 5	920 ± 70 (550 ± 44)	1960 ± 61 (178 ± 5.5)
2b[a]	F	2β,3β	13.9 ± 2.0	835 ± 45 (503 ± 27)	692 ± 27 (63 ± 2.5)
2c[a]	Cl	2β,3β	1.1 ± 0.1	37 ± 2.1 (22 ± 1.3)	44.5 ± 1.3 (4.0 ± 0.12)
2d[a]	Br	2β,3β	1.7 ± 0.2	37.4 ± 5.2 (23 ± 3.1)	10.6 ± 0.24 (0.96 ± 0.02)
2e[a]	I	2β,3β	1.3 ± 0.01	36 ± 2.7 (22 ± 1.6)	4.21 ± 0.30 (0.38 ± 0.03)
2f[a]	CH_3	2β,3β	1.7 ± 0.3	60 ± 0.53 (36 ± 0.32)	240 ± 27 (23 ± 2.5)
3a	H	2β,3α	101 ± 16	541 ± 69 (271 ± 34)	5700 ± 720 (518 ± 66)
3b[b]	F	2β,3α	21.0 ± 0.5	1200 ± 90 (741 ± 55)	5060 ± 490 (460 ± 44)
3c	Cl	2β,3α	3.1 ± 0.6	5.14 ± 1.08 (3.1 ± 0.60)	53 ± 3 (4.8 ± 0.26)
3d	Br	2β,3α	1.7 ± 0.4	32.4 ± 3.5 (16.2 ± 1.7)	84 ± 13.5 (20.6 ± 3.3)
3e	I	2β,3α	2.9 ± 0.2	52.4 ± 4.9 (32 ± 3.0)	64.9 ± 1.97 (5.9 ± 0.18)
3f	CH_3	2β,3α	10.2 ± 0.8	270 ± 24 (160 ± 14)	4250 ± 420 (390 ± 38)
4a	H	2α,3β	670 ± 90	>10000	>10000
4b	F	2α,3β	325 ± 8	7200 ± 810 (4340 ± 480)	>10000
4c	Cl	2α,3β	25.0 ± 5	444 ± 29 (222 ± 15)	1450 ± 160 (356 ± 40)
4d	Br	2α,3β	15.7 ± 0.9	272 ± 25 (136 ± 15)	570 ± 80 (140 ± 20)
4e	I	2α,3β	22.7 ± 0.9	760 ± 49 (458 ± 30)	66.3 ± 1.8 (6.0 ± 0.16)
4f	CH_3	2α,3β	207 ± 21	2230 ± 380 (1120 ± 190)	>10000)
5b	Cl	2α,3α	11.7 ± 4.2	559 ± 27 (280 ± 13)	1800 ± 210 (441 ± 52)
5c	CH_3	2α,3α	23.4 ± 4.2	3850 ± 1270 (1930 ± 630)	>2000

[a] Data taken from ref 16. [b] Data taken from ref 17. [c] DAT, [^{3}H]WIN 35,428; NET, [^{3}H]nisoxetine; 5-HTT, [^{3}H]paroxetine.

Brasofensine (**6**), which has the 2α,3β-stereochemistry, was reported to be less potent than its 2β,3β-isomers in both DAT in vitro and in vivo binding assays.[15,29] Nevertheless, brasofensine (**6**) is reported to show excellent efficacy, with a favorable adverse effect profile after oral administration in relevant animal models of Parkinson's disease.[15] In addition, a clinical study designed to investigate the safety of brasofensine in patients with Parkinson's disease receiving levodopa/carbidopa treatment indicated that no serious adverse effects were experienced at doses of 0.5−4.0 mg/d.[13,14]

In this study, we compared the monoamine transporter binding properties of the 2β,3β-, 2β,3α-, 2α,3β-, and 2α,3α-isomers of several 3-(4-substituted-phenyl)-tropane-2-carboxylic acid methyl esters. The results are listed in Table 1. The 2β,3β-isomers (**2a**−**f**) showed the highest affinity (IC_{50} = 1.1−23 nM) for the DAT. The 4′-hydrogen, 4′-fluoro, and 4′-methyl 2α,3β-isomers, **4a**, **4b**, and **4f**, possessed the lowest affinity for the DAT. None of the compounds evaluated showed selectivity for one transporter over the other two transporters. The order of potency at the DAT for the 2β,3β-isomers is 4′-chloro (**2c**) = 4′-iodo (**2e**) > 4′-bromo (**2d**) = 4′-methyl (**2f**) > 4′-fluoro (**2b**), > 4′-hydrogen (**2a**). The 2β,3α-isomer showed a somewhat different order of potency. In this case, the 4′-bromo (**3d**) > 4′-iodo (**3e**) = 4′-chloro (**3c**) > 4′-methyl (**3f**) > 4′-fluoro (**3b**) > 4′-hydrogen (**3a**). The order of potency for the 2α,3β-isomers at the DAT was more like the 2β,3α-isomers. In this case, the order of potency was 4′-bromo (**4d**) > 4′-iodo (**4e**) > 4′-chloro (**4c**) > 4′-methyl (**4f**) > 4′-fluoro (**4b**) > 4′-hydrogen (**4a**). In the case of the 2α,3α-isomer, the 4′-chloro (**5b**) was more potent at the DAT than the 4′-methyl (**5c**) analogue.

With the exception of the 4′-bromo and 4′-iodo-2β,3β-isomers (**2d**−**e**) and the 4′-iodo 2α,3β-isomer (**4e**), which showed their highest K_i values for the 5-HTT, all other compounds showed their highest affinity at the DAT. Only the 2α,3β-isomers **4a**, **4b**, and **4f**, which have a 4′-hydrogen, 4′-fluoro, and 4′-methyl group, respectively, and the 4′-hydrogen 2β,3α-isomer **3a** have lower affinity than cocaine at the DAT.

Table 2. Gross Signs[a] over 4 h for Compounds **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c** in Mice (ip)

compd	1 mg/kg	10 mg/kg	100 mg/kg
cocaine		HA	C,Sy,HA
2a	Sy,HA	Sy,HA,Cc	C,Sy,ST
2b	Sy(sl),HA(sl)	Sy,HA	C,Sy,ST
2c	Sy,HA,Cc	Sy	Sy,HL,ST
2d	Sy,HA	Sy,Cc	Sy,Cc
2e	Sy,HA,Cc	Sy,HA(sl),Cc	D,Sy,HA(sl),HL
2f		Sy	Sy
3a		Sy,HA(sl)	Sy,HA,Cc
3b		Sy.HA(sl),Cc	Sy,HA,Cc
3c		HA	Sy,Cc,Sn
3d	Sn	Sy,HA	Sy,Cc
3e			HA(sl)
3f		HA(sl)	Sy,HA,Cc
4a			C,Cc,Sn,HL,T,A
4b		Sy(sl)	C,Sy,Ho,Cc,T
4c		Sn	D,C,Sy,Cc,T,A,ST
4d		Sy,Ho,P	D,C,Sy,Ho,Cc,P,ST
4e		Sy(sl)	D,C,Sy,HL,T,ST
4f		Sn(sl)	C,Ho,Cc,HL,A
5b			D,C,Ho,FBP
5c			D,C,HL,P,FBP

[a] A, ataxia; C, convulsions; Cc, circling; D, death; EG, excessive grooming; FBP, flattened body posture; HA, hyperactivity; HL, hind limb splay; Ho, hypoactivity; MR, muscle relaxation; P, ptosis; (sl), slight or intermittent; Sn, stimulation; ST, Straub tail; Sy, stereotypy; T, tremor.

The gross observation and locomotor activity results in mice and the drug discrimination results in cocaine-trained rats for compounds **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c** and cocaine are shown in Tables 2−4. In addition to providing dosing guidance for the locomotor activity studies, gross observation (Table 2) allowed an overall determination of the effects on mice at three doses, with two mice per dose. At 1 mg/kg, the 2β,3β-isomers **2a**−**e** produced stereotypy and hyperactivity; **2c** and **2e** also caused circling. The only non-2β,3β-compound to cause any effect at 1 mg/kg was the 2β,3α-4′-bromo isomer **3d**, which produced signs of stimulation/agitation. At 10 mg/kg, all 2β,3β-compounds (**2a**−**f**) caused stereotypy. Stereotypy was also seen with compounds **3a**, **3b**, **3d**, **4b**, **4d**, and **4e**. Hyperactivity was seen with compounds **2a**, **2b**, **2e**, **3a**−**d**, **3f**, and cocaine. The lack of hyperactivity with the 2α,3β-isomers **4c**, **4d**, and **4f** was probably due to the rapid onset of intense stereotypy. Circling behavior was seen with **2a**, **2d**, **2e**, and **3b**. Some stimulation was seen with compounds **4c** and **4f**. Compound **4d** caused hypoactivity and ptosis before the onset of stereotypy. At 100 mg/kg, deaths occurred following **2e**, **4c**−**e**, and **5b,c**. Convulsions occurred following cocaine, **2a**, **2b**, **4a**−**f**, and **5b,c**. Stereotypy was seen with all compounds except **3e**, **4a**, **4f**, **5b**, and **5c**. Cocaine, **2e**, **3a**, **3b**, **3e**, and **3f** caused hyperactivity and **4b**, **4d**, **4f**, and **5b** hypoactivity. Compound **2d** and all the 2β,3α- and 2α,3β-compounds caused circling behavior except **3e** and **4e**, **2a**−**c**. Compounds **4c**−**e** produced Straub tail. A variety of other gross signs such as ptosis, hind limbs play, tremor, and ataxia were seen almost exclusively with the 2α-compounds. The 2α,3α-compounds caused a flattened body posture. In summary, the 2β,3β-compounds were potent stimulants (1 mg/kg) with at least a 100-fold spread between stimulation and other symptoms. The 2β,3α-compounds were stimulants at 10 mg/kg and only stimulation was seen at a 10-fold higher dose. The 2α,3β-compounds were stimulants at 10 mg/kg, but a host of other symptoms occurred at 100 mg/kg. The 2α,3α-compounds were not stimulants and caused symptoms at 100 mg/kg.

Table 3 shows locomotor activity as percent change from vehicle in mice in 1-h bins over 4 h. Cocaine produced its greatest stimulation in hour 1; in hours 2 and 3 only the 100 mg/kg dose caused significant stimulation. There were no significant effects in hour 4. Three of the six 2β,3β-compounds, **2a**, **2b**, and **2f**, caused their greatest stimulation in hour 1. Compound **2c** produced its greatest effect in hour 3 and **2d** and **2e** in hour 4. All of the 2β,3β-compounds had a longer duration of action than cocaine, with at least one dose producing significant stimulation in hour 4. Four of the six 2β,3α-compounds, **3a**−**c** and **3f**, had their greatest effect in hour 1 and the other two, **3d** and **3e**, in hour 3. In general, the duration of locomotor stimulation was less than that produced by the 2β,3β-compounds. Three of the six compounds, **3a**, **3c**, and **3d**, had significant increases in hour 4; however, **3e** and **3f**, like cocaine, had a significant increase in hour 3. Compound **3b** produced significant increases only through hour 2. Of the 2α,3β-compounds only **4a** produced its greatest effect in hour 1. Compounds **4b**−**e** had their greatest effect in hour 3. Compound **4f** had its greatest effect in hour 4, but no dose produced a significant increase. In general, the 2α,3β-compounds seemed to have a slower onset (among the six compounds, only two, **4a** and **4c**, had a significant effect in hour 1) and a short duration of action (only two, **4c** and **4e**, had a significant effect in hour 4). The 2α,3α-compounds did not cause significant increases in activity at any time point.

The ED_{50}s for the hour of peak effect show the 2β,3β-compounds to be very potent with values ranging from 0.1 to 1.2 mg/kg. The ED_{50}s for the 2β,3α-compounds were 6.6−21.8 mg/kg, and for the 2α,3β-compounds, 2.4−11.7 mg/kg (but **4f** did not have an ED_{50}); thus, they were approximately equipotent and at least 10-fold weaker than the 2β,3β-compounds. The compounds can also be examined for their effects during the first hour after administration, where cocaine exerts its peak effect. Five of the six 2β,3β-compounds produced greater effects than cocaine during hour 1, with ratios ranging from 1.6 to 4.4. Compound **2d** actually produced less activity stimulation than cocaine, with a ratio of 0.8; however, the rapid onset of intense stereotypy probably artificially lowered this ratio. The 2β,3α-compounds produced equivalent or greater activity than cocaine in hour 1, with ratios ranging from 1 to 2.6. The 2α,3β-compounds **4a**−**f** produced less stimulation in hour 1 than cocaine, with ratios ranging from 0.1 to 0.7. The 2α,3α-compounds were almost without effect in hour 1, with ratios of 0.0 and 0.2.

Table 4 shows effects in rats trained to discriminate cocaine. All compounds produced greater than 80% generalization to cocaine except the 2α,3α-compounds **5b,c**, which showed a complete lack of generalization. Again the 2β,3β-compounds were potent, with ED_{50}s ranging from 0.13 to 0.34 mg/kg. The 2β,3α-compounds were somewhat weaker than cocaine, with ED_{50}s ranging from 2.55 to 7.73 mg/kg. The 2α,3β-compounds had ED_{50}s ranging from 4.07 to 13.51 mg/kg. Thus, again the 2β,3β-compounds were at least 10-fold more potent than cocaine and their 2β,3α-, 2α,3β-, and 2α,3α-tropane isomers.

Table 3. Locomotor Activity in Mice (ip) over 4 h for Compounds **2a**–**f**, **3a**–**f**, **4a**–**f**, and **5b**,**c**

compd	dose, mg/kg	hour 1	hour 2	hour 3	hour 4	ED_{50} ± SEM in peak hour, mg/kg	ratio to cocaine for hour 1
cocaine	3	+27	+14	−17	−9	10.0 ± 3.0	
	10	+127[a]	+24	+10	−21		
	30	+341[a]	+100	−10	+16		
	100	+269[a]	+391[a]	+255[a]	+91		
2a	0.3	+44	+56	+24	+62	1.2 ± 1.5	2.7
	1	+253[a]	+192	+102	+151		
	3	+660[a]	+506[a]	+276	+199		
	10	+904[a]	+1080[a]	+964[a]	+190[a]		
2b	0.3	+5	−33	−66	−47	0.7 ± 2.9	1.9
	1	+333[a]	+232[a]	+28	−29		
	3	+633[a]	+558[a]	+199[a]	+140		
	10	+330[a]	+220[a]	+594[a]	+739[a]		
2c	0.1	−26	+53	+91	+17	0.2 ± 4.6	1.9
	0.3	+462[a]	+1426[a]	+1201[a]	+235		
	1	+645[a]	+958[a]	+1892[a]	+1507[a]		
	3	+416[a]	−36	+589	+1643[a]		
2d	0.03	−30	−39	+9	−43	0.1 ± 2.4	0.8
	0.1	−38	+29	+81	+116		
	0.3	+259[a]	+598[a]	+869[a]	+834[a]		
	1	+258[a]	+35	+302	+656[a]		
2e	0.1	−12	+38	−25	+62	0.4 ± 3.9	4.4
	0.3	+415	+1765[a]	+1289[a]	+900[a]		
	1	+1489[a]	+1721[a]	+2099[a]	+2546[a]		
	3	+770	+145	+327	+794[a]		
2f	0.3	+19	−3	−61	−28	0.6 ± 2.9	1.6
	1	+305[a]	+298[a]	+41	−4		
	3	+533[a]	+460[a]	+157	+30		
	10	+283[a]	+476[a]	+343[a]	+273[a]		
3a	3	−30	−39	−35	+12	21.8 ± 1.6	1.0
	10	+43	+6	−13	+6		
	30	+161[a]	+17	+47	+97		
	100	+338[a]	+191[a]	+234[a]	+390[a]		
3b	3	+26	−23	+16	−41	15.6 ± 1.4	1.7
	10	+137	+105	+146	+58		
	30	+362[a]	+187[a]	+215	+66		
	100	+575[a]	+340[a]	+194	+73		
3c	3	+49	+64	+135	+38	9.1 ± 2.6	2.6
	10	+340[a]	+322	+373	+11		
	30	+868[a]	+1127[a]	+1002[a]	+158		
	100	+487[a]	+217	+1065[a]	+598[a]		
3d	1	+35	−26	−56	−12	6.6 ± 1.7	1.5
	3	+60	+88	+100	+109		
	10	+353[a]	+457[a]	+686[a]	+781[a]		
	30	+518[a]	+861[a]	+1275[a]	+883[a]		
3e	3	+14	−72	+64	−42	7.7 ± 1.9	1.0
	10	+210	+100	+457	+49		
	30	+308[a]	+121	+391	+25		
	100	+328[a]	+168	+692[a]	+124		
3f	3	+28	+29	−6	−41	14.7 ± 1.4	2.5
	10	+294[a]	+149	+108	+22		
	30	+482[a]	+147	+5	−66		
	100	+843[a]	+436[a]	+263[a]	+18		
4a	1	+9	+35	+12	−60	11.7 ± 2.2	0.4
	3	−14	−20	−9	−50		
	10	+35	+16	−16	−27		
	30	+138[a]	+122	+69	+12		
4b	1	−10	+5	+52	+28	2.4 ± 1.4	0.2
	3	+15	+58	+120	+19		
	10	+58	+107	+260[a]	+196		
	30	+24	+207[a]	+298[a]	+127		
4c	1	+15	+50	+83	+62	4.3 ± 2.5	0.7
	3	+68	+188	+92	+15		
	10	+248[a]	+1003[a]	+1275[a]	+667		
	30	+154	+493	+911[a]	+794[a]		
4d	1	+9	+26	+56	−5	10.0 ± 1.9	0.1
	3	+19	+123	+100	+29		
	10	+49	+268	+267	+74		
	30	−33	+640[a]	+901[a]	+373		

Table 3. Locomotor Activity in Mice (ip) over 4 h for Compounds **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b**,**c**

compd	dose, mg/kg	hour 1	hour 2	hour 3	hour 4	ED_{50} ± SEM in peak hour, mg/kg	ratio to cocaine for hour 1
4e	1	0	+44	+99	+41	9.9 ± 2.6	0.1
	3	+19	+17	+96	+13		
	10	+17	+49	+85	+40		
	30	+7	+150[a]	+268[a]	+255[a]		
4f	1	−42	−76	+39	−68	no	0.1
	3	+20	+10	−1	+39	significant	
	10	+24	−11	+6	+55	stimulation	
	30	−23	+7	+17	+42		
5b	1	+6	+182	+154	+45	no	0.2
	3	+16	+128	+107	+69	significant	
	10	+51	+169	+114	+41	stimulation	
	30	−46	−27	+125	−27		
5c	1	−15	+110	+118	−51	no	0.0
	3	+6	+132	+47	+56	significant	
	10	+8	+96	+120	−36	stimulation	
	30	−57	−36	+78	−35		

[a] Different from vehicle by Newman−Kuels following one−way analysis of variance, $p < 0.05$. Boxes indicate hour of peak activity.

Table 4. Drug Discrimination Effects of Compounds **2a**−**f**, **3a**−**f**, **4a**−**f**, and **5b,c** in Rats (ip)

	percent of rats choosing the cocaine lever at the dose (mg/kg) specified											
compd	0.1	0.17	0.3	0.56	1	1.7	3	5.6	10	17	30	ED_{50}, mg/kg
cocaine					21	32	59	82	97			2.44
2a		14	36	88	100							0.34
2b		0	57	86	100							0.29
2c	50	44	100									0.13
2d	14	50	100									0.17
2e	22	25	100									0.20
2f	0	25	57	71	86							0.31
3a							13	100[a]	100[a]			3.19
3b					0		29	38	86			5.66
3c					25	25	67	67	100			2.55
3d					13	13	50	88				2.98
3e					0	14	43	44	86			4.72
3f					0	14	29	43	43	75	100	7.73
4a								29	67	100		7.66
4b							0	33	57	100[a]		8.01
4c							13	88	67[a]			4.07
4d							25	17	86			7.29
4e							0		14	80[a]		13.51
4f							13	43	71	71	100[a]	7.11
5b					13		0	13	13[b]			
5c					13		0	13	0[b]			

[a] Because some of the rats in the group did not make a lever choice, the value represents the percent of rats that made a choice. [b] Dose−effect curve terminated because of severe toxicity at higher doses.

In summary, with few exceptions (**2d**, **2e**, and **4e**) the isomers had greater affinity at the DAT than at the 5-HTT and NET transporters. The potency of the 2β,3β-isomers for the DAT was slightly greater than that of the 2β,3α-isomers, which were considerably more potent than the 2α,3β- and 2α,3α-isomers. The 2β,3β-isomers were also much more potent in the locomotor activity and cocaine discrimination assays than the 2β,3α- and 2α,3β-isomers. The 2α,3α-isomers did not show either locomotor or cocaine discrimination activity. It is particularly important to note that 2α,3β-4′-chloro, -bromo, and -iodo isomers (**4c**−**e**) and the 2α,3α-4′-chloro and -methyl (**5b,c**) isomers produced death at 100 mg/kg. In contrast, none of the 2β,3α-isomers and only the 4′-iodo isomer **2e** produced death at 100 mg/kg.

Thus, the higher affinity for monoamine transporter binding and greater potency and duration of action in the locomotor activity and drug discrimination assays, as well as lower toxicity as judged by observed gross effects, suggest that the 2β,3β- and 2β,3α-isomers are more likely to be candidates for pharmacotherapies to treat cocaine abuse, Parkinson's disease, and other central nervous system disorders than the 2α,3β- and 2α,3α-isomers.

Experimental Section

Nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded on either a 250 MHz (Bruker AM-250) or a 300 MHz (Bruker AVANCE 300) spectrometer. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal $(CH_3)_4Si$ (δ 0.0). Optical rotations were measured on an AutoPol III polarimeter, purchased from Rudolf Research. Elemental analyses were performed by Atlantic Microlab, Norcross, GA. Analytical thin-layer chromatography (TLC) was carried out on plates pre-coated with silica gel GHLF (250 μm thickness). TLC visualization was accomplished with a UV lamp or in an iodine chamber. All moisture sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct

line from a nitrogen source. THF was distilled just prior to its use (sodium benzophenone ketyl) or was purchased.

(1*R*)-3α-Phenyltropane-2*β*-carboxylic Acid Methyl Ester Tosylate (3a). Sodium borohydride (2.55 g, 67.3 mmol) in MeOH (75 mL) was added to a solution of nickel(II) acetate (16.75 g, 67.3 mmol) in MeOH (75 mL) at room temperature. (1*R*)-3α-Phenyl-2*β*-(3-methyl-[1,2,4]oxadiazole-5-yl)tropane (**7a**)[20] (3.80 g, 13.46 mmol) in MeOH (50 mL) was then added to the mixture followed by concentrated HCl (5.6 mL, 67 mmol). The black suspension was heated at reflux for 4 h, cooled to room temperature, filtered through Celite, and concentrated under reduced pressure. Water and ammonium hydroxide were added, and the green suspension was extracted with Et_2O (3 × 100 mL). The Et_2O extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified using medium-pressure column chromatography on silica, with 50% (Et_2O/TEA, 9:1), 50% (hexane), to provide an oil (0.77 g, 22%). The oil was dissolved in EtOAc and 1.1 equiv of *p*-toluenesulfonic acid was added. The solution was heated until the solid dissolved, the solution cooled, and the solid precipitate was collected to provide **3a** as a white solid. Mp: 172−173 °C. $[\alpha]^{22}_D$: −31.6° (*c* 1.16, EtOH). ^{1}H NMR (DMSO-d_6): *δ* 1.90 (m, 3H), 2.19, (m, 1H), 2.24 (s, 3H), 2.26 (m, 1H), 2.42 (m, 1H), 2.66 (s, 3H), 3.25 (m, 2H), 3.52 (s, 3H), 3.85 (m, 1H), 4.09 (m, 1H), 7.08 (m, 2H), 7.31 (m, 2H), 7.42 (m, 2H), 9.38 (brs, 1H). Anal. ($C_{23}H_{29}NSO_5$): C, H, N.

(1*R*)-3α-(4-Bromophenyl)-2*β*-(3-methyl-[1,2,4]oxadiazole-5-yl)tropane (7b). A 2.5 M solution of *n*-butyllithium (14 mL, 35 mmol) in hexane was added at −78 °C under N_2 to 1-bromo-4-iodobenzene (9.65 g, 34 mmol) in THF (250 mL). The solution was allowed to stir for 15 min and (1*R*)-2-(3-methyl-[1,2,4]oxadiazole-5-yl)trop-2-ene[30] (3.5 g, 17 mmol) in THF (25 mL) was slowly added. The reaction was then allowed to stir for 3 h at −78 °C and a solution of TFA (4.4 mL, 57 mmol) in Et_2O (10 mL) was added in a dropwise manner. The slurry was concentrated under reduced pressure and ammonium hydroxide (100 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an orange oil. The oil was purified using medium-pressure column chromatography on silica (Et_2O/TEA, 9:1) to provide a mixture of the 3α,2*β*- and 3α,3α-isomers (2.12 g) which were used in the next step without further purification.

(1*R*)-3α-(4-Bromophenyl)tropane-2*β*-carboxylic Acid Methyl Ester Tosylate (3d). Sodium borohydride (1.0 g, 27.6 mmol) in MeOH (30 mL) was added to a solution of nickel(II) acetate (6.9 g, 27.6 mmol) in MeOH (30 mL) at room temperature. A mixture of (1*R*)-3α-(4-bromophenyl)-2*β*-(3-methyl-[1,2,4]oxadiazole-5-yl)tropane (**7b)** and (1*R*)-3α-(4-bromophenyl)-2α-(3-methyl-[1,2,4]oxadiazole-5-yl)tropane, (2.0 g, 5.5 mmol) in MeOH (50 mL) was then added to the mixture followed by concentrated HCl (2.4 mL, 27.6 mmol). The black suspension was heated at reflux for 4 h, cooled to room temperature, filtered through Celite, and concentrated under reduced pressure. Water and ammonium hydroxide were added, and the green suspension was extracted with Et_2O (3 × 100 mL). The Et_2O extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified using medium-pressure column chromatography on silica, with 75% (Et_2O/TEA, 9:1), 30% (hexane), to provide an oil (0.36 g, 20%). The oil was dissolved in EtOAc and 1.1 equiv of *p*-toluenesulfonic acid was added. The solution was heated until the solid dissolved, the solution cooled, and the solid precipitate was collected to provide **3d** as a white solid. Mp: 191−192 °C. $[\alpha]^{22}_D$: −40.12° (*c* 0.81, MeOH). ^{1}H NMR (CD_3OD): *δ* 2.0 (m, 2H), 2.32 (m, 3H), 2.39 (s, 3H), 2.57 (m, 1H), 2.82 (s, 3H), 3.34 (m, 2H), 3.68 (s, 3H), 3.93 (m, 1H), 4.18 (m, 1H), 7.22 (d, *J* = 9 Hz, 2H), 7.35 (d, *J* = 9 Hz, 2H), 7.49 (d, *J* = 9 Hz, 2H), 7.72 (d, *J* = 9 Hz, 2H). Anal. ($C_{23}H_{28}BrNSO_5$): C, H, N.

(1*R*)-3*β*-(4-Bromophenyl)tropane-2α-carboxylic Acid Methyl Ester Fumarate (4d). Sodium metal (0.70 g, 30 mmol) was added in small portions to MeOH (25 mL) at 0 °C under N_2. (1*R*)-3*β*-(4-Bromophenyl)tropane-2*β*-carboxylic acid methyl ester **2d**[16] (2.07 g, 6.12 mmol) was then added and the reaction was heated at reflux for 27 h. The solution was cooled and concentrated under reduced pressure and water was added. The mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the organic extracts were combined, dried ($NaSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified using medium-pressure column chromatography on silica (EtOAc/TEA/hexane, 9:1:10) to provide a white solid (1.37 g, 66%). The solid was converted to the fumarate salt with fumaric acid in MeOH. The mixture was heated until all of the solid dissolved, the solution cooled, and Et_2O was added slowly until a precipitate formed. The solid precipitate was collected and dried to provide **4d** as a white powder. Mp: 152−153 °C. $[\alpha]^{22}_D$: +9.05° (*c* 0.42, MeOH). ^{1}H NMR (D_2O): *δ* 2.08 (m, 2H), 2.20 (m, 1H), 2.31 (m, 2H), 2.40 (m, 1H), 2.86 (s, 3H), 3.40 (m, 2H), 3.55 (s, 3H), 4.02 (m, 1H), 4.34 (m, 1H), 7.30 (d, *J* = 9 Hz, 2H), 7.55 (d, *J* = 9 Hz, 2H). Anal. ($C_{20}H_{26}BrNO_8$): C, H, N.

(1*R*)-3α-(4-Chlorophenyl)tropane-2α-carboxylic Acid Methyl Ester tosylate (5b). (1*R*)-3-(4-Chlorophenyl)trop-2-ene-2-carboxylic acid methyl ester (**8a**) (0.40 g, 1.37 mmol) in anhydrous MeOH (20 mL) was added to a 100 mL Parr hydrogenation bottle containing Raney nickel (0.40 g, washed free from water with MeOH) in MeOH (5 mL). The suspension was hydrogenated at 50 psi for 14 h. The suspension was filtered through a Celite pad and the filtrate was concentrated under reduced pressure to provide a colorless oil. The oil was purified using medium-pressure column chromatography (CH_2Cl_2/MeOH 9.5:0.5) to provide a colorless oil (0.17 g, 0.58 mmol, 42%). The oil was dissolved in EtOAc (20 mL) and *p*-toluenesulfonic acid (0.10 g, 0.58 mmol) was added. The suspension was heated until the solid dissolved and concentrated under reduced pressure. The semisolid residue was then recrystallized from 2-propanol/Et_2O to provide **5b** as a white solid. Mp: 189−190 °C. $[\alpha]^{22}_D$: +7.8° (*c* 0.43, MeOH). ^{1}H NMR (CD_3OD): *δ* 1.6 (m, 1H), 2.15 (m, 1H), 2.30 (m, 1H), 2.32 (s, 3H), 2.48 (m, 2H), 2.65 (m, 1H), 2.85 (s, 3H), 3.60 (s, 3H), 3.61 (m, 1H), 3.83 (dd, *J* = 7.5, 14.5 Hz, 1H), 3.90 (m, 1H), 4.25 (m, 1H), 7.23 (d, *J* = 6 Hz, 2H), 7.30 (m, 4H), 7.70 (d, *J* = 6 Hz, 2H). Anal. ($C_{23}H_{28}ClNSO_5$): C, H, N.

(1*R*)-3α-(4-Methylphenyl)tropane-2α-carboxylic Acid Methyl Ester Tosylate (5c). (1*R*)-3-(4-Methylphenyl)trop-2-ene-2-carboxylic acid methyl ester (**8b**)[21] (0.69 g, 2.53 mmol) in anhydrous MeOH (20 mL) was added to a 100 mL Parr hydrogenation bottle containing Raney nickel (0.69 g, washed free from water with MeOH) in MeOH (5 mL). The suspension was hydrogenated at 50 psi for 14 h. The suspension was filtered through a Celite pad and the filtrate was concentrated under reduced pressure to provide a colorless oil. The oil was purified using medium-pressure column chromatography (CH_2Cl_2/MeOH 9.5:0.5) to provide a colorless oil (0.39 g, 1.43 mmol, 55%). The oil was dissolved in EtOAc (20 mL), and *p*-toluenesulfonic acid (0.28 g, 1.62 mmol) was added. The suspension was heated until the solid dissolved and concentrated under reduced pressure. The semisolid residue was then recrystallized from 2-propanol/Et_2O to provide **5c** as a white solid. Mp: 145−146 °C. $[\alpha]^{22}_D$: +10.7° (*c* 0.59, MeOH). ^{1}H NMR (CD_3OD): *δ* 1.6 (m, 1H), 2.15 (m, 1H), 2.28 (m, 1H), 2.30 (s, 3H), 2.39 (s, 3H), 2.50 (m, 2H), 2.68 (m, 1H), 2.85 (s, 3H), 3.60 (m, 1H), 3.61 (s, 3H), 3.8 (m, 1H), 3.93 (m, 1H), 4.25 (m, 1H), 7.20 (m, 6H), 7.71 (d, *J* = 8 Hz, 2H). Anal. ($C_{24}H_{31}NSO_5$): C, H, N.

(1*R*)-3-(4-Chlorophenyl)trop-2-ene-2-carboxylic Acid Methyl Ester (8a). 4-(Chlorophenyl)boronic acid (0.65 g, 4.13 mmol) was added to a suspension of LiCl (0.29 g, 6.84 mmol), $Pd(Ph_3)_4$ (0.13 g, 0.08 mmol), and (1*R*)-3-trifluoromethanesulfonyloxy-trop-2-ene-2-carboxylic acid methyl ester[31] (1.11 g, 3.18 mmol) in 1,2-dimethoxyethane (13 mL). The suspension was allowed to stir for 5 min and Na_2CO_3 (2.0 M, 1.65 mL) was added. The reaction was heated at reflux (2 h) and allowed to cool to room temperature. Water (20 mL) and concentrated NH_4OH (25 mL) were added, and the suspension was extracted with $CHCl_3$ (3 × 50 mL). The extracts were combined, dried

($MgSO_4$), and concentrated under reduced pressure to provide a brown oil. The oil was purified using medium-pressure column chromatography on silica (petroleum ether/EtOAc/TEA, 10:9:1) to provide a pale yellow oil (0.87 g, 94%). $[\alpha]^{22}_D$: −55.7° (*c* 0.82, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 1.62 (m, 1H), 1.95 (m, 2H), 2.18 (m, 2H), 2.45 (s, 3H), 2.75 (m, 1H), 3.31 (m, 1H), 3.49 (s, 3H), 3.83 (m, 1H), 7.03 (d, J = 8 Hz, 2H), 7.35 (d, J = 8 Hz, 2H).

Gross Observation. Subjects. Male CD-1 mice, 19−28 g, from Charles River Laboratories, Raleigh, NC, were habituated to the Animal Research Facility for at least 5 days.

Apparatus and Procedure. Observations were made in six clear plexiglass chambers, 4" × 6" × 4". Compounds were homogenized in 0.5% methyl cellulose and injected at 1, 10, or 100 mg/kg ip in a volume of 10 mL/kg of body weight (N = 2 per dose in the same chamber). Control mice received vehicle. Subjects were observed continuously for the first 15 min and thereafter at 0.5, 1, 2, 4, and 24 h after injection. Gross signs such as hyperactivity, hypoactivity, stereotypy, blepharoptosis, seizure, and death were recorded in narrative form with indications of presence and intensity.

Locomotor Activity. Subjects. Male CD-1 mice, 19−28 g, from Charles River Laboratories, Raleigh, NC, were habituated to the Animal Research Facility for at least 5 days.

Apparatus and Procedure. Activity was measured in 24 plexiglass chambers, 16" × 8" × 8", each set in an array of four photocells in a cage rack system (San Diego Instruments, San Diego, CA). Initial doses were selected on the basis of gross observation results, with 30 mg/kg being the upper limit, except for cocaine and the 2β,3α-compounds, where 100 mg/kg was the upper limit. Compounds were prepared as for gross observation. Mice (N = 5 or 6 per dose and vehicle, with a replication to increase the N for compounds of particular interest or to extend the dose−effect curve) were habituated to the activity chambers for 0.5 h and then removed individually, injected ip, and replaced. Photobeam interruptions were recorded in 10-min bins for 4 h.

Analyses. Data were grouped into 1-h time bins and subjected to one-way analysis of variance, with the Newman−Kuels test applied post hoc at each time point where a main effect of dose or a dose × time interaction was significant (p < 0.05). An ED_{50} was determined for the hour showing the greatest change from control by using a sigmoidal dose−response curve-fitting procedure (GraphPad Prism, GraphPad Software, Inc., San Diego, CA).

Drug Discrimination. Subjects. Adult male CD albino rats (Sprague Dawley derived) from Charles River Laboratories, Raleigh, NC, were maintained one or two per cage on a reverse light/dark cycle (lights on from 1800 to 0600) in an animal housing room with controlled temperature (69−75 °F) and humidity (40−70%).

Apparatus and Procedure. Training and testing were done in 16 standard rat operant chambers inside sound-attenuating enclosures (Coulbourn Instruments, Allentown, PA). Each chamber was equipped with a house light, two response levers, a food trough between the levers, and a dispenser for 45-mg food pellets (BioServ, Frenchtown, NJ). Programming of contingencies and data acquisition were done by an L2T2 system (Coulbourn Instruments).

Initial Training. Subjects were deprived of food for 24 h and given at least two 1-h sessions on an autoshaping schedule and at least two 15-h nightly sessions on a progressive ratio schedule.

Cocaine Discrimination Training. When subjects readily emitted 10 lever presses for each food pellet (FR10), they were put on daily 10-min sessions on FR10 for 5 days per week, with access to standard lab chow postsession and on Saturday and Sunday in sufficient quantity to maintain good health and stable body weight. Fifteen min before each session, either cocaine (10 mg/kg) or saline vehicle was injected ip in a volume of 1 mL/kg of body weight. For half of the subjects, after cocaine injection, every 10 presses on the left lever delivered a pellet and presses on the right lever had no programmed consequence; after saline injection, every 10 presses on the right lever delivered a pellet and presses on the left lever had no programmed consequence. For the other half of the subjects, this contingency was reversed. A correct lever choice for a session was defined as earning the first pellet with 12 or fewer presses (i.e., no more than 2 on the incorrect lever). Cocaine (C) or saline (S) was assigned such that each was given on two consecutive days (e.g., for the 5 days of 1 week, C−C−S−S−C, and for the next week, C−S−S−C−C). The criterion for stability on the discrimination was at least nine correct choices for the first FR10 in 10 consecutive sessions. To maintain stability, a subject had to have made the correct choice in the most recent drug-lever-correct and the most recent saline-lever-correct sessions.

Compound Testing for Generalization with the Cocaine Cue. Days were assigned to cocaine, saline, and test compound (T) in a double alternation sequence such that a T day occurred three times every 2 weeks, with sequential T days separated by at least 1 C and 1 S day. A compound was tested initially at doses that produced stimulation in mouse locomotor activity, and then a full dose−effect curve from 20% or less to 80% or greater generalization was generated. The highest dose was that which reduced lever pressing by >50% or produced significant side effects or 30 mg/kg, whichever was lower. The degree of generalization was defined as the percentage of subjects that chose the cocaine-appropriate lever. In most cases, all doses of a compound were tested in seven or eight subjects. Compounds were prepared as for the mouse experiments and injected ip in a volume of 1 mL/kg 15 min before the session.

Analyses. Lever choice on a test day was defined as the first lever on which 10 presses occurred. Total presses for the session also were recorded. Results for a compound were tabulated as the percent of subjects choosing the cocaine lever. Where cocaine-lever choice reached 75%, an ED_{50} was calculated with GraphPad Prism.

Acknowledgment. This research was supported by the National Institute on Drug Abuse Grants DA05477, DA11530, and DA00418. The authors express their appreciation to Larry Brieaddy and Dori Shand for technical assistance.

Supporting Information Available: Analysis data for **3a**, **3d**, **4d**, **5b**, **5c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Cocaine receptor: Biochemical characterization and structure−activity relationships for the dopamine transporter. *J. Med. Chem.* **1992**, *35*, 969−981.
(2) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* **1991**, *14*, 299−302.
(3) 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.
(4) Carroll, F. I.; Howell, L. L.; Kuhar, M. J. Pharmacotherapies for treatment of cocaine abuse: Preclinical aspects. *J. Med. Chem.* **1999**, *42*, 2721−2736.
(5) Spanagel, R.; Weiss, F. The dopamine hypothesis of reward: Past and current status. *Trends Neurosci.* **1999**, *22*, 521−527.
(6) Newman, A. H. Novel pharmacotherapies for cocaine abuse 1997−2000. *Expert Opin. Ther. Patents* **2000**, *10*, 1095−1122.
(7) Carroll, F. I.; Lewin, A. H.; Mascarella, S. W. Dopamine Transporter Uptake Blockers: Structure−Activity Relationships. In *Neurotransmitter Transporters: Structure, Function, and Regulation,* 2nd ed.; Reith, M. E. A., Ed.; Humana Press: Totowa, NJ, 2001, pp 381−432.
(8) Carroll, F. I.; Pawlush, N.; Kuhar, M. J.; Pollard, G. T.; Howard, J. L. Synthesis, monoamine transporter binding properties, and behavioral pharmacology of a series of 3β-(substituted phenyl)-2β-(3′-substituted isoxazol-5-yl)tropanes. *J. Med. Chem.* **2004**, *47*, 296−302.
(9) Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. Synthesis and ligand binding of cocaine isomers at the cocaine receptor. *J. Med. Chem.* **1991**, *34*, 883−886.
(10) Clarke, R. L. The tropane alkaloids. In *The Alkaloids*; Manske, R. H. F. Ed.; Academic Press: New York, 1977; Vol. 16, Chapter 2.

(11) Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cumiskey, W. R.; Bogado, E. F. Compounds affecting the central nervous system. 4. 3β-Phenyltropane-2-carboxylic esters and analogues. *J. Med. Chem.* **1973**, *16*, 1260−1267.

(12) Carroll, F. I. 2002 Medicinal Chemistry Division Award address: Monoamine transporters and opioid receptors. Targets for addiction therapy. *J. Med. Chem.* **2003**, *46*, 1775−1794.

(13) Frackiewicz, E. J.; Jhee, S. S.; Shiovitz, T. M.; Webster, J.; Topham, C.; Dockens, R. C.; Whigan, D.; Salazar, D. E.; Cutler, N. R. Brasofensine treatment for Parkinson's disease in combination with levodopa/carbidopa. *Ann. Pharmacother.* **2002**, *36*, 225−230.

(14) Yu, P. Brasofensine NeuroSearch. *Curr. Opin. Investig. Drugs* **2000**, *1*, 504−507.

(15) Moldt, P.; Wåtjen, F.; Scheel-Krüger, J., Tropane-2-aldoxine derivatives as neurotransmitter reuptake inhibitors, WO9528401, 1995.

(16) Carroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis, ligand binding, QSAR, and CoMFA study of 3β-(p-substituted phenyl)tropan-2β-carboxylic acid methyl esters. *J. Med. Chem.* **1991**, *34*, 2719−2927.

(17) Holmquist, Christopher R.; Keverline-Frantz, Kathryn I.; Abraham, P.; Boja, John W.; Kuhar, Michael J. K.; Carroll, F. I. 3α-(4′-Substituted phenyl)tropane-2β-carboxylic Acid Methyl Esters: Novel Ligands with High Affinity and Selectivity at the Dopamine Transporter. *J. Med. Chem.* **1996**, *39*, 4139−4141.

(18) Xu, L.; Trudell, M. L. Stereoselective synthesis of 2β-Carbomethoxy-3β-phenyltropane derivatives. Enhanced stereoselectivity observed for the conjugate addition reaction of phenylmagnesium bromide derivatives with anhydro dichloromethane. *J. Heterocycl. Chem.* **1966**, *33*, 2037−2039.

(19) Neumeyer, J. L.; Wang, S.; Milius, R. A.; Baldwin, R. M.; Zea-Ponce, Y.; Hoffer, P. B.; Sybirska, E.; Al-Tikriti, M.; Charney, D. S.; Malison, R. T.; Laruelle, M.; Innis, R. B. [^{123}I]-2β-Carbomethoxy-3β-(4-iodophenyl)tropane: High affinity SPECT radiotracer of monoamine reuptake sites in brain. *J. Med. Chem.* **1991**, *34*, 3144−3146.

(20) 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 transporters. *J. Med. Chem.* **1993**, *36*, 6, 2886−2890.

(21) Jiang, S.; Chang, A.-C.; Abraham, P.; Kuhar, M. J.; Carroll, F. I. Synthesis and Transporter Binding Properties of (R)-2β,3β- and (R)-2α,3α-Diaryltropanes. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3689−3692.

(22) 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.

(23) Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. *Biochem. Pharmacol.* **1986**, *35*, 1123−1129.

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

(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) Damaj, M. I.; Slemmer, J. E.; Carroll, F. I.; Martin, B. R. Pharmacological characterization of nicotine's interaction with cocaine and cocaine analogues. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1229−1236.

(27) Blough, B. E.; Holmquist, C. R.; Abraham, P.; Kuhar, M. J.; Carroll, F. I. 3α-(4-Substituted phenyl)nortropane-2β-carboxylic acids methyl esters show selective binding at the norepinephrine transporter. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2445−2447.

(28) Meltzer, P. C.; Blundell, P.; Madras, B. K. Structure activity relationships of inhibition of the dopamine transporter by 3-arylbicyclo[3.2.1]octanes. *Med. Chem. Res.* **1998**, *8*, 12−34.

(29) Pearce, R. K.; Smith, L. A.; Jackson, M. J.; Banerji, T.; Scheel-Kruger, J.; Jenner, P. The monoamine reuptake blocker brasofensine reverses akinesia without dyskinesia in MPTP-treated and levodopa-primed common marmosets. *Mov. Disord.* **2002**, *17*, 877−886.

(30) Triggle, D. J.; Kwon, Y. W.; Abraham, P.; Rahman, M. A.; Carroll, F. I. Synthesis of 2-(3-Substituted-1,2,4-oxadiazol-5-yl)-8-methyl-8-azabicyclo[3.2.1]octanes and 2α-(3-substituted-1,2,4-oxadiazol-5-yl)-8-methyl-8-azabicyclo[3.2.1]oct-2-enes as potential muscarinic agonist. *Pharm. Res.* **1992**, *9*, 1474−1479.

(31) Keverline, K. I.; Abraham, P.; Lewin, A. H.; Carroll, F. I. Synthesis of the 2β,3α- and 2β,3β-isomers of 3-(*p*-substituted phenyl)tropane-2-carboxylic acid methyl esters. *Tetrahedron Lett.* **1995**, *36*, 3099−3102.

JM0401311