Cocaine and 3β-(4′-Substituted phenyl)tropane-2β-carboxylic Acid Ester and Amide Analogues. New High-Affinity and Selective Compounds for the Dopamine Transporter

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Several 2β-carboxylic acid ester and amide analogues of cocaine and of 3β-(4′-substituted phenyl)tropane-2β-carboxylic acid were prepared. The binding affinities of these compounds, and of some previously prepared analogues, at the dopamine (DA), norepinephrine (NE), and serotonin (5-HT) transporters were determined. The phenyl esters of 3β-(4′-methylphenyl)- and 3β-(4′-chlorophenyl)tropane-2β-carboxylic acid are highly potent and highly selective for the DA transporter. The isopropyl esters of 3β-(4′-chlorophenyl)- and 3β-(4′-iodophenyl)tropane-2β-carboxylic acid also possess high DA affinity and show significant DA transporter selectivity. Similarly, the phenyl and isopropyl ester analogues of cocaine are much more selective for the DA transporter than cocaine. Tertiary amide analogues of cocaine and of 3β-(4′-substituted phenyl)tropane-2β-carboxylic acids are more potent inhibitors of radioligand binding at the DA transporter than the primary and secondary amide analogues. In particular, 3β-(4′-chlorophenyl)tropane-2β-*N*-morpholinocarboxamide as well as the 3β-(4′-chlorophenyl)- and 3β-(4′-iodophenyl)tropane-2β-*N*-pyrrolidinocarboxamides possess high affinity and selectivity for the DA transporter. The *N*,*N*-dimethylamide cocaine analogue is the most selective cocaine amide derivative for the DA transporter. High correlation between the inhibition of radioligand binding and inhibition of uptake at the DA, NE, and 5-HT transporter was found for a selected group of analogues. Within this group, one compound, the isopropyl ester of 3β-(4′-iodophenyl)-tropane-2β-carboxylic acid, was found to be more potent in the inhibition of radioligand binding than in the inhibition of DA uptake. Taken together with its high potency and selectivity at the DA transporter, this suggests that this compound may be a lead in the development of a cocaine antagonist.

Introduction

Several lines of evidence suggest that the reinforcing and locomotor properties of cocaine (**1a**) are related to its ability to inhibit dopamine (DA) reuptake by binding to a specific site on the dopamine transporter.[1-7] The putative role of this site in addiction has prompted investigations of structure–activity relationships for binding at the DA transporter. Studies from our laboratory, as well as from other laboratories, have addressed the structural features of cocaine (**1a**), and of 3β-phenyltropane-2β-carboxylic acid methyl ester (**2a**), required for binding to the dopamine transporter. Specifically, it was found that the overall molecular configuration, the nature of the 2β-substituent, an aromatic ring at the 3 position, the tropane nitrogen, and the N-substituent are important in recognition site interactions.[8-19] For analogues of **2a**, we, and others, have reported that binding affinities for the dopamine transporter were highly dependent upon the substituent on the 3β-phenyl ring.[9,13,14,19] For example, the 4′-methyl- (**2b**), 4′-chloro- (**2c**), 4′-iodo- (**2d**), 4′-bromo- (**2e**), 4′-amino-3′-iodo- (**2f**), 3′,4′-dichloro- (**2g**), and 4′-chloro-3′-methyl- (**2h**) analogues were 13–29 times more potent than **2a**. In contrast, we discovered that replacement of the methyl group in the 2β-carbomethoxy functionality of cocaine (**1a**) with other groups to give cocaine analogues **1b**–i had only small effects on potency at the DA transporter.[10]

Cocaine (**1a**), **2a**, and **2i** had also been shown to inhibit the uptake of norepinephrine (NE) and serotonin (5-HT), as well as DA, into presynaptic terminals.[12,20,21] Indeed, a modulatory role has been ascribed to the inhibition of 5-HT uptake.[22] Investigation of the contributions of the various neurotransmitters to the neurochemical mode of action of cocaine is hampered by the unavailability of transporter-selective cocaine and **2a** analogues. Such analogues would be valuable for the study of pharmacological and behavioral effects associated with transporter-specific binding and could lead to therapeutic agents useful in the treatment of cocaine abuse. In this study, we report the binding potency of previously prepared compounds **1a**–i and **2a**–i at the norepinephrine and serotonin transporters and present the syntheses and binding affinities at the DA, NE, and 5-HT transporters of 3β-(4′-substituted phenyl)tropane-2β-carboxylic esters and amides **4a**–**y** and **5a**–**t**, respectively, as well as data for the new cocaine amide analogues **6a**–**c** and for the previously reported cocaine *N*-methylamide (**6d**).[10] Some of the analogues possess high affinity and selectivity for the cocaine binding site on the DA transporter as well as for inhibition of dopamine uptake into synaptosomes. Parts of these studies have been described in preliminary communications.[23-25]

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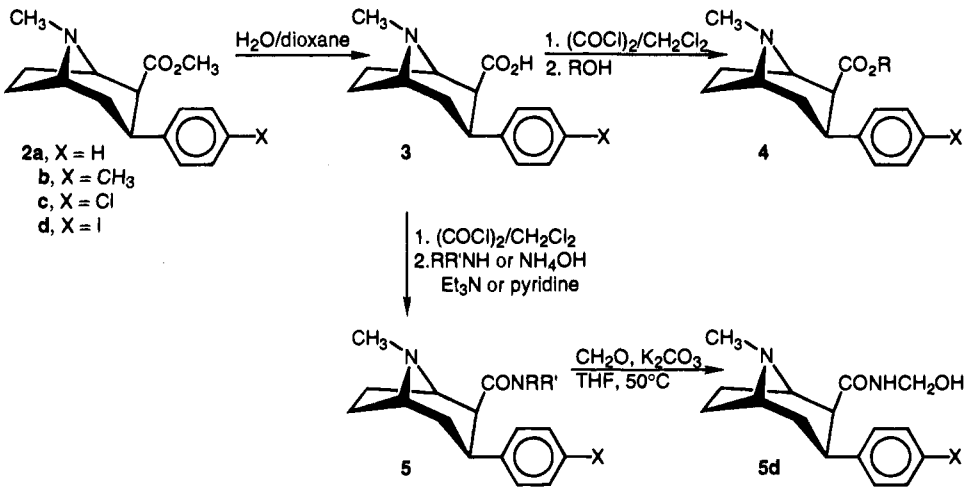
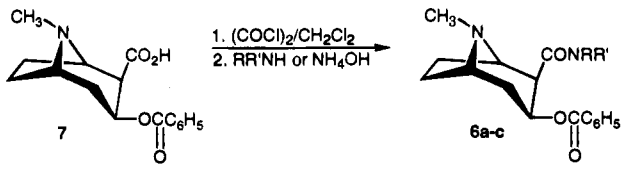
Scheme 1



Scheme 2



Results

Chemistry. Scheme 1 outlines the general synthetic approach used to prepare the ester and amide analogues of 3β-(4′-substituted phenyl)tropane-2β-carboxylic acids. Hydrolysis of **2a**–**d** in aqueous dioxane gives the corresponding acids **3a**–**d**. Treatment of **3a**–**d** with oxalyl chloride gives the acid chlorides which were converted to the esters and amides, **4** and **5**, respectively, by treatment with the appropriate alcohol, phenol, amine, aniline, or ammonia. Amide analogue **5d** was prepared by treating a THF solution of **5a** with 37% aqueous formaldehyde in the presence of potassium carbonate. The cocaine carboxamide analogues **6a**–**c** were prepared by treating benzoylecgonine (**7**)[24-26] with oxalyl chloride in CH_2Cl_2 to give the corresponding acid chloride, followed by reaction of the acid chloride with ammonium hydroxide or the appropriate amine (Scheme 2). The structures and physical properties of the ester and amide analogues are shown in Tables 1 and 2, respectively. The 3β-(substituted phenyl)tropane-2β-carboxylic acid methyl esters (**2a**–i), the cocaine ester analogues (**1b**–i), and the cocaine *N*-methylamide analogue (**6d**) were prepared as previously reported.[9,10,13,27]

Biochemical. Binding affinities at the DA, NE, and 5-HT transporters represent inhibition of 0.5 nM [^{3}H]-WIN 35,428, 0.5 nM [^{3}H]nisoxetine, and 0.2 nM [^{3}H]-paroxetine binding, respectively, determined as described previously.[28] The IC_{50} values for the ester and amide analogues of cocaine (**1a**), and of 3β-(4′-substituted phenyl)tropane-2β-carboxylic acid, **4a**–**y**, **5a**–**t**, and **6a**–**d**, and for several previously reported 3β-(substituted phenyl)tropane-2β-carboxylic acid esters and cocaine ester analogues are listed in Tables 3–6.

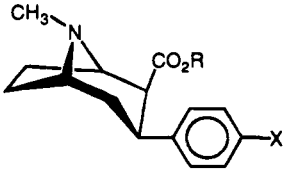
Inhibition of [^{3}H]DA, [^{3}H]NE, and [^{3}H]5-HT uptake into synaptosomes prepared from rat striatum, midbrain, and frontal cortex, respectively, were measured as described in the experimental section, and the K_i values for several of the ligands were calculated using the Cheng and Prusoff equation[29] and are listed in Table 7.

Discussion

Clarke et al.[30] reported the syntheses of **2a** and **2i** and showed them to be inhibitors of NE reuptake. We have previously described the synthesis of cocaine analogues **2b**–**h** and reported the potencies of **2a**–i at the cocaine binding site on the DA transporter.[9,13,14] We now report the binding affinity of the same compounds at the NE and 5-HT transporters (Table 3). With the exception of the effect of substitution on the 4′-amino-3′-iodophenyl analogue **2f** at the NE transporter, the addition of substituents to **2a** serves to enhance potency at all three transporters. The unsubstituted and *p*-fluoro analogues **2a** and **2i** possess IC_{50} values of 23 and 13.9 nM, respectively, for the DA transporter. The 3β-(4′-substituted phenyl) analogues **2b**–**h** are 14–30 times more potent at the DA transporter than **2a**, with analogues **2c**, **2g**, and **2h** showing the highest affinities. The 4′-iodo analogue **2d** and the 3′,4′-dichloro analogue **2g** also possess high affinities for the 5-HT transporter with IC_{50} values of 4.21 and 3.13 nM, respectively. Although the analogues **2a**–i show increased affinity for the NE transporter relative to cocaine (**1a**), none are highly potent, and all have NE/DA and/or 5-HT/DA ratios similar to those for cocaine (**1a**), which is not selective for the DA transporter. The most selective compound is **2f** with 984- and 89-fold selectivities for the DA transporter relative to the NE and 5-HT transporters. The 4′-methyl analogue **2b** with a 5-HT/DA ratio of 140 possesses the highest selectivity for the DA transporter relative to the 5-HT transporter.

We had previously reported that the effect of replacement of the methyl group in the 2-position ester of cocaine with an ethyl, propyl, isopropyl, phenyl, benzyl, phenylethyl, phenylpropyl, or *trans*-cinnamyl group (compounds **1b**–i in Table 4) resulted in only small decreases (1.3–4-fold) in potency at the dopamine transporter. Determination of the effects on binding at the NE and 5-HT transporters show two of the analogues, the isopropyl and phenyl esters **1d** and **1e**, to be reasonably selective for the DA transporter. Thus, relative to cocaine (**1a**), these analogues exhibit 9.2- and 9.4-fold decreases in potency at the NE transporter and 24- and 32-fold decreases in potency at the 5-HT

Table 1. Physical Properties of 3β-(4′-Substituted phenyl)tropane-2β-carboxylic Acid Esters



	compd	X	R	molecular formula[a]	% yield	mp, °C	$[\alpha]_D$, deg (c, CH_3OH)
4a[b]	RTI-135	H	$CH(CH_3)_2$	$C_{18}H_{26}ClNO_2$	61	236–237	−103.7 (1.08)
4b	RTI-134	H	C_6H_5	$C_{25}H_{27}NO_6$	43	199–200	−119.4 (0.65)
4c	RTI-117	CH_3	$CH(CH_3)_2$	$C_{19}H_{28}ClNO_2$[c]	65	238 dec	−99.3 (0.983)
4d	RTI-127	CH_3	$CH(C_2H_5)_2$	$C_{21}H_{32}ClNO_2$[d]	90	188–192	−77.6 (1.06)
4e	RTI-149	CH_3	c-C_5H_{11}	$C_{21}H_{30}ClNO_2$[c]	62	203–205	−75.4 (1.09)
4f	RTI-150	CH_3	c-C_4H_9	$C_{20}H_{28}ClNO_2$[c]	57	201–202	−80.6 (1.35)
4g	RTI-193	CH_3	c-C_3H_5	$C_{19}H_{26}ClNO_2$[c]	8	174–176	−68.75 (0.24)
4h	RTI-120	CH_3	C_6H_5	$C_{22}H_{26}ClNO_2$	81	234–235	−158.6 (0.49)
4i	RTI-205	CH_3	3-$CH_3C_6H_4$	$C_{23}H_{28}ClNO_2$[e]	79	191–192	−154.4 (0.16)
4j	RTI-209	CH_3	4-$CH_3C_6H_4$	$C_{23}H_{28}ClNO_2$[f]	75	229–231	−163 (0.27)
4k	RTI-207	CH_3	2-$CH_3C_6H_4$	$C_{23}H_{28}ClNO_2$[f]	79	166–168	−162 (0.15)
4l	RTI-211	CH_3	4-ClC_6H_4	$C_{22}H_{25}Cl_2NO_2$[c]	69	228–229	−172 (0.15)
4m	RTI-213	CH_3	4-$CH_3OC_6H_4$	$C_{23}H_{28}ClNO_3$[f]	68	228–230	−165 (0.245)
4n	RTI-114	Cl	$CH(CH_3)_2$	$C_{18}H_{25}Cl_2NO_2$	38	248–249	−90.1 (0.81)
4o	RTI-190	Cl	c-C_3H_5	$C_{18}H_{23}Cl_2NO_2$[g]	16	241–242	−65.5 (0.53)
4p	RTI-113	Cl	C_6H_5	$C_{21}H_{23}Cl_2NO_2$	90	232–233	−157.6 (1.015)
4q	RTI-116	Cl	4-IC_6H_4	$C_{21}H_{22}Cl_2INO_2$[c]	45	218–221	−158.2 (1.582)
4r	RTI-203	Cl	3-$CH_3C_6H_4$	$C_{22}H_{25}Cl_2NO_2$[f]	68	217–218	−154.6 (0.15)
4s	RTI-206	Cl	4-$CH_3C_6H_4$	$C_{22}H_{25}Cl_2NO_2$[h]	71	227–228	−168.7 (0.15)
4t	RTI-204	Cl	2-$CH_3C_6H_4$	$C_{22}H_{25}Cl_2NO_2$[f]	74	193–194	−147.2 (0.175)
4u	RTI-212	Cl	4-ClC_6H_4	$C_{21}H_{22}Cl_3NO_2$	69	210–212	−162 (0.215)
4v	RTI-210	Cl	4-$CH_3OC_6H_4$	$C_{22}H_{25}Cl_2NO_3$	76	191–193	−138 (0.195)
4w	RTI-121	I	$CH(CH_3)_2$	$C_{18}H_{25}ClINO_2$	63	250–251	−86.4 (0.235)
4x	RTI-191	I	c-C_3H_5	$C_{18}H_{23}ClINO_2$[e]	8	242–244	−49.6 (0.25)
4y	RTI-122	I	C_6H_5	$C_{21}H_{23}ClNO_2$[e]	81	254–255	−135.8 (0.985)

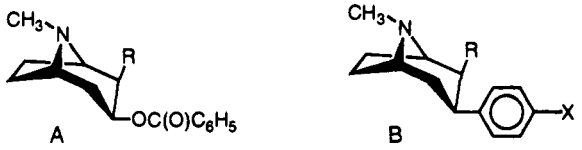
[a] All compounds exhibit ^1H NMR spectral data in agreement with assigned structures. All the compounds were characterized and tested as their hydrochloride salts with the exception of **4b**, which was characterized and tested as the fumarate salt. All salts with the exception of **4d**, **4e**, **4f**, **4h**, and **4x** were recrystallized from a methanol and ethyl ether mixture. Compound **4d** was recrystallized from a methylene chloride and hexane mixture. Compounds **4e**, **4f**, and **4g** were recrystallized from a 2-propanol and ethyl ether mixture. Compound **4h** was recrystallized from a chloroform and ethyl ether mixture. Compound **4x** was recrystallized from a methylene chloride and acetone mixture. Elemental analyses were within ±0.4% of the theoretical values for C, H, N, and Cl. [b] Reference 30, mp 244–245 °C dec; $[\alpha]^{25}_D = -105.4°$ (1%, H_2O). [c] This salt was hydrated with 0.5 mol of water. [d] This salt was crystallized with 0.5 mol of HCl. [e] This salt crystallized with 1.0 mol of water. [f] This salt was hydrated with 0.75 mol of water. [g] This salt is hydrated with 0.25 mol of water. [h] This salt is hydrated with 1.5 mol of water.

transporter, respectively, resulting in NE/DA ratios of 138 and 277 and 5-HT/DA ratios of 115 and 301 for **1d** and **1e**, respectively. Somewhat surprisingly, the benzyl, phenylethyl, phenylpropyl, and cinnamyl esters **1f–i** show increased potency (1.7–3.5-fold) at the 5-HT transporter relative to cocaine (**1a**).

The increased selectivity for the DA transporter observed for **1d** and **1e** relative to cocaine (**1a**) suggested that changing the methyl of the ester group of **2a** to an isopropyl or phenyl group might similarly provide DA-selective compounds in this series of cocaine analogues. Indeed, we found that both the isopropyl and phenyl esters **4a** and **4b**, respectively, showed increased selectivity for the DA transporter relative to **2a** or cocaine (Table 5). Unfortunately, the binding affinities of **4a** and **4b** at the DA transporter decreased relative to **2a**. Since compound **2b** has greater affinity for the DA transporter than **2a** and is reasonably selective for the DA transporter relative to the 5-HT transporter, the isopropyl (**4c**) and phenyl (**4h**) ester analogues of **2b** were prepared and evaluated. 3β-(4′-Methylphenyl)-tropane-2β-carboxylic acid phenyl ester (**4h**) with an IC_{50} of 3.26 nM for the DA transporter and NE/DA and 5-HT/DA ratios of 1789 and 7406, respectively, is the most DA transporter-selective analogue of **2a** found (Table 5). The analogous isopropyl ester **4c** with NE/DA and 5-HT/DA ratios of 299 and 944, respectively, and an IC_{50} value of 6.45 nM also possesses reasonable selectivity with only slightly lower affinity than **4h** at the DA transporter. Changing the isopropyl group of **4c** to the larger 3′-pentyl or cyclopentyl groups to give the esters **4d** and **4e**, respectively, results in loss of affinity for the DA transporter. In contrast, the cyclobutyl and cyclopropyl esters, **4f** and **4g**, respectively, both show greater potency at the DA transporter than **4c**, with **4g** having an IC_{50} value (1.68 nM) equal to that of the methyl ester **2b**. Moreover, with NE/DA and 5-HT/DA ratios of 383 and 634, compound **4g** is relatively selective for the DA transporter. The addition of 4-methyl, 2-methyl, 4-chloro, or 4-methoxy substituents to the phenyl of the ester group (**4j–m**, respectively) results in substantially lower potency at the DA transporter, while the 3-methylphenyl analogue **4i** is a little over 2-fold less potent than **4h**. None of the substituted phenyl analogues in the series **4i–m** show DA transporter selectivity equal to or greater than **4h**.

As in the 3β-(4′-methylphenyl) series, the isopropyl (**4n** and **4w**), cyclopropyl (**4o** and **4x**), and phenyl (**4p** and **4y**) esters of 3β-(4′-chlorophenyl)- and 3β-(4′-iodophenyl)tropane-2β-carboxylic acids are similar in binding affinity at the DA transporter to their parent methyl esters **2c** and **2d**. In contrast, large decreases in potency for the NE (21–105-fold) and 5-HT (16–53-fold) transporters, relative to the parent methyl esters

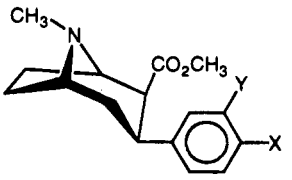
Table 2. Physical Properties of 3β-(4′-Substituted phenyl)tropane-2β-carboxylic Amides and Cocaine Analogues



	compd	structure	X	R	molecular formula[a]	% yield	mp, °C	$[\alpha]_D$, deg (c, CH_3OH)
5a	RTI-118	B	Cl	$CONH_2$	$C_{15}H_{20}Cl_2N_2O$[d]	25	309 dec	−85.0 (0.14)
5b	RTI-106	B	Cl	$CONHCH_3$	$C_{16}H_{22}Cl_2N_2O$[e]	37	138 dec	−99.0 (0.16)
5c	RTI-196	B	Cl	$CONH(OCH_3)$	$C_{16}H_{22}Cl_2N_2O_2$	58	234−235	−63.1 (0.14)
5d	RTI-146	B	Cl	$CONH(CH_2OH)$	$C_{16}H_{22}Cl_2N_2O_2$[f]	93	209 dec	−109 (0.10)
5e	RTI-129	B	Cl	$CON(CH_3)_2$	$C_{17}H_{24}Cl_2N_2O$[d]	72	238 dec	−85.2 (0.12)
5f	RTI-215	B	Cl	$CON(C_2H_5)_2$	$C_{19}H_{28}Cl_2N_2O$[f]	65	110−112	−46.5 (0.17)
5g	RTI-183	B	Cl	$CON(OCH_3)CH_3$	$C_{21}H_{27}ClN_2O_6$	98	76 dec	−32.7 (0.30)
5h	RTI-147	B	Cl	$CONCH_2CH_2CH_2CH_2$	$C_{19}H_{26}Cl_2N_2O$[g]	84	214 dec	−66.0 (0.10)
5i	RTI-156	B	Cl	$CON(CH_2)_4CH_2$	$C_{20}H_{28}Cl_2N_2O$[f]	96	154 dec	−81.0 (0.10)
5j	RTI-198	B	Cl	$CONCH_2CH_2CH_2$	$C_{18}H_{24}Cl_2N_2O$[g]	78	197−201	−78.2 (0.13)
5k	RTI-208	B	Cl	$CONOCH_2CH_2CH_2$	$C_{18}H_{24}Cl_2N_2O_2$[h]	63	154 dec	−73.1 (0.14)
5l	RTI-214	B	Cl	$CONCH_2CH_2OCH_2CH_2$	$C_{19}H_{26}Cl_2N_2O_2$[f]	79	112−114	−44.7 (0.265)
5m	RTI-226	B	Cl	$CON(CH_3)C_6H_5$	$C_{26}H_{31}ClN_2O_7$[d]	97	105−110	−30.8 (0.13)
5n	RTI-133	B	CH_3	$CONH_2$	$C_{20}H_{26}N_2O_5$[h]	82	211 dec	−69.5 (0.095)
5o	RTI-221	B	CH_3	$CON(C_2H_5)_2$	$C_{20}H_{31}ClN_2O$[c]	83	97−100	−53.6 (0.28)
5p	RTI-186	B	CH_3	$CON(OCH_3)CH_3$	$C_{22}H_{30}N_2O_6$[f]	95	77−80	−43.1 (0.24)
5q	RTI-222	B	CH_3	$CONCH_2CH_2OCH_2CH_2$	$C_{20}H_{29}ClN_2O_2$[d]	90	107−110	−50.4 (0.32)
5r	RTI-229	B	I	$CONCH_2CH_2CH_2CH_2$	$C_{23}H_{31}IN_2O_7$	50	115 dec	−51.1 (0.11)
5s	RTI-228	B	I	$CON(OCH_3)CH_3$	$C_{21}H_{29}IN_2O_8$[g]	68	112 dec	−25.9 (0.11)
5t	RTI-227	B	I	$CONOCH_2CH_2CH_2$	$C_{22}H_{29}IN_2O_8$[d]	54	112−118	−58.0 (0.10)
6a	RTI-128	A		$CONH_2$	$C_{16}H_{21}ClN_2O_3$[b]	66	242 dec	−24.0 (0.10)
6b	RTI-160	A		$CON(CH_3)_2$	$C_{18}H_{25}ClN_2O_3$[c]	43	155 dec	−5.0 (0.10)
6c	RTI-192	A		$CON(OCH_3)CH_3$	$C_{18}H_{25}ClN_2O_4$[b]	56	221−224	+33.2 (0.28)

[a] All compounds exhibit ^1H NMR spectral data in agreement with assigned structures. All the compounds were characterized and tested as their hydrochloride salts with the exception of **5g**, **5n**, **5o**, and **5p**, which were characterized and tested as the fumarate salt, and **5m**, **5r**, **5s**, and **5t**, which were tested as tartrate salts. All salts were recrystallized from a methanol and ethyl ether mixture. Elemental analyses were within ±0.4% of theoretical values for C, H, N, and Cl. [b] This salt was hydrated with 0.25 mol of water. [c] This salt was hydrated with 1.25 mol of water. [d] This salt is hydrated with 1.0 mol of water. [e] This salt was hydrated with 0.75 mol of water. [f] This salt is hydrated with 1.5 mol of water. [g] This salt is hydrated with 2.0 mol of water. [h] This salt is hydrated with 0.5 mol of water.

Table 3. Comparison of Transporter Binding Potencies for Previously Reported 3β-(4-Substituted Phenyl)tropane-2β-carboxylic Acid Methyl Esters



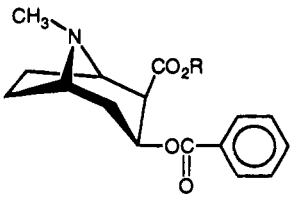
				IC_{50} (nM)				
	compd	X	Y	DA[a,b] [^3H]WIN 35,428	NE[a] [^3H]nisoxetine	5-HT[a] [^3H]paroxetine	NE/DA[c] ratio	5-HT/DA[c] ratio
2a	WIN 35,065-2	H	H	23 ± 5	920 ± 73	1962 ± 61	40	85
2i	WIN 35,428	F	H	13.9 ± 2.0	835 ± 45	692 ± 71	60	50
2b	RTI-32	CH_3	H	1.71 ± 0.31	60 ± 0.53	240 ± 27	35	140
2c	RTI-31	Cl	H	1.12 ± 0.1	37 ± 2.1	44.5 ± 1.3	33	40
2d	RTI-55[d]	I	H	1.26 ± 0.04	36 ± 2.7	4.21 ± 0.30	29	3.3
2e	RTI-51	Br	H	1.69 ± 0.23	37.4 ± 5.2	10.6 ± 0.24	22	6
2f	RTI-88	NH_2	I	1.35 ± 0.11	1329 ± 124	120 ± 4.4	984	89
2g	RTI-111	Cl	Cl	0.79 ± 0.09	17.96 ± 0.85	3.13 ± 0.36	67	4
2h	RTI-112	Cl	CH_3	0.81 ± 0.05	36.2 ± 1.0	10.5 ± 0.05	45	13

[a] Data are mean ± standard error of three or four experiments performed in triplicate. [b] The [^3H]WIN 35,428 binding data are from ref 14. [c] NE/DA are 5-HT/DA are ratios of IC_{50} values. [d] This compound has been named β-CIT by RBI.

2c and **2d**, are observed for the isopropyl esters **4n** and **4w** and the phenyl esters **4p** and **4y**. 3β-(4′-Chlorophenyl)tropane-2β-carboxylic acid phenyl ester (**4p**), with an IC_{50} of 1.98 nM for the DA transporter and NE/DA and 5-HT/DA ratios of 1492 and 1179, is both highly potent and reasonably selective for the DA transporter.

As parts of other earlier studies, we reported that [^{125}I]-3β-(4′-iodophenyl)tropane-2β-carboxylic acid methyl ester, which is the iodine-125 analogue of **2d** ([^{125}I]-**2d**), was a useful ligand for examining the DA and 5-HT transporters in vitro and in vivo.[31−33] Since these early reports, other researchers have reported the use of [^{125}I]-

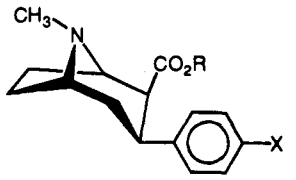
Table 4. Comparison of Transporter Binding Potencies for Cocaine and Ester Analogues



	compd	R	IC_{50} (nM) DA[a] [^{3}H]WIN 35,428	NE[a] [^{3}H]nisoxetine	5-HT[a] [^{3}H]paroxetine	NE/DA[b] ratio	5-HT/DA[b] ratio
1a	cocaine	CH_3	89 ± 4.8	3298 ± 293	1045 ± 89	37	12
1b	RTI-12	C_2H_5	195 ± 45	10000 ± 751	5801 ± 493	51	30
1c	RTI-13	C_3H_7	196 ± 46	6124 ± 262	4517 ± 430	31	23
1d	RTI-14	$CH(CH_3)_2$	219 ± 48	30384 ± 1685	25224 ± 1498	138	115
1e	RTI-15	C_6H_5	112 ± 31	31024 ± 1909	33666 ± 3330	277	301
1f	RTI-52	$CH_2C_6H_5$	257 ± 14	20794 ± 950	302 ± 23	81	1.2
1g	RTI-53	$(CH_2)_2C_6H_5$	181 ± 10	19944 ± 1026	615 ± 52	110	3.4
1h	RTI-49	$(CH_2)_3C_6H_5$	147 ± 19	4893 ± 344	374 ± 15	33	2.5
1i	RTI-74	$CH_2CH{=}CHC_6H_5$	371 ± 15	68931 ± 3476	368 ± 6.3	186	1

[a] Data are mean ± standard error of three or four experiments performed in triplicate. [b] NE/DA are 5-HT/DA are ratios of IC_{50} values.

Table 5. Comparison of Transporter Binding Potencies for 3β-(4′-Substituted phenyl)tropane-2β-carboxylic Acid Esters **4a–y**



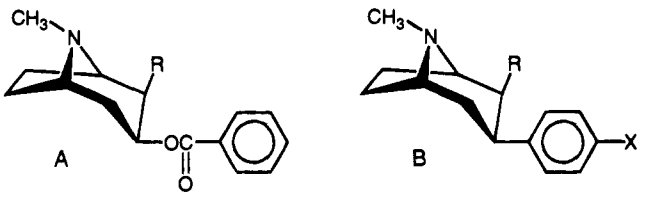
	compd	X	R	IC_{50} (nM) DA[a] [^{3}H]WIN 35,428	NE[a] [^{3}H]nisoxetine	5-HT[a] [^{3}H]paroxetine	NE/DA[b] ratio	5-HT/DA[b] ratio
4a	RTI-135	H	$CH(CH_3)_2$	85.1 ± 2.5	32047 ± 1491	23121 ± 3976	377	272
4b	RTI-134	H	C_6H_5	76.7 ± 3.6	19262 ± 593	106149 ± 7256	251	1384
4c	RTI-117	CH_3	$CH(CH_3)_2$	6.45 ± 0.85	1926 ± 38	6090 ± 488	299	944
4d	RTI-127	CH_3	$CH(C_2H_5)_2$	19.1 ± 1	3444 ± 44	4499 ± 557	180	236
4e	RTI-149	CH_3	c-C_5H_9	17.8 ± 0.76	2628 ± 252	485 ± 21	148	27
4f	RTI-150	CH_3	c-C_4H_7	3.74 ± 0.52	4738 ± 322	2019 ± 133	1267	540
4g	RTI-193	CH_3	c-C_3H_5	1.68 ± 0.14	644 ± 28	1066 ± 109	383	634
4h	RTI-120	CH_3	C_6H_5	3.26 ± 0.06	5833 ± 373	24471 ± 1515	1789	7406
4i	RTI-205	CH_3	3-$CH_3C_6H_4$	8.19 ± 0.90	2136 ± 208	5237 ± 453	261	639
4j	RTI-209	CH_3	4-$CH_3C_6H_4$	81.2 ± 16	4096 ± 121	15954 ± 1614	50	197
4k	RTI-207	CH_3	2-$CH_3C_6H_4$	23.2 ± 0.97	25695 ± 1394	11040 ± 504	1107	476
4l	RTI-211	CH_3	4-ClC_6H_4	117 ± 7.9	9519 ± 864	42761 ± 2399	81	366
4m	RTI-213	CH_3	4-$CH_3OC_6H_4$	95.6 ± 8.8	3151 ± 282	82316 ± 7852	33	861
4n	RTI-114	Cl	$CH(CH_3)_2$	1.40 ± 0.13	778 ± 21	1404 ± 7	556	1002
4o	RTI-190	Cl	c-C_3H_5	0.96 ± 0.10	235 ± 8.39	168 ± 1.8	245	175
4p	RTI-113	Cl	C_6H_5	1.98 ± 0.05	2955 ± 223	2336 ± 176	1492	1179
4q	RTI-116	Cl	4-IC_6H_4	32.6 ± 3.9	967.6 ± 26.3	1227 ± 176	30	38
4r	RTI-203	Cl	3-$CH_3C_6H_4$	9.37 ± 0.52	2744 ± 140	2153 ± 143	293	230
4s	RTI-206	Cl	4-$CH_3C_6H_4$	27.4 ± 1.5	1277 ± 118	1203 ± 42	45	44
4t	RTI-204	Cl	2-$CH_3C_6H_4$	3.91 ± 0.23	4783 ± 487	3772 ± 384	1223	965
4u	RTI-212	Cl	4-ClC_6H_4	55 ± 2.3	4883 ± 288	16914 ± 1056	89	308
4v	RTI-210	Cl	4-$CH_3OC_6H_4$	71 ± 5.6	1522 ± 94	19689 ± 1843	21	277
4w	RTI-121	I	$CH(CH_3)_2$	0.43 ± 0.05	285 ± 7.6	66.8 ± 6.53	662	155
4x	RTI-191	I	c-C_3H_5	0.61 ± 0.08	102 ± 11	15.5 ± 0.72	167	25
4y	RTI-122	I	C_6H_5	1.50 ± 0.35	3791 ± 149	184 ± 22	2577	123

[a] Data are mean ± standard error of three or four experiments performed in triplicate. [b] NE/DA are 5-HT/DA are ratios of IC_{50} values.

2d for studies of the DA and 5-HT transporters.[34,35] We also showed that [^{123}I]-**2d** was a highly useful single photon emission computed tomography (SPECT) ligand for noninvasive study of DA and 5-HT transporters in brain.[36] Innis and co-workers, and other researchers, have conducted clinical studies which show that [^{123}I]-**2d** is a promising SPECT ligand for early diagnosis and treatment monitoring of Parkinsons disease patents.[37–44] However, **2d** is not very selective for the DA transporter, showing NE/DA and 5-HT/DA ratios of 29 and 3.3, respectively (Table 3). The isopropyl ester (**4w**) analogue of **2d** shows much higher selectivity for the DA transporter with NE/DA and 5-HT/DA ratios of 662 and 155, respectively. Recently, we and others reported that [^{125}I]-**4w** and [^{123}I]-**4w** were more selective radioligands for the DA transporter.[39–41]

In order to gain additional information concerning the effect of the 2β-substituent on binding affinity at monoamine transporters, we synthesized several 3β-(substituted phenyl)-2β-carboxamides and cocaine amide analogues and evaluated their binding affinities at the DA, NE, and 5-HT transporters. In earlier studies, we reported that replacement of the 2β-carbomethoxy group of cocaine with an *N*-methylcarboxamido group (com-

Table 6. Comparison of Binding Potencies for 3β-(4′-Substituted phenyl)tropane-2β-carboxylic Amides and Cocaine Amide Analogues



	compd	structure	X	R	IC50 (nM) DA[a] [3H]WIN 35,428	IC50 (nM) NE[a] [3H]nisoxetine	IC50 (nM) 5-HT[a] [3H]paroxetine	NE/DA[b] ratio	5-HT/DA[b] ratio
5a	RTI-118	B	Cl	$CONH_2$	11.5 ± 1.6	4270 ± 359	1621 ± 110	371	141
5b	RTI-106	B	Cl	$CONHCH_3$	12.4 ± 1.17	1584 ± 62	1313 ± 46	128	106
5c	RTI-196	B	Cl	$CONH(OCH_3)$	10.7 ± 1.25	9907 ± 632	43700 ± 1960	826	4084
5d	RTI-146	B	Cl	$CONH(CH_2OH)$	2.05 ± 0.23	144 ± 3	97.8 ± 10	47	48
5e	RTI-129	B	Cl	$CON(CH_3)_2$	1.38 ± 0.1	942 ± 48	1079 ± 102	683	782
5f	RTI-215	B	Cl	$CON(CH_2CH_3)_2$	5.48 ± 0.19	5532 ± 229	9433 ± 770	1010	1733
5g	RTI-183	B	Cl	$CON(OCH_3)CH_3$	0.85 ± 0.06	549 ± 18.5	724 ± 94	646	852
5h	RTI-147	B	Cl	$CONCH_2CH_2CH_2CH_2$	1.38 ± 0.03	3950 ± 72	12400 ± 1207	2862	8985
5i	RTI-156	B	Cl	$CON(CH_2)_4CH_2$	6.95 ± 1.21	1752 ± 202	3470 ± 226	252	499
5j	RTI-198	B	Cl	$CONCH_2CH_2CH_2$	6.57 ± 0.67	990 ± 4.8	814 ± 57	151	124
5k	RTI-208	B	Cl	$CONOCH_2CH_2CH_2$	1.47 ± 0.13	1083 ± 76	2470 ± 56	736	1680
5l	RTI-214	B	Cl	$CONCH_2CH_2OCH_2CH_2$	2.90 ± 0.3	8545 ± 206	88769 ± 1855	2946	30610
5m	RTI-226	B	Cl	$CON(CH_3)C_6H_5$	45.5 ± 3	2202 ± 495	23610 ± 2128	48	519
5n	RTI-133	B	CH_3	$CONH_2$	41.8 ± 2.45	4398 ± 271	6371 ± 374	105	152
5o	RTI-221	B	CH_3	$CON(CH_2CH_3)_2$	24.7 ± 1.93	6222 ± 729	33928 ± 2192	252	1373
5p	RTI-186	B	CH_3	$CON(OCH_3)CH_3$	2.55 ± 0.43	442 ± 26	3402 ± 353	173	1334
5q	RTI-222	B	CH_3	$CONCH_2CH_2OCH_2CH_2$	11.7 ± 0.87	23601 ± 1156	>100000	2017	>8547
5r	RTI-229	B	I	$CONCH_2CH_2CH_2CH_2$	0.37 ± 0.04	991 ± 20.9	1728 ± 39.3	2678	4670
5s	RTI-228	B	I	$CON(OCH_3)CH_3$	1.08 ± 0.15	103 ± 6.2	73.9 ± 8.1	95	68
5t	RTI-227	B	I	$CONOCH_2CH_2CH_2$	0.75 ± 0.02	357 ± 42	130 ± 15.8	476	173
6a	RTI-128	A		$CONH_2$	753 ± 41.3	3981 ± 229	13725 ± 1256	5.3	18
6b	RTI-160	A		$CON(CH_3)_2$	127 ± 6.36	7329 ± 158	143713 ± 8854	58	1131
6c	RTI-192	A		$CON(OCH_3)CH_3$	60 ± 6.4	3935 ± 266	28162 ± 2565	66	469
6d	RTI-66	A		$CONHCH_3$	2424 ± 118	4213 ± 206	44798 ± 2105	1.7	18

[a] Data are mean ± standard error of three or four experiments performed in triplicate. [b] NE/DA and 5-HT/DA are ratios of IC_{50} values.

Table 7. Inhibition of Monoamine Transport by Cocaine Analogues

	compd	K_i (nM)[d] DA uptake[a]	K_i (nM)[d] NE uptake[b]	K_i (nM)[d] 5-HT uptake[c]	NE/DA[e] ratio	5-HT/DA[e] ratio
1a	cocaine	241.30 ± 18.01	160.55 ± 14.81	112.11 ± 1.91	0.69	0.46
2a	WIN 35,065-2	49.80 ± 2.25	37.20 ± 5.23	172.79 ± 12.91	0.75	3.47
2i	WIN 35,428	22.95 ± 0.45	38.59 ± 9.93	100.56 ± 13.02	1.68	4.38
2b	RTI-32	7.02 ± 0.30	8.42 ± 1.53	19.38 ± 0.65	1.20	2.76
2c	RTI-31	3.68 ± 0.09	5.86 ± 0.67	5.00 ± 0.05	1.59	1.36
2d	RTI-55[f]	1.96 ± 0.09	7.51 ± 0.82	1.74 ± 0.23	3.83	0.89
4c	RTI-117	15.31 ± 2.08	73.43 ± 11.58	917.11 ± 54.20	4.80	59.90
4h	RTI-120	9.13 ± 0.79	277.66 ± 23.43	1537.14 ± 101.43	30.41	168.36
4n	RTI-114	6.04 ± 0.31	250.32 ± 0.93	128.62 ± 15.55	3.36	21.29
4p	RTI-113	5.25 ± 0.76	242.29 ± 29.78	390.68 ± 34.26	46.15	74.42
4w	RTI-121	2.79 ± 0.13	41.22 ± 3.00	12.54 ± 1.05	14.77	4.49
4y	RTI-122	6.85 ± 0.93	32.73 ± 4.45	51.64 ± 6.23	4.78	7.54

[a] Inhibition of [3H]dopamine. [b] Inhibition of [3H]norepinephrine. [c] Inhibition of [3H]serotonin. [d] Calculated using the Cheng and Prusoff equation,[29] K_m values for dopamine, norepinephrine, and serotonin uptake were 105, 19, and 49 nM,[25] respectively. The data ± standard error represent the mean of three or four independent experiments, each performed in triplicate. [e] NE/DA and 5-HT/DA are ratios of K_i values. [f] This compound has been named β-CIT by RBI.

pound **6d** in Table 6) resulted in a significant loss of affinity for the DA transporter.[10] The results obtained with the primary and secondary amides (**5a–c**, **6a**, and **6d**) in the present investigations confirm and extend the previous observations. Thus, the primary amide **6a** is 8.5 times less potent than cocaine, and the 2β-carboxamido-, *N*-methyl-2β-carboxamido-, and *N*-methoxy-2β-carboxamido-3β-(4′-chlorophenyl)tropanes **5a–c**, respectively, are all approximately 10 times less potent than the parent, 3β-(4′-chlorophenyl)tropane-2β-carboxylic acid methyl ester (**2c**). The primary amide 3β-(4′-methylphenyl)tropane-2β-carboxamide (**5n**) is 24 times less potent than **2b**. Interestingly, potency could be restored to a secondary amide by an *N*-hydroxymethyl substituent (**5d** has an IC_{50} value of 2.05).

In contrast to the decreased potency of primary and secondary amides, the tertiary *N*,*N*-dimethylamide analogue **6b** of cocaine is almost equipotent to cocaine (**1a**), and the *N*-methyl-*N*-methoxyamide **6c** (IC_{50} 60 nM) is slightly more potent than cocaine (**1a**). In the series of 3β-(4′-chlorophenyl)tropane-2β-carboxamides, the tertiary *N*,*N*-dimethylamide **5e** and the pyrrolidine amide **5h**, both with IC_{50} values of 1.38 nM, are almost as potent as the parent ester **2c** while the *N*-methyl-

N-methoxyamide **5g**, with an IC_{50} of 0.85 nM, has the highest affinity for the DA transporter of all the amide analogues reported in this study and, in fact, is more potent than **2c**. The sensitivity of the system to steric effects is clearly manifested in the 4–5-fold loss in potency resulting from changing the size of the ring substituent of **5h** from pyrrolidine to the larger piperidine or the smaller azetidine analogues (**5i** and **5j**, respectively). Replacement of a methylene of amides **5h** and **5i** with an oxygen gives analogues **5k** and **5l** which retain high affinity for the DA transporter. The *N*-methyl-*N*-anilinoamide **5m** possesses low affinity for the DA transporter. The 3β-(4′-methylphenyl)tropane-2β-*N*-methyl-*N*-methoxycarboxamide (**5p**) as well as all three amide analogues (**5r–t**) of 3β-(4′-iodophenyl)-tropane-2β-carboxylic acid show high potency for the DA transporter.

As discussed above, replacement of the carbomethoxymethyl group in 3β-(substituted phenyl)tropane-2β-carboxylic acid methyl esters by either an isopropyl or a phenyl group (**4a–c**, **4h**, **4n**, **4p**, **4w**, and **4y**) had only small effects on affinity at the DA transporter but caused large decreases in affinity for both the NE and 5-HT transporters, suggesting a highly specific interaction at the 2β-position. A similar situation exists with the tertiary amide analogues. The potency of the tertiary amide analogues (**6b** and **6c**) of cocaine is close to that of cocaine at the DA and NE transporters but is 137- and 27-fold less at the 5-HT transporter. Most importantly, 3β-(4′-chlorophenyl)-tropane-2β-*N*-morpholinocarboxamide (**5l**), 3β-(4′-chlorophenyl)tropane-2β-*N*-pyrrolidinocarboxamide (**5h**), and 3β-(4′-iodophenyl)tropane-2β-*N*-pyrrolidinocarboxamide (**5r**) all exhibit high selectivity for the DA transporter and thus may have important applications as biochemical probes for the DA transporter binding site. In addition, the iodine-125 and iodine-123 analogues of **5r** are likely to be useful high-specific activity radioligands for the DA transporter.

The data given in Tables 3–6 were obtained in competitive binding assays using radioligands for the DA, NE, and 5-HT transporters. They provide information useful to the correlation of structure with binding potency. It has been demonstrated that the binding of cocaine to its site on the DA transporter leads to the inhibition of dopamine reuptake and, consequently, to its accumulation in the synaptic cleft, which, in turn, may be responsible for behaviors associated with cocaine addiction and abuse. Since the binding data provide relative rather than direct information regarding transport inhibition, we examined the effect of selected analogues in the inhibition of [^{3}H]DA, [^{3}H]NE, and [^{3}H]5-HT uptake into synaptosomes (see Table 7). The results show highly significant correlations between the K_i values for uptake inhibition and the IC_{50} values for the inhibition of radioligand binding, for all three transporters (Figure 1). High correlation coefficients are obtained for the DA and 5-HT plots (0.96 and 0.98, respectively); a somewhat less robust correlation is obtained for NE (0.85). Thus, it appears that the indirect measurement of the inhibition of uptake by determination of the binding potency provides useful functional information. Two compounds appear as outliers on the DA plot: 3β-(4′-iodophenyl)tropanecarboxylic acid isopropyl ester (**4w**) and 3β-(4′-iodophenyl)-tropanecarboxylic acid methyl ester (**2d**).

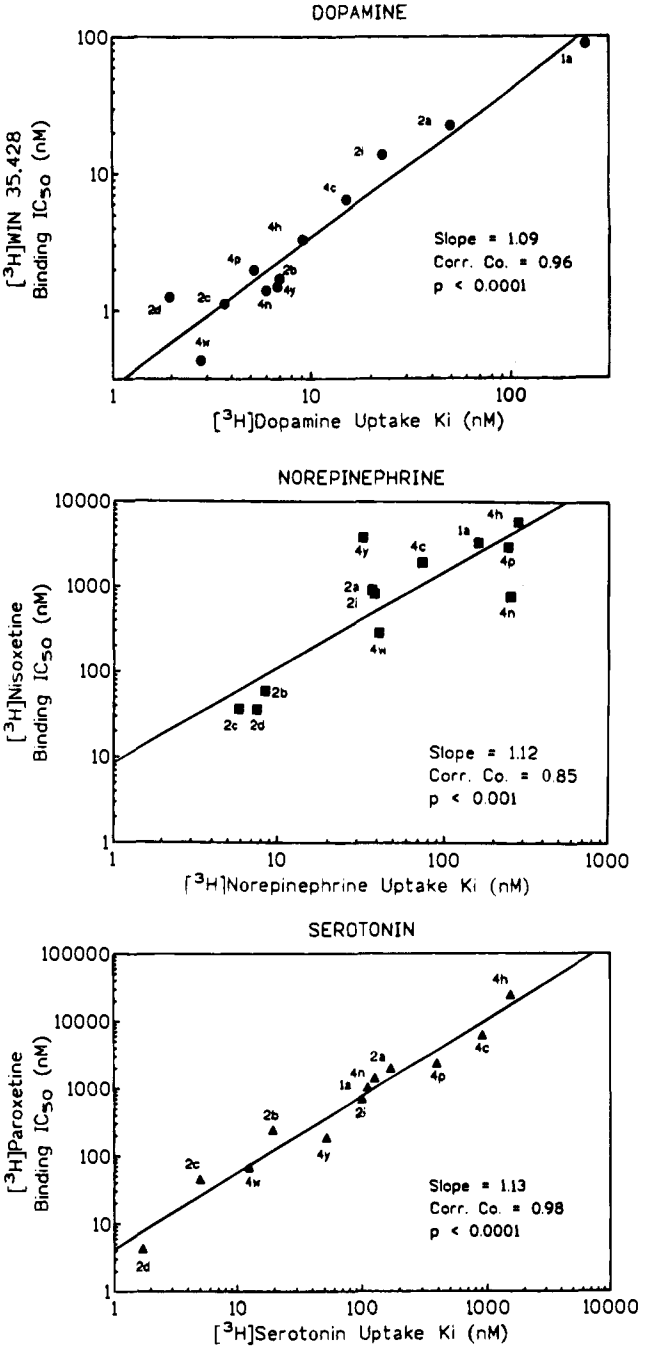


Figure 1. Inhibition of radioligand binding (IC_{50}) vs inhibition of monoamine uptake (K_i) at the DA (top panel), NE (middle panel), and 5-HT (bottom panel) transporters. The IC_{50} data are from Tables 3 and 4, and the K_i values are from Table 7.

As expected, the K_i values for uptake are different from the binding IC_{50} values. Ratios of the K_i values for inhibition of [^{3}H]dopamine uptake to the IC_{50} values for inhibition of [^{3}H]WIN 35,428 binding (shown $\times 10^3$ in Table 8) vary from 1.6 (**2i** and **2d**) to 6.5 (**4w**), with an average of 3.3. Excluding **4w** since it is an outlier on the K_i vs IC_{50} plot for DA (Figure 1) causes the range to narrow to 1.6–4.6 (**4y**), and the average becomes 3. Since **4w** is more potent in inhibiting [^{3}H]WIN 35,428 binding than [^{3}H]dopamine uptake compared to other compounds tested, **4w** may be a lead compound as a cocaine antagonist. Compounds which are more potent in inhibition of [^{3}H]mazindol binding of the cocaine site

Table 8. Ratio of K_i for Inhibition of Monoamine Transport to IC_{50} for Inhibition of Radioligand Binding by Cocaine Analogues

		10^3K_i (nM)/IC_{50} (nM)[a]		
	compd	DA	NE	5-HT
1a	cocaine	2700	48.7	107
2a	WIN 35,065-2	2165	40.4	88.1
2i	WIN 35,428	1651	46.2	145
2b	RTI-32	4105	140	80.8
2c	RTI-31	3286	158	112
2d	RTI-55	1556	207	414
4c	RTI-117	2374	38.1	151
4h	RTI-120	2801	4.7	62.8
4n	RTI-114	4314	321	91.6
4p	RTI-113	2652	82	167
4w	RTI-121	6488	14.4	188
4y	RTI-122	4567	8.6	281

[a] K_i values from Table 7; IC_{50} values from Tables 3 and 5.

at the DA transporter than DA transport have previously been reported.[48] However, all had low affinity and would therefore probably not be suitable as potential medications. By contrast, the analogue **4w** has subnanomolar potency at the DA transporter as well as modest DA selectivity. Additional work is needed to evaluate this compound. Uptake/binding ratios for norepinephrine and serotonin are 1 order of magnitude smaller and vary more widely than the ratios for dopamine. This variation suggests that cocaine antagonism might be more readily obtained at the norepinephrine and serotonin transporters. However, such conclusions must be made with great caution since uptake and binding differences could be due in part to kinetic differences in the two different assays as the conditions for uptake measurement differ considerably from conditions for binding measurements.

Conclusions

In general, potency at and selectivity for the DA transporter are important considerations for potential rehabilitation medications for cocaine abuse. Under our experimental conditions, although there is general rank-order agreement between inhibition of dopamine uptake and binding potencies, DA selectivity as indicated by NE/DA and 5-HT/DA ratios is 1 order of magnitude smaller in uptake assays than in binding assays.

Examination of the effects of replacement of the 2β-position of cocaine (**1a**), 3β-phenyltropane-2β-carboxylic acid methyl ester (**2a**), and analogues with other ester and amide groups on the binding affinities at the DA, NE, and 5-HT transporters leads to the following conclusions:

(1) Replacement of the carbomethoxymethyl group at the 2-position of cocaine (**1a**) or of 3β-phenyltropane-2β-carboxylic acid methyl ester (**2a**) and its analogues has only small effects on potency at the DA transporter; usually decreases of 20-45-fold are obtained for the NE and 5-HT transporters, respectively, relative to the relevant parent methyl ester.

(a) The phenyl (**4b**,**h**,**p**,**y**) and isopropyl (**4a**,**c**,**n**,**w**) ester analogues of each of the 3β-(substituted phenyl)-tropane-2-carboxylic acids studied possess high selectivity for the DA transporter; the analogous phenyl (**1e**) and isopropyl (**1d**) ester analogues of cocaine have the highest DA selectivity among cocaine esters.

(b) The esters combining highest potency and selectivity for the DA transporter are 3β-(4'-methylphenyl)- and 3β-(4'-chlorophenyl)tropane-2β-carboxylic acid phenyl esters (**4h** and **4p**), respectively, and 3β-(4'-chlorophenyl)- and 3β-(4'-iodophenyl)tropane-2β-carboxylic acid isopropyl esters (**4n** and **4w**).

(2) Replacement of the carbomethoxy group at the 2-position of cocaine (**1a**) or of 3β-phenyltropane-2β-carboxylic acid methyl ester (**2a**) and its analogues by a tertiary amide, particularly with oxygen-bearing substituents, leads to 33-fold increased potency at the DA transporter, relative to the parent methyl ester. The effects at the NE and 5-HT transporters are varied, with large decreases in potency dominating the picture.

(a) The *N*-pyrrolidine (**5h** and **5t**) and *N*,*N*-dimethyl (**5e**) analogues of 3β-(substituted phenyl)tropane-2-carboxamide possess high affinity and selectivity for the DA transporter.

(b) The most potent analogue of **2a** is 3β-(4'-iodophenyl)tropane-2β-*N*-pyrrolidinocarboxamide (**5r**) and the most selective is 3β-(4'-chlorophenyl)tropane-2β-*N*-morpholinocarboxamide (**5l**). The most potent analogue of **1a** is the *N*-methyl-*N*-methoxyamide **6c** and the most DA-selective cocaine amide derivative is the *N*,*N*-dimethylamide **6b**.

(3) Overall, the 5-HT transporter seems to be the most sensitive to substitution at the 2-position.

These conclusions provide a basis for the design of DA-selective ligands with optimized potency that may be useful as substitute medications for cocaine abuse. They may be tagged with radioisotopes and/or other groups for use as biochemical and/or pharmacological probes. Appropriate modifications could lead to compounds and conjugates useful in immunoassays and enzyme assays.

The strong correlations between the inhibition of radioligand binding and monoamine uptake suggests that it is unlikely that cocaine antagonists would be structurally similar or analogous to cocaine and to the 3β-phenyltropane-2β-carboxylic acid esters. A possible exception which may lead to other compounds is the isopropyl ester of 3β-(4'-iodophenyl)tropane-2β-carboxylic acid (**4w**), which requires further investigation.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III Polarimeter (1 dm cell). NMR spectra were recorded on a Bruker WM-250 or AM-500 spectrometer using tetramethylsilane as an internal standard; all were consistent with the structural assignments. Thin layer chromatography was carried out on Whatman silica gel 60 TLC plates using hexane-Et_2O-Et_3N (10:9:1). Flash chromatography was conducted on silica gel 60 (230-400 mesh) using hexane-Et_2O-Et_3N (30:9:1) as the eluent. Visualization was accomplished under UV or in an iodine chamber. Since all of the compounds described were prepared starting from natural cocaine, they are all optically active and have the absolute configuration of natural cocaine. Microanalyses were carried out by Atlantic Microlab, Inc. Cocaine was provided by the National Institute on Drug Abuse. [^{3}H]-3β-(*p*-Fluorophenyl)-tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN 35,428), and [^{3}H]paroxetine were purchased from Dupont-New England Nuclear (Boston, MA), and [^{3}H]nisoxetine was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

General Procedure for 3β-(substituted phenyl)tropane-2β-carboxylic acids (3). A solution or a partial suspension of the corresponding ester in 50%/H_2O (25 mL/mmol) was hydrolyzed by refluxing for 6 h. The clear solution

was evaporated to dryness and the residue recrystallized from MeOH–Et_2O.

3β-Phenyltropane-2β-carboxylic acid (3a) hydrochloride salt: mp 274 °C (lit.[30] mp 273–274 °C).

3β-(4′-Methylphenyl)tropane-2β-carboxylic acid (3b): mp 115–116 °C; $[\alpha]^{25}_D$ −104.99° (*c* 0.54, MeOH). Anal. Calcd for $C_{16}H_{21}NO_2$: C, H, N.

3β-(4′-Chlorophenyl)tropane-2β-carboxylic acid (3c): mp 300–301 °C; $[\alpha]^{25}_D$ −108.0° (*c* 0.10, MeOH). Anal. Calcd for $C_{15}H_{18}NO_2 \cdot 0.25\ H_2O$: C, H, N.

3β-(4′-Iodophenyl)tropane-2β-carboxylic acid (3d): mp 318–320 °C; $[\alpha]^{25}_D$ −79.3° (*c* 0.55, MeOH). Anal. Calcd for $C_{15}H_{18}NO_2$: C, H, N.

General Procedure for the Synthesis of 3β-(Substituted phenyl)tropane-2β-carboxylic Acid and Benzoylecgonine Ester and Amide Analogues (4–6). To a solution of 1 mmol of the appropriate 3β-(4′-substituted phenyl)tropane-2β-carboxylic acid (**2a–d**) or benzoylecgonine[26] in 5 mL of CH_2Cl_2 was added dropwise with stirring under nitrogen 2.0 equiv of oxalyl chloride (2 M solution in CH_2Cl_2). The resulting solution was stirred at room temperature for 1 h after evolution of gas had ceased. The solvent was removed in vacuo at room temperature and then at high vacuum to remove residual traces of oxalyl chloride. The resulting residue of acid chloride was suspended in 5 mL of CH_2Cl_2 under nitrogen at 0 °C and treated with 3 equiv of the appropriate alcohol or phenol or with 2.0 equiv of the appropriate amine hydrochloride containing 4.0 equiv of triethylamine, 2.5 equiv of the amine free base, or an aqueous solution (concentrated NH_4OH or 50% solution of methylamine or dimethylamine). The mixture was stirred at room temperature overnight. The reaction mixture was basified with 3 N NaOH or concentrated NH_4OH, the organic layer was separated, and the aqueous layer extracted with 3 × 10 mL of $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo to give crude product. The esters and amides were purified by flash column chromatography or crystallization. The physical properties of the esters and amides are listed in Tables 1 and 2.

N-3β-(4-Chlorophenyl)tropane-2β-*N*-(hydroxymethyl)carboxamide Hydrochloride (5d). To a solution of 0.15 g (0.54 mmol) of amide **5a** in 6 mL of THF were added 1 mL of 37% aqueous formaldehyde and 0.03 g of K_2CO_3. The reaction mixture was heated with stirring at 50 °C for 2 h and then poured into water and 3 N NaOH. The aqueous layer was extracted with $CHCl_3$ and dried (Na_2SO_4). Removal of solvent afforded 0.15 g (93%) of the product as free base, which was converted to the hydrochloride salt. Physical data are listed in Table 2.

Binding Assays. Inhibition of 0.5 nM [^{3}H]WIN 35,428, 0.5 nM [^{3}H]nisoxetine, and 0.2 nM [^{3}H]paroxetine binding was carried out as described previously.[28]

[^{3}H]Dopamine Uptake Assay. Striata were rapidly dissected from male Sprague–Dawley rats (Harlan Labs, Indianapolis, IN) and homogenized in ice-cold 0.32 M sucrose using a glass–Teflon homogenizer. The resulting homogenate was centrifuged for 10 min at 800*g*. The supernatant was decanted into another centrifuge tube and was centrifuged for 10 min at 20000*g*. The resulting pellet was resuspended in 0.32 M sucrose to a concentration of 15 mg/mL. Monoamine transport assays were conducted in modified Kreb's phosphate buffer (126 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.4 mM $MgSO_4$, 16.0 mM sodium phosphate, pH = 7.4) containing 2 mg/mL dextrose and 0.2 mg/mL ascorbic acid. Each assay tube contained 1 mL of buffer containing 0.5 nM [^{3}H]dopamine (Amersham, Arlington Heights, IL, specific activity 47 Ci/mmol), 10 μM pargyline, and 1.5 mg of crude striatal synaptosomes. Nonspecific uptake was measured using 1 μM mazindol. The assay was initiated by the addition of tissue and incubated for 3 min at 30 °C. The reaction was terminated by the addition of 5 mL of ice-cold 0.32 M sucrose, followed by immediate filtration using GF/B filters soaked in 0.05% polyethylenimine. The filters were washed twice with 5 mL of 0.32 M sucrose, and radioactivity was counted using a Beckman 5200 liquid scintillation counter.

[^{3}H]Norepinephrine Uptake Assay. The frontal cortex was utilized as a tissue source of the norepinephrine transporter. All assay conditions were similar to those utilized for the dopamine uptake assay except the tissue concentration was 10 mg/mL, the nonspecific uptake was measured using 1 μM desipramine, and the final concentration of [^{3}H]norepinephrine (New England Nuclear, Boston, MA, specific activity 56.9 Ci/mmol) was 5 nM.

[^{3}H]Serotonin Uptake Assay. The midbrain was utilized as a tissue source of the serotonin transporter. Again the assay conditions were identical to those utilized above with the following modifications: the tissue concentration was 1 mg/mL, the nonspecific uptake was measured using 1 μM citalopram, and the final concentration of [^{3}H]serotonin (New England Nuclear, Boston, MA, 27.4 Ci/mmol) was 5 nM.

Correlation of Inhibition of Radioligand Binding and Monoamine Uptake Parameters (Figure 1). Multiple linear regressions were performed using the Implot software from Graphplot.

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