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Perspective

2002 Medicinal Chemistry Division Award Address: Monoamine Transporters and Opioid Receptors. Targets for Addiction Therapy[†]

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In 2001, an estimated 15.9 million Americans aged 12 or older were current illicit drug users.¹ This estimate represents 7.1% of the population aged 12 years or older.¹ The percentage of the population using illicit drugs increased from 6.3% in 1999 and 2000 to 7.1% in 2001.¹ Among youths 12–17 years of age, 10.8% were current illicit drug users, which is higher than the rate observed in 2000 (9.7%). An estimated 1.2 million Americans are current users of cocaine, and 0.1% of the population aged 12 and older are current users of heroin.¹ In addition, approximately 957 000 persons aged 12 and older had used oxycontin nonmedically at least once in their lifetime.¹ These facts, together with the expanding social costs of increased street violence and drug-abuse-related deaths, the proliferation of children and adolescents working in the drug abuse trade, the suffering caused to innocent victims of crime, and the intravenous injection of the drug, which increases the risk of AIDS, speak to the need for solutions to this public health problem. It is now recognized that cocaine and heroin addictions are diseases of the brain with specific neurobiological characteristics resembling those of many other diseases.^{2,3} Thus, very similar to other diseases, the development of pharmacotherapies effective in treating cocaine and opiate addictions is one way of addressing this health problem. The initial event that leads to addiction is the interaction of a drug of abuse at a specific site (receptor) on a target protein. In this perspective, I present our research directed

toward monoamine transporters and opioid receptors as targets for the development of such pharmacotherapies.

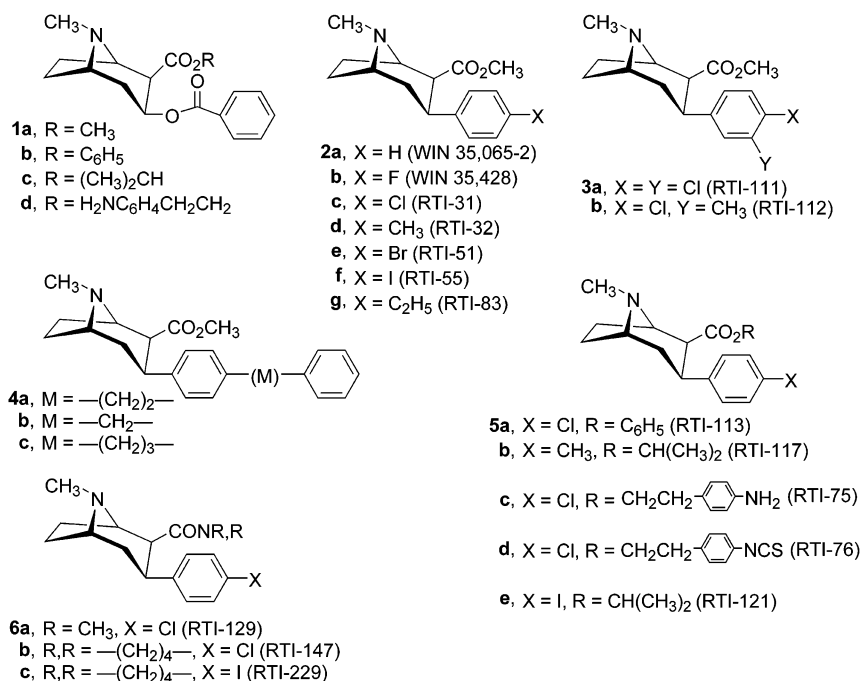
(1) Monoamine Transporters as Target for Addiction Therapy

(1.1) Introduction. The molecular site where cocaine interacts to produce its physiological effects had to first be identified in order to understand the biochemical mechanisms related to its addictive properties. Cocaine has several sites of action in the central nervous system (CNS). It has been shown to block the reuptake of dopamine (DA), serotonin (5-HT), and norepinephrine (NE) as well as to exert effects on the cholinergic muscarinic and σ receptors and sodium channels.^{4,5} However, over the past several years, substantial evidence has accumulated from animal studies that strongly suggests that the dopamine transporter (DAT) is a key target (receptor) for cocaine regarding the reinforcing effects of the drug.^{4,5} The DAT is a protein located on presynaptic nerve terminals that plays a major role in the uptake of synaptic DA. The cDNA for the DAT has been cloned from rat,^{6–8} mouse,^{9,10} bovine,¹¹ and human brains.¹² The predominant theoretical base for the reinforcing properties of cocaine is the so-called “dopamine hypothesis.”^{4,5} This hypothesis assumes that cocaine binds to the dopamine transporter site in a way that inhibits dopamine transport. This inhibition of dopamine uptake results in a buildup of dopamine in the synaptic cleft, leading to significant potentiation of dopaminergic transmission. This potentiation, which presumably does not occur under natural circumstances, is responsible for the reinforcing proper-

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Chart 1. Compounds 1–6



ties of cocaine and perhaps for some of its euphorogenic effects as well.

In a 1992 perspectives paper,⁴ we summarized the information that strongly suggested the DAT as the cocaine receptor responsible for abuse and presented what was known about the site at that time. We presented the structure–activity relationship (SAR) information, speculated on a preliminary pharmacological model, and provided perspectives for future research. Many studies by numerous investigators have been reported since that review. Even though the DA hypothesis has received strong support from a large number of preclinical investigations,^{4,13} there is evidence that the serotonin transporter (5-HTT) or the norepinephrine transporter (NET) may also play some type of modulatory role in cocaine addiction.¹⁴

To develop high-affinity and selective ligands for each of these transporters, an understanding of the SAR was required. This may be accomplished experimentally by correlation of the structural variation with inhibition of [³H]WIN 35,428, [³H]paroxetine, and [³H]nisoxetine binding at the DAT, 5-HTT, and NET, respectively. This information was used as an indication of the compound's potential ability to inhibit uptake of the DA, 5-HT, and NE neurotransmitters.

(1.2) Structure–Activity Relationship Studies.

(1.2.1) Cocaine Analogues. Since cocaine (**1a**, Chart 1) was the drug of interest, we started our SAR efforts by studying cocaine analogues. The cocaine structure has three sites of asymmetry; thus, there are eight possible isomers. We found that only natural cocaine has appreciable affinity for the DAT; the other seven isomers were 60–600 times weaker.¹⁵ In addition to providing information about the stereochemical requirements for binding at the DAT, this information provided additional support for the dopamine hypothesis. In subsequent studies, we found that while a 2 β -substituent was required for high affinity, the position was tolerant to a group possessing a variety of structural

features and sizes.^{16,17} The results obtained from these early studies suggested that an electrostatic interaction might occur between the 2 β -carbomethoxy group of cocaine and amino acid residues in the DAT protein. However, we also pointed out that an additional effect of the 2 β -substituent might be to distort the 8-azabicyclo-[3.2.1]octane skeleton by flattening of the seven-membered ring, particularly at the 8-aza end, to relieve steric strain between the 2 β -substituent and the aza bridge.¹⁶

One of our most important findings from our SAR studies on cocaine analogues was that the phenyl and isopropyl esters, **1b** and **1c**, respectively, while similar to cocaine in their affinity for the DAT, showed much lower affinity at the 5-HTT and NET.^{18,19} This information was used to design and prepare DAT-selective analogues in the 3-phenyltropane series (see below). Another important finding was that compounds with very large ester groups, such as the *p*-aminophenylethyl group in **1d**, retained affinity for the DAT similar to that of cocaine. This SAR information led to the design, synthesis, and evaluation of a number of cocaine and 3-phenyltropanes with diverse groups in the 2-position of the tropane ring.¹⁷

(1.2.2) 3-Phenyltropane Analogues. With the goal of separating the stimulant and depressant actions of cocaine from its toxicity and dependence liability, Clarke and co-workers^{20,21} synthesized and determined the biological properties of WIN 35,065-2 (**2a**) and its 4'-fluoro analogue WIN 35,428 (**2b**). These compounds were 5- to 60-fold more potent than cocaine in behavioral studies, including locomotor activities and preventing or reversing reserpine-induced eyelid ptosis in mice. Subsequent investigators made similar observations with other drug-induced behaviors.^{22–25} Drug discrimination studies using pigeons showed that WIN 35,428 and WIN 35,065-2 were fully generalized to cocaine and were more potent.²⁶ WIN 35,065-2 and WIN 35,428 were 3–10 times more potent than cocaine in squirrel mon-

keys responding under a multiple-fixed-ratio, fixed-interval schedule of termination of a stimulus, associated with electric shock or food presentation.²⁷ In squirrel monkey fixed-interval schedule of drug self-administration studies, WIN 35,065-2 was three to 10 times more potent than cocaine.²⁸ The SARs found in the self-administration study paralleled the SAR found in the drug-induced locomotor activity studies, suggesting a common pharmacological mechanism for the different behaviors. These advances prompted our interest in the further development of the 3-phenyltropane class of DA uptake inhibitors.

The pharmacological properties of the 3-phenyltropane class of compounds, like those of cocaine, are centered around their interaction with the DAT, 5-HTT, and NET. As part of our program to study the biochemical mechanism of action of cocaine, we conducted an SAR study to investigate the monoamine transporter binding properties of a series of the 3-phenyltropane analogues. These studies have led to the discovery of new, nonselective monoamine uptake inhibitors, as well as structurally unique 3-phenyltropane analogues selective for the DAT, 5-HTT, and NET.

(1.2.3) Analogues Nonselective for the Monoamine Transporter. We first reported that the 4'-chloro (**2c**, RTI-31) and 4'-methyl (**2d**, RTI-32) analogues of WIN 35,065-2 were 20 times more potent than the parent WIN 35,065-2, showing that DAT binding affinity was profoundly affected by 3 β -phenyl ring substituents.²⁹ This was followed by the synthesis and evaluation of a number of phenyl ring substituted analogues.¹⁷ Some of the more interesting and widely studied analogues initially synthesized were the 4'-chloro-, 4'-methyl-, 4'-bromo-, and 4'-iodophenyl compounds **2c** (RTI-31), **2d** (RTI-32), **2e** (RTI-51), and **2f** (RTI-55), respectively.^{29,30} Later studies showed that these analogues also possessed good affinity for the 5-HTT and NET (Table 1).¹⁹

On the basis of the results from our initial studies, we investigated a series of 12 WIN 35,065-2 analogues where the 4'-substituent was varied with respect to size, electronic character, and hydrophobic/hydrophilic properties and to minimize covariance among QSAR variables.^{30,31} The binding affinities of the 12 analogues were correlated with the structural features using comparative molecular field analysis (CoMFA).³² The relative contributions of steric interaction and electrostatic potential to the CoMFA regression equation for binding to the DAT were found to be 62% and 38%, respectively. The positive and negative steric and electrostatic regions identified in the standard deviation coefficient contour maps were helpful in understanding the DAT binding affinities. Most importantly, the CoMFA information was useful in predicting new structures to be investigated. On the basis of these studies, the 3',4'-dichloro and the 3'-methyl-4'-chloro analogues **3a** (RTI-111) and **3b** (RTI-112) were prepared and found to possess 29 and 28 times greater affinity, respectively, for the DAT than WIN 35,065-2 and were 112 and 110 times more potent than cocaine (Table 1).^{19,33} Later studies showed that these analogues, as well as other 3',4'-dihalosubstituted 3-phenyltropane analogues, also possessed high affinity for the 5-HTT and NET as well as for the DAT. This strategy was used

Table 1. Comparison of Monoamine Binding Properties of RTI 3 β -Phenyltropane Analogues to Cocaine and WIN 35,065-2

compd	IC ₅₀ , nM (K _i , nM)		
	DAT [³ H]WIN 35,428	5-HTT [³ H]paroxetine	NET [³ H]nisoxetine
cocaine ^a	89	1050 (96)	3300 (2000)
WIN 35,065-2 ^a	23	1962 (178)	920 (554)
RTI-31 ^a	1.12	44.5(4.1)	37 (22)
RTI-32 ^a	1.71	240 (22)	60 (36)
RTI-51 ^a	1.69	10.6 (0.96)	37.4 (23)
RTI-55 ^a	1.26	4.21 (0.38)	36 (21.6)
RTI-75 ^b	2.16		
RTI-76 ^b	5.3	7.10 (0.65)	1400 (843)
RTI-83 ^c	55	28.4 (2.6)	3907 (2353)
RTI-92 ^d	26	39 (3.6)	1600 (964)
RTI-111 ^a	0.79	3.13 (0.28)	18 (10.8)
RTI-112 ^a	0.81	10.5 (0.95)	36.2 (2.8)
RTI-113 ^a	1.98	2340 (214)	2960 (1780)
RTI-117 ^a	6.45	6090 (554)	1930 (1160)
RTI-129 ^a	1.38	1080 (98)	940 (566)
RTI-147 ^a	1.38	12,400 (1127)	3940 (2370)
RTI-173 ^c	50	8.1 (0.74)	122 (74)
RTI-229 ^a	0.37	1730 (157)	990 (596)
RTI-177 ^e	1.28	2420 (220)	504 (304)
RTI-422 ^f	1.96	11,000 (1000)	480 (289)
RTI-353 ^d	329	0.69 (0.063)	148 (89)
RTI-357 ^g	23	0.60 (0.055)	144 (87)
RTI-362 ^h	33.6	500 (46)	9.0 (5.4)
RTI-336 ⁱ	4.09	5740 (522)	1710 (1030)
RTI-539 ⁱ	9.0	97.4 (23.8)	0.86 (0.43)

^a Taken from ref 19. ^b Taken from ref 36. ^c Taken from ref 55. ^d Taken from ref 56. ^e Taken from ref 39. ^f Taken from ref 40. ^g Taken from ref 57. ^h Taken from ref 58. ⁱ Unpublished.

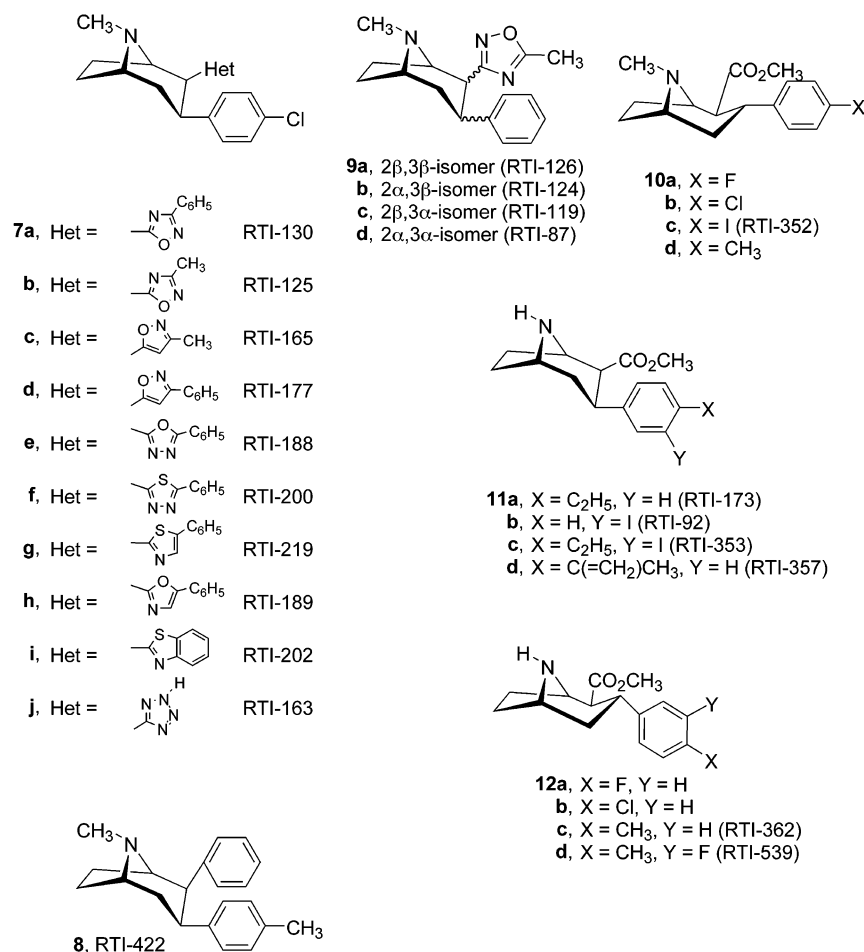
for obtaining a number of high-affinity, nonselective monoamine transporter analogues (unpublished results).

We conducted a second QSAR and CoMFA study on 25 3 β -(substituted phenyl) tropanes.³⁴ The classical QSAR model suggested that hydrophobicity was an important contributor to the DAT binding affinity. The CoMFA models showed that some steric bulk extending from and above the 4'-position contributes to enhanced binding affinity, but excessive bulk leads to reduced affinity. In addition, the models suggested that electrostatic forces accounted for approximately one-quarter of the binding affinity. The studies also revealed that 3'-halo analogues were less potent than 4'-halo analogues.

More recent studies showed that there was a remote aromatic ring binding site approximately two carbons extended from the 4'-position.³⁵ For example, the 4'-phenylethyl analogue **4a** has high affinity for the DAT (IC₅₀ = 5.14 nM), whereas 4'-benzyl- and 4'-phenylpropyl analogues **4b** and **4c**, with shorter or longer linker chains, possessed IC₅₀ values of 526 and 351 nM, respectively.

(1.2.4) Analogues Selective for the Dopamine Transporter. Since we had found that modification of the 2-substituent on the cocaine skeleton afforded compounds showing increased selectivity for the DAT, we expected that similar modification of the 3-phenyltropane structure would lead to DAT-selective analogues in this series.^{18,19} As in the cocaine series, the phenyl and isopropyl esters, such as **5a** (RTI-113) and **5b** (RTI-117), have higher affinity and are selective for the DAT relative to binding at the 5-HTT and NET (Table 1).^{18,19} Similar to the cocaine series, replacement of the methyl group in the 2 β -carbomethoxy substituent with larger groups such as the 2-(4'-aminophenyl)ethyl

Chart 2. Compounds 7–12



group **5c** (RTI-75) had only small effects on binding at the DAT (Table 1).³⁶ This information was used to design the useful irreversible binding ligand **5d** (RTI-76).³⁶ Its use will be discussed in Neurochemical Tools.

To obtain analogues with increased metabolic stability, we studied a series of 3-phenyltropanes where the 2 β -carbomethoxy group was replaced with a carboxamide group.^{19,37} Tertiary amides were found to have higher affinity at the DAT than primary and secondary amides. The analogue 3 β -(4'-cholophenyl)-2 β -dimethylcarboxamide **6a** (RTI-129), with an IC₅₀ value of 1.38 nM at the DAT and much larger IC₅₀ values at the NET and 5-HTT, is a potent and highly selective DAT ligand (Table 1). The pyrrolidinocarboxamides **6b** (RTI-147) and **6c** (RTI-229) also were potent and selective for the DAT (Table 1). We also replaced the metabolically labile 2 β -carbomethoxy group by stable bioisosteric heterocyclic groups possessing properties similar to those of the 2 β -carbomethoxy group such as 1,2-isoxazoles, 1,2,4-oxadiazoles, 1,3-thiazoles, 1,3,5-oxadiazoles, 1,3,5-thiadiazoles, and 1,3-oxazoles (**7a–i**, Chart 2) and found that several of these analogues possessed high affinity and selectivity for the DAT.^{38,39} RTI-177 and RTI-336 with IC₅₀ values of 1.28 and 4.09 nM for DAT and reasonable selectivity for the DAT are two of the more studied analogues (Table 1).

Computational correlation of the electrostatic (molecular electrostatic potential, MEP), hydrophobic (calculated log *P*), and steric (substituent volume) properties of the 2 β -heterocyclic moiety of 3 β -(4'-chlorophenyl)-

tropane analogues **7a–j**, as well as of the parent ester RTI-31 with their DAT binding affinities, revealed a high correlation with the MEP of one of the heteroatoms in the heterocyclic rings.^{38,39} Neither the ClogP nor the substituent volume of the 2 β -substituents provided a satisfactory correlation. These molecular modeling studies strongly suggested a predominantly electrostatic interaction for these 2 β -heterocyclic analogues.³⁹

In another study, we replaced the 2 β -carbomethoxy group of WIN 35,065-2 with aryl groups. From this study, *R*-3 β -(4'-methylphenyl)-2 β -phenyltropane (**8**, RTI-422) was found to be a potent and selective ligand for the DAT (Table 1).⁴⁰ This shows that a phenyl ring as well as a heterocyclic group can replace the 2 β -carbomethoxy group in 3 β -phenyltropane-2 β -carboxylic acid methyl esters without loss of affinity at the DAT.

A possible explanation for the wide range of substituents that can be accommodated at the C-2 position of the 3-phenyltropanes is the existence of several different binding modes for the C-2 group.¹⁷ Apparently, C-2 substituents can interact with the DAT binding site via an electrostatic, hydrophobic, or some other process depending on the nature of the C-2 group. Our results with the 3-phenyltropane analogues bearing heterocyclic groups at the C-2 position^{38,39} showed that the electrostatic interactions predominate over hydrophobic interactions when both types of interactions are possible.^{38,39}

Like cocaine, WIN 35,065-2 has three sites of asymmetry and eight possible isomers. To our knowledge, no study of all eight isomers of WIN 35,065-2 has been

reported. The data available suggest that the $2\beta,3\beta$ -isomer (WIN 35,065-2) has the highest affinity for the DAT.¹⁷ To gain information about stereoselectivity, we prepared all four isomers (**9a–d**) of the (1*R*,5*S*)-3-phenyl-2-(3'-methyl-1',2',4'-oxadiazol-5'-yl)tropane bioisostere of WIN 35,065-2 and evaluated their binding affinity at the DAT.^{41,42} The $2\beta,3\beta$ -isomer (RTI-126) with an IC₅₀ value of 100 nM possessed the highest affinity for the DAT. However, the IC₅₀ values of 167 and 204 nM for the $2\beta,3\alpha$ - and $2\alpha,3\alpha$ -isomers, respectively, were only slightly larger than that of the $2\beta,3\beta$ -isomer. These interesting results led us and other investigators to study ($2\beta,3\alpha$)- and ($2\alpha,3\alpha$)-3-phenyltropane-2-carboxylic acid methyl ester analogues.¹⁷ For example, we developed two novel synthetic routes to the 3α -(4'-substituted phenyl)tropane-2- β -carboxylic acid methyl esters such as **10a–d** and showed that the $2\beta,3\alpha$ -isomers existed in the boat conformation.^{43,44} This was not unexpected because we had already shown that the $2\beta,3\alpha$ -oxadiazole compound **9c** possessed a boat conformation.^{41,42} In a study of the monoamine transporter binding properties of the $2\beta,3\alpha$ -isomers of 3-(substituted phenyl)tropane-2-carboxylic acid methyl esters, we found that in many cases this isomer was more selective for the DAT and with only slightly lower DAT affinity than the corresponding $2\beta,3\beta$ -isomers.⁴³

(1.2.5) Analogues Selective for the Serotonin Transporter. Several reported studies suggest that the serotonin system may be involved in the neurochemical and behavioral effects of cocaine. It is well-known that cocaine shows greater inhibition of 5-HT uptake than DA and thus influences 5-HT neurotransmission.⁴⁵ Both DAT and 5-HTT knock-out mice have been generated, as well as combined DAT and 5-HTT knock-out mice. Whereas conditioned place preference was achieved with cocaine in the DAT and 5-HTT knock-out mice,⁴⁶ no place preference for cocaine was observed in the combined DAT and 5-HTT knock-out mice.⁴⁷ This suggests that in the absence of DAT, the interaction of cocaine at the 5-HTT might be sufficient to induce cocaine reward.

In animal behavioral studies, 5-HT uptake inhibitors were reported not to be self-administered.^{48–50} In addition, some of the inhibitors are reported to decrease iv self-administration of cocaine in rats and squirrel monkeys.^{51–54} Even though the importance of the 5-HTT in mediating the neurochemical and behavioral action of cocaine is now recognized, the biochemical mechanism of action and regulation of this transporter are not well understood. To help in studies involving the 5-HTT, we designed and synthesized 3-phenyltropane analogues that were selective for the 5-HTT. In our SAR studies to develop the monoamine nonselective and DAT-selective compounds, we noted that the 3β -(4'-ethylphenyl) analogue (**2g**, RTI-83), which differs from the 3β -(4'-methylphenyl) analogue RTI-32 by one methylene group, was 32 and 65 times less potent than RTI-32 at the DAT and NET, respectively, but was 9 times more potent at the 5-HTT (Table 1).⁵⁵ We also noted that N-demethylation of cocaine and of 3-phenyltropanes to give the nortropane analogues led to relatively small effects at the DAT.⁵⁵ In contrast, affinity at both the 5-HTT and the NET was enhanced.⁵⁵ Particularly important findings were that 3β -(4'-ethylphenyl)nortro-

pane-2- β -carboxylic acid methyl esters (**11a**, RTI-173), the nortropane analogue of RTI-83, possessed 5-fold greater binding selectivity for the 5-HTT relative to the DAT (Table 1).⁵⁵ In addition, in our studies of the substituted aromatic ring analogues, we noted that 3β -(3-iodophenyl)tropane-2- β -carboxylic acid methyl ester (**11b**, RTI-92) showed a 50-fold increase in affinity at the 5-HTT relative to WIN 35,065-2 and only a slight affinity increase at the DAT.⁵⁶ Combining these SAR findings led to the synthesis of the 5-HTT-selective 3β -(4'-ethyl-3'-iodophenyl)nortropane-2- β -carboxylic acid methyl ester (**11c**, RTI-353), which had a K_i value of 0.06 nM at the 5-HTT and much lower affinity for the DAT and NET (Table 1).⁵⁶ In a separate study, 3β -(4'-isopropenylphenyl)tropane-2- β -carboxylic acid methyl ester (**11d**, RTI-357) was also found to be a 5-HTT-selective analogue (Table 1).⁵⁷

(1.2.6) Analogues Selective for the Norepinephrine Transporter. As mentioned in the previous section, the SAR studies on the 3β -(4'-substituted phenyl)nortropane-2- β -carboxylic acid methyl esters revealed that replacement of the *N*-methyl group by a hydrogen on the tropane ring increased binding affinity at the NET and 5-HTT with little effect on binding affinity at the DAT. The study of 3α -(4'-substituted phenyl)tropane-2- β -carboxylic acid methyl esters revealed that for several of these analogues the NET affinity was reduced less than that of the DAT and 5-HTT. Combinations of these findings suggested that 3α -(4'-substituted phenyl)nortropane-2- β -carboxylic acid methyl esters might be more potent and selective for the NET. Indeed, we found that 3α -(4'-fluoro-, 4'-chloro-, and 4'-methylphenyl)nortropane-2- β -carboxylic acid methyl esters (**12a–c**) possessed increased affinity at the NET relative to the corresponding $2\beta,3\beta$ -tropane analogues.⁵⁸ The 4'-methyl analogue **12c** (RTI-362) was the first 3-phenyltropane analogue to show selectivity for the NET (Table 1). The addition of a 3'-fluoro substituent to RTI-362 provided **12d** (RTI-539), which possessed a K_i value of 0.43 nM at the NET and was even more NET-selective (Table 1).

(1.3) Behavioral Studies. In the previous SAR section, the development of a number of 3-phenyltropane analogues, which possessed desirable in vitro monoamine transporter binding properties, was presented. In order for one or more of these analogues to be a preclinical candidate for treating cocaine abuse, they need to also possess desirable pharmacological properties. Important properties for a cocaine substitute pharmacotherapy would include high potency in various in vivo tests. Since the success of methadone as a substitute agonist for heroin addiction has been attributed in part to its long duration and slow onset of action,^{59–61} we reasoned that a cocaine-like compound such as a 3-phenyltropane analogue with a slower onset and a longer duration of action might be an effective pharmacotherapy for cocaine dependence.

It would be desirable to have efficacy demonstrated in animal models predictive of clinical outcomes. Unfortunately, no single animal model has yet been shown to be predictive of clinical efficacy for cocaine abuse. Hence, several behavioral animal models are used in the development of pharmacotherapies for cocaine abuse. In our development of the 3-phenyltropanes, compound

Table 2. Summary of Locomotor Activity, Drug Discrimination, and Self-Administration Studies of RTI 3-Phenyltropane Analogues^a

compd	locomotor activity, ip (po)				drug discrimination, ^f ED ₅₀	blocks cocaine SA	
	peak time, ^b min	ED ₅₀ , ^c mg/kg	% cocaine ^d	duration, ^e h		rat	monkey
cocaine	0–30	21	100	<2	2.50		
WIN 35,065-2	10–40	2.1	146	2–4	0.35		
RTI-112	30–60	2.2	75	>4	0.21	yes	yes
RTI-113	30–60	0.93	128	>4	2.28	yes	yes
RTI-177	10–40 (100–130)	3.1 (4.7)	103 (116)	>4 (>4)	4.40 (5.7)	yes	yes
RTI-336	70–100 (60–90)	4.8 (14.4)	71 (77)	>4 (>4)	5.60 (3.54)	yes	yes

^a Unpublished. ^b 30-min period during which the compound produced its maximal effect. ^c Dose to produce 50% of the compound's maximal effect. ^d Compound's maximal effect as a percent of cocaine's maximal effect. ^e Time during which activity returned to baseline level. ^f Generalization testing in cocaine-trained rats; dose necessary to produce 50% of the compound's maximal effect if greater than 80% generation.

selection is initially based on monoamine binding properties followed by rodent studies of locomotor activity and drug discrimination. Compounds that pass these tests are then subjected to drug self-administration studies in rats and monkeys. Information concerning the potency, as well as the onset and duration of action, of the 3-phenyltropanes relative to cocaine was gained using both locomotor activity and drug discrimination tests. The ratio of the level of stimulation of the test 3-phenyltropane to that of cocaine was used as an efficacy measure. It is thought by some that the discrimination stimulus properties of cocaine and cocaine-like compounds may be related to their subjective effects in humans. The self-administration studies were used to relate the abuse potential of the 3-phenyltropane analogues to that of cocaine and to determine the effect of the test 3-phenyltropanes on cocaine intake of rat and monkeys trained to self-administer cocaine. Cocaine substitute pharmacotherapies would be expected to reduce cocaine intake.

In 1992, we reported that the 4'-Cl, Br, I, and CH₃ analogues RTI-31, -51, -55, and -32, respectively, were all significantly more potent than cocaine in locomotor activity. Moreover, *in vivo* binding studies revealed that the dose that elicited maximal behavioral effects was very similar to the dose that produced maximal occupancy of the DAT.⁶² In another study intravenous injection of RTI-55, -121, and -130 affected dose-related increases in locomotor activity in mice with potencies at least 10-fold greater than that of cocaine. The increased locomotor activity was observed at least 10 h after *iv* administration.⁶³ More recently, we determined the locomotor activity of a number of DAT-selective and monoamine transporter nonselective analogues, all of which possessed affinity at the DAT greater than that of cocaine. All of these analogues were more potent than cocaine and possessed at least equivalent efficacy.⁶⁴

RTI-55, -31, and -32 also substituted for cocaine in rats trained to discriminate cocaine from saline with potencies 13-, 10.3-, and 6.6-fold greater than that of cocaine.⁶² In a separate rat drug discrimination study, RTI-31 and -32 were found to be 26.8 and 6 times more potent than cocaine.⁶⁵ Even though the potency in behavioral studies did not exactly parallel the DAT binding and uptake inhibition data, there was a rank order correlation. Thus, the more potent compounds in the behavioral studies tended to be more potent in DAT binding. In a more recent study, we demonstrated that several monoamine transporter nonselective 3-phenyltropane analogues such as RTI-112 and DAT selective analogues such as RTI-113 produced cocaine-like dis-

Table 3. Comparison of Pharmacological Potencies of RTI-112 to Bupropion, a Drug To Treat Smokers (Nicotine Addiction)

compd	nicotinic antagonism ^a		reuptake ^b IC ₅₀ (nM)		
	AD ₅₀ , mmol/kg analgesia	AD ₅₀ , mmol/kg hypothermia	DA	NE	5-HT
bupropion	9	16	2900	1450	47000
RTI-112	3.5	1.5	0.6	0.8	1.0

^a Antagonism of nicotine-induced antinociception in mice. Data taken from ref 67. ^b Monoamine reuptake data using cloned human transporter. Data supplied by NIDA through their CTD program.

crimination stimulus effects that were mediated by the DAT but not by the 5-HTT.⁶⁶ In addition, 3-phenyltropane analogues had slower onset of action and much longer duration of action. For example, the 3-phenyltropane analogue **3b** (RTI-112) showed a 2–4 times slower onset of action and a 6–8 times longer duration of action relative to cocaine. In drug discrimination tests, RTI-112 showed potencies 10 times that of cocaine, but its maximal stimulant effect was slightly less than that of cocaine (Table 2). On the basis of its interesting pharmacological profile, the monoamine nonselective RTI-112 was evaluated in self-administration tests. Pretreatment of rats, squirrel monkeys, and rhesus monkeys trained to self-administer cocaine with RTI-112 resulted in a reduced intake of cocaine (Table 2, unpublished results), properties expected of a substitute pharmacotherapy.

In another study, RTI-112 was shown to be more potent than bupropion as a noncompetitive nicotinic antagonist⁶⁷ (Table 3). By combination of these favorable pharmacological profiles with low acute and chronic toxicity tests in rats and dogs, the lack of any interactions in a 61 assay NovaScreen and lack of adverse indications in a MDS Pharma screen led to the selection of RTI-112 as a clinical candidate for possible treatment of both cocaine and nicotine (smoking) addiction. The GMP sample has been prepared, and advanced pharmacological and toxicological studies are underway. The studies with RTI-112 showed that 3-phenyltropane analogues that possessed high affinity for all three monoamine transporters possess pharmacological properties needed for a substitute pharmacotherapy.

We were interested in determining if a DAT-selective 3-phenyltropane analogue might also possess pharmacological properties desirable for a substitute pharmacotherapy. As already mentioned, RTI-113 produces a long-lasting cocaine-like discriminative effect and inhibited self-administration of cocaine in rat at doses that

did not alter responding maintained by food.⁶⁸ The dose that inhibited 92% of cocaine intake produced 60% levels of DAT occupancy. This was the first reported case of a highly DAT-selective 3-phenyltropane analogue inhibiting self-administration of cocaine, showing that interaction at the DAT is sufficient to inhibit cocaine self-administration (Table 2).

Similarly, in squirrel and rhesus monkeys, systemically administered doses of RTI-113 decreased cocaine self-administration and exhibited a duration of action longer than that of cocaine as determined in fixed-interval stimulus termination procedures^{69,70} (Table 2). RTI-113 is self-administered in rhesus monkeys but at levels generally lower than those obtained with cocaine.⁷⁰ Taken together with the long-lasting effects of RTI-113 seen in cocaine-like discrimination studies, these data showed that RTI-113 or other DAT-selective 3-phenyltropane analogues may be useful in furthering the development of a cocaine substitute pharmacotherapy for cocaine-dependent disorders.

In recent studies, the DAT-selective 3-phenyltropane analogue **13** (RTI-336) (Table 1) has been shown to possess locomotor activity (by both ip and po administration) and to possess cocaine discrimination and self-administration properties expected of a substitute pharmacotherapy (Table 2). This information, combined with favorable results in the AMES, HERG, NOVA, and P-450 enzyme *in vitro* assays and the low acute toxicity, has led to the selection of RTI-336 as a clinical candidate for a pharmacotherapy for treating cocaine addiction (Table 2, unpublished results).

Anticocaine catalytic antibodies, which serve as circulating peripheral blockers of cocaine that prevent its action in the brain, provide an alternative medication for cocaine abuse.^{13,71} We found that several 3-phenyltropane analogues did not possess affinity for the anticocaine catalytic antibody 15A10, suggesting that the two types of treatments could be used together.⁷² Thus, the 3-phenyltropane analogues could give the benefits associated with the substitution therapy, while the antibody would simultaneously reduce the effects of cocaine in the event of a relapse.

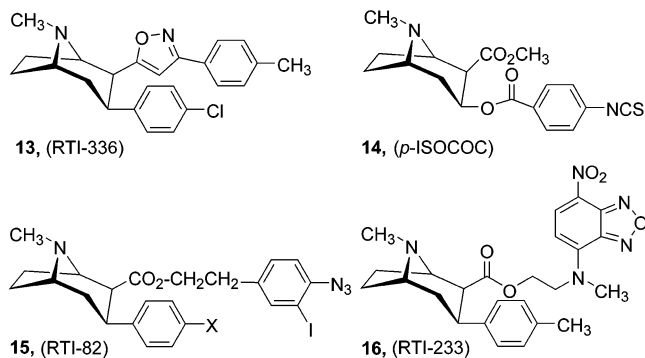
(1.4) Neurochemical Tools. In previous sections, we pointed out that cocaine exerts its behavioral effects in part by inhibiting synaptic DA uptake, resulting in potentiation of dopaminergic neurotransmission. However, the serotonin and norepinephrine systems also play an important part. In addition to the development of pharmacotherapies for cocaine addiction discussed in previous sections, we have devoted considerable effort toward developing 3-phenyltropane analogues designed as neurochemical tools for studying the monoaminergic systems. These efforts have included tritium, carbon-11, iodine-125, and iodine-123 radiolabeled ligands, 3-phenyltropane analogues that bind irreversibly to the cocaine binding site on the DAT and 5-HTT, and fluorescent ligands.

(1.4.1) Radiolabeled Probes. Since the DAT is a marker for DA neurons, there is interest in studying regulatory or drug-induced changes in DAT levels as well as the loss of DA neurons caused by neurodegenerative diseases such as Parkinson's. During the late 1980s and early 1990s, there was substantial progress in the imaging of the DAT.⁷³ Initial studies used [¹¹C]-

nomifensine and [¹¹C]cocaine; unfortunately, the usefulness of these ligands was limited because of the low signal-to-noise ratio (low-specific binding).⁷³ We synthesized [³H]WIN 35,065-2 and showed that, like [³H]cocaine, it bound to both high- and low-affinity sites and possessed a much better specific-to-nonspecific binding ratio.^{74,75} An important advance in imaging the DAT occurred when *in vivo* binding studies in mice using [³H]-WIN 35,065-2 showed substantially improved specific-to-nonspecific binding when compared to [³H]cocaine. These studies were followed by the syntheses of [³H]-RTI-31 and [¹²⁵I]RTI-55 (also referred to as [¹²⁵I]βCIT), both of which were shown to possess pharmacological properties similar to those of [³H]cocaine and [³H]WIN 35,065-2.⁷⁶ [¹²⁵I]RTI-55 was particularly unique, since it was the first radioiodinated ligand available for studying the DAT. Its highly favorable specific-to-nonspecific binding ratio and its ideal pharmacological properties made it the radioligand of choice for *in vitro* and *in vivo* radioligand binding and autoradiographic studies.^{73,77–80} Particularly important was that in *in vivo* preimaging studies RTI-55 was observed to possess a greater than 15 to 1 striatal to cerebellum binding ratio, a measure of specific binding. This was followed by the syntheses and development of [¹²³I]RTI-55 as the first single photon emission computed tomography (SPECT) ligand for imaging DAT uptake sites in primate brain.^{81,82} We showed that 21 h after iv injection of [¹²³I]RTI-55 into baboon only striatal binding was observed. If the baboon was unilaterally injected with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), [¹²³I]RTI-55 was absent from the pretreated striatum. These and other studies led to the development of [¹²³I]-RTI-55 (DOPASCAN) as a diagnostic ligand for Parkinson's disease.^{83–85} In clinical trials on over 1600 patients worldwide, [¹²³I]RTI-55 has been shown to provide a high degree of specificity and accuracy in the diagnosis of Parkinson's disease. [¹²³I]RTI-55 is in phase III clinical trials as a diagnostic agent for Parkinson's disease in Japan, and phase II trials have been completed in the U.S.

Since SAR studies had shown that changing the 2β-carbomethoxy group of RTI-32 to the 2β-carboisopropoxy group to give RTI-117 resulted in increased selectivity for the DAT, we developed [¹²⁵I]-3β-(4'-iodophenyl)-tropane-2β-carboxylic acid isopropyl ester ([¹²⁵I]RTI-121) as a DAT-selective radioligand.^{18,19,86,87} We showed that the pharmacological profile of [¹²⁵I]RTI-121 in rat striatum was consistent with that of the DAT. Imaging studies using [¹²³I]RTI-121 in rodents and nonhuman primates also showed the DAT selectivity of this radioligand.⁸⁸ There was a strong correlation between the potencies of drugs that displaced specific [¹²⁵I]RTI-121 binding and the potencies of drugs that inhibit the uptake of [³H]dopamine.⁸⁸ In contrast, no correlation was found for the potencies of drugs to inhibit the uptake of either [³H]norepinephrine or [³H]serotonin. The autoradiographic distribution of [¹²⁵I]RTI-121 binding in rat⁸⁶ and human brain^{89,90} was shown to be consistent with a DAT-selective ligand.

Since autoradiographic studies using [¹²⁵I]RTI-55 revealed the distribution of both DAT and 5-HTT with a distribution pattern resembling that of cocaine, it is useful for examining the overall distribution of cocaine

Chart 3. Compounds **13**–**16**

binding sites. On the other hand, the high-affinity, low-background binding, and superior selectivity of [¹²⁵I]-RTI-121 make it an excellent ligand for the study of the DAT. More recently we developed [¹²⁵I]RTI-229 as an even more DAT-selective radioligand.⁹¹

RTI-352 (**10c**), the 3 α -isomer of RTI-55, was also shown to be an effective *in vivo* binding ligand for the DAT.⁹² Studies with [³H]RTI-352 showed that it possessed greater selectivity for the DAT relative to 5-HTT, and there was also more rapid achievement of apparent equilibrium in the striatal-to-cerebellum binding ratio compared to RTI-55. We also showed that several [¹²⁵I]-labeled *N*-substituted (*N*-allyl, *N*-butyl, and *N*-fluoropropyl) 3-phenyltropanes possessed good *in vivo* binding properties for rapid imaging studies of the DAT but overall were no better than RTI-55.⁹³

The iodine-125 radiolabeled analogue of 3 β -(4'-ethyl-3'-iodophenyl)nortropine-2 β -carboxylic acid methyl ester ([¹²⁵I]-RTI-353) with high specific activity and high specificity for the 5-HTT^{94,95} is a highly useful radioligand for studying the 5-HTT, especially in tissues with mixed transporter populations and low levels of 5-HTT or in assays where small amounts of tissue are desirable, such as in high-throughput assays.

(1.4.2) Irreversible Binding Ligand. Progress in the characterization of the cocaine receptor protein was aided by the use of irreversible ligands. Since irreversible ligands can bond covalently to or near the ligand recognition site of a receptor, these agents are particularly valuable as molecular probes. As part of our program, we synthesized *p*-ISOCOC (**14**), RTI-76 (**5d**), and RTI-82 (**15**) for studying the DAT^{36,96} (Chart 3).

We reported that *p*-ISOCOC inhibited binding at the DAT in a wash-resistant fashion and blocked dopamine uptake by the DAT. Moreover, since *p*-ISOCOC blocked the high-affinity site in preference to the low-affinity site, it was a useful probe for studying the role of the high- versus the low-affinity site.⁹⁶ Following the success with *p*-ISOCOC, we developed RTI-76 as the first 3-phenyltropane analogue showing irreversible binding to the DAT at rat striatal brain tissue and to expressed human DAT in human embryonic kidney-293 cells.⁹⁷

The steady-state levels of the 5-HTT and DAT proteins at any given time are due to a balance of rates of production and degradation of the protein. The production rate and degradation constant reflect the net effect of a number of intracellular processes. We used RTI-76 to determine the production rate *r*, degradation rate constant *k*, and half-life of recovery *t*_{1/2} of both the 5-HTT and DAT proteins.^{98,99} The half-life of recovery

Table 4. Recovery of DAT after Intraventricular Injection of 100 nmol of RTI-76^a

kinetic measure	striatum	nucleus accumbens
<i>t</i> _{1/2} , day	2.1	2.1
<i>k</i> , day ⁻¹	0.34	0.3
<i>r</i> , fm mg ⁻¹ day ⁻¹	202	106

^a Taken from ref 98.

for the DAT protein in the striatum and the nucleus accumbens after intraventricular injection of RTI-76 was found to be 2.1 days in both the striatum and the nucleus accumbens (Table 4).⁹⁸ The degradation rate constant was similar for the two regions, but the synthesis rate was higher in the striatum, reflecting a higher density of DAT in this brain region. In a similar study, we found that the half-life of recovery of the 5-HTT protein in the hippocampus was 3.4 days.⁹⁹

The methods developed for measuring the production rate, degradation rate, and the half-life of the DAT protein were used to determine how stimulation and blockade of the D1 and D2 dopamine receptor families affect the degradation rate constant and the production rate of DAT and thus its half-life in the rat striatum and nucleus accumbens.¹⁰⁰ In the striatum, DAT protein half-life was decreased by D2 dopamine receptor agonists and increased by D2 dopamine receptor antagonists (Table 5). Both D1 dopamine receptor agonists and antagonists had no effects on DAT kinetics in the striatum. In contrast, in the nucleus accumbens, D1 and D2 dopamine receptor agonists increased the half-life of the DAT protein, whereas the D1 dopamine receptor antagonists decreased the protein half-life, and the D2 dopamine receptor antagonist had no effect. Even though DAT protein turnover was influenced in some cases, there was no significant change in the DAT density following treatment with any of the dopaminergic ligands.

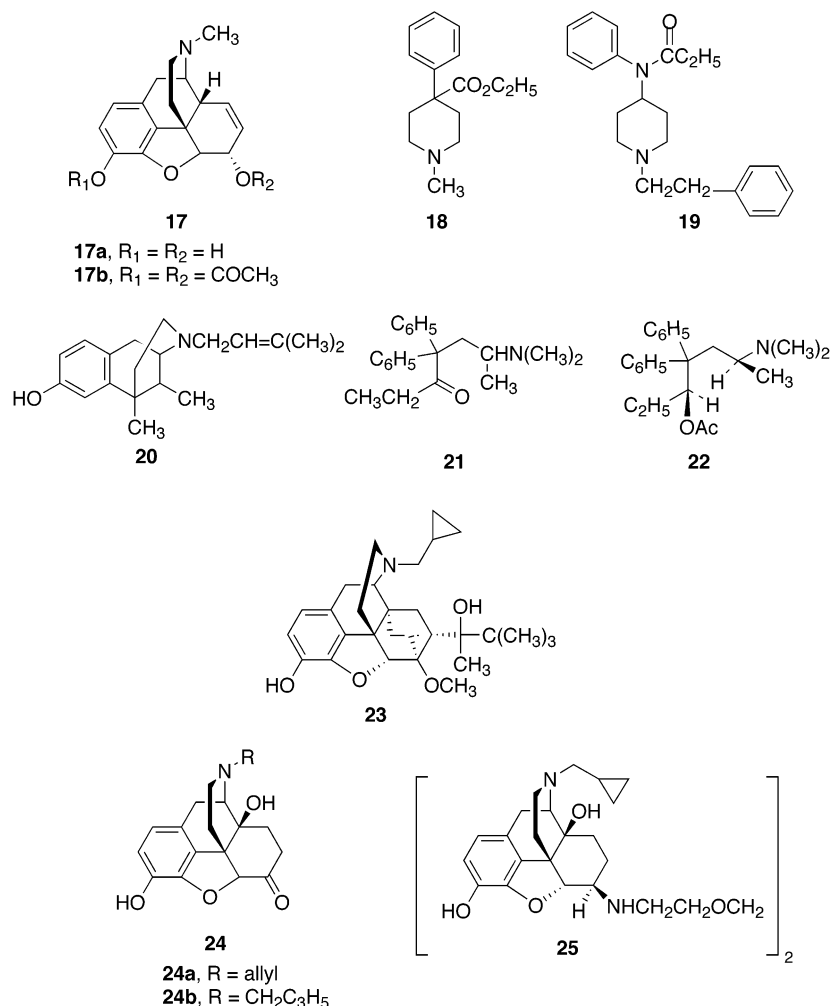
We synthesized¹⁰¹ and used the photoaffinity ligand [¹²⁵I]RTI-82 to show that it labeled a DAT protein having the same molecular weight, a similar pharmacological profile, and similar sensitivity to a neuroaminidase labeled by [¹²⁵I]DEEP, a GBR analogue.¹⁰² These studies showed that it was likely that both a 3-phenyltropane analogue and a GBR analogue labeled the same protein. This work was done before the DAT was cloned and thus was highly useful in the characterization of the DAT. [¹²⁵I]RTI-82 also was used as a probe for the identification of binding domain on the DAT. Epitope-specific immunoprecipitation of proteolytic fragments generated from SDS-solubilized DATs showed that [¹²⁵I]-RTI-82 was localized to the C-terminal half of the protein near transmembrane domain (TM) 4–7.^{103,104} Similar studies with the GBR photoaffinity DAT ligand [¹²⁵I]DEEP showed that it was incorporated near the 1–2 TM domain. This suggests that the DAT might be folded into a structure where the TM 1–2 and TM 4–7 domains are in proximity.

(1.4.3) Fluorescent Probes. We synthesized **16** (RTI-233) as the first fluorescent 3-phenyltropane analogue and used the compound for biophysical characterization of the cocaine binding pocket at the 5-HTT.¹⁰⁵ The data obtained using steady-state fluorescence anisotropy and collisional quenching experiments with aqueous and hydrophobic quenchers were consistent with a highly hydrophobic microenvironment in the

Table 5. Influence of D₁ and D₂ Receptors on Synthesis and Degradation of DAT. Summary of DAT Kinetic Parameters in the Striatum and the Nucleus Accumbens^a

systemic drug treatment	for $t_{1/2}$ (day), ^b %		for k (day ⁻¹), ^b %		for r (fm mg ⁻¹ day ⁻¹), ^b %	
	Str	NAC	Str	NAC	Str	NAC
SKF38393 (D ₁ agonist)	NC ^c	+40	NC ^c	-27	NC ^c	-20
SCH23390 (D ₁ antagonist)	NC ^c	-35	NC ^c	+62	NC ^c	+52
quinpirole (D ₂ agonist)	-45	+42	+74	-35	+68	-41
eticlopride (D ₂ antagonist)	+24	NC ^c	-18	NC ^c	-14	NC ^c
cocaine (DAT inhibition)	+22	+43	-17	-30	-16	-27

^a Calculated from data in ref 100. ^b Percentage change relative to control. ^c NC = no significant change.

Chart 4. Compounds 17–25

binding pocket for cocaine-like uptake inhibitors. However, the bound cocaine analogue was still accessible for aqueous quenching and thus partially exposed to solvent.

(2) Opioid Receptors as Targets for Addiction Therapy

(2.1) Introduction. The concept of cellular receptors as mediators of drug action was recognized shortly after the turn of the century.¹⁰⁶ Investigation of opioid receptor SARs was reported in the early 1970s following the initial identification of specific opioid receptors by radioligand binding methods.^{107–109} With the subsequent discovery of endogenous opioid peptides,^{110–112} this complex family of endogenous ligands and their receptors has been widely studied and implicated in a diverse range of physiological processes.^{113–121} Even

before these studies, Beckett and Casy hypothesized that opioid drugs exert their effects via specific receptors,¹²² and Portoghese¹²³ and Martin,¹²⁴ using SAR and behavioral information, respectively, proposed the existence of separate opioid receptors. It is now well established that opioid receptors belong to the superfamily of G-protein-coupled receptors (GPCRs). Distinct cDNAs encoding μ , δ , and κ receptors for animals and humans have now been identified, sequenced, and cloned, definitively establishing the heterogeneity of opioid receptors.^{125–135} Much effort has been directed toward the development of small-molecule opioid agonists structurally related to morphine (**17a**, Chart 4). These efforts have led to the development of drugs such as meperidine (**18**), fentanyl (**19**), and pentazocine (**20**) as important drugs for treatment of pain¹³⁶ as well as drugs such as methadone (**21**), LAAM (**22**), and buprenorphine (**23**)

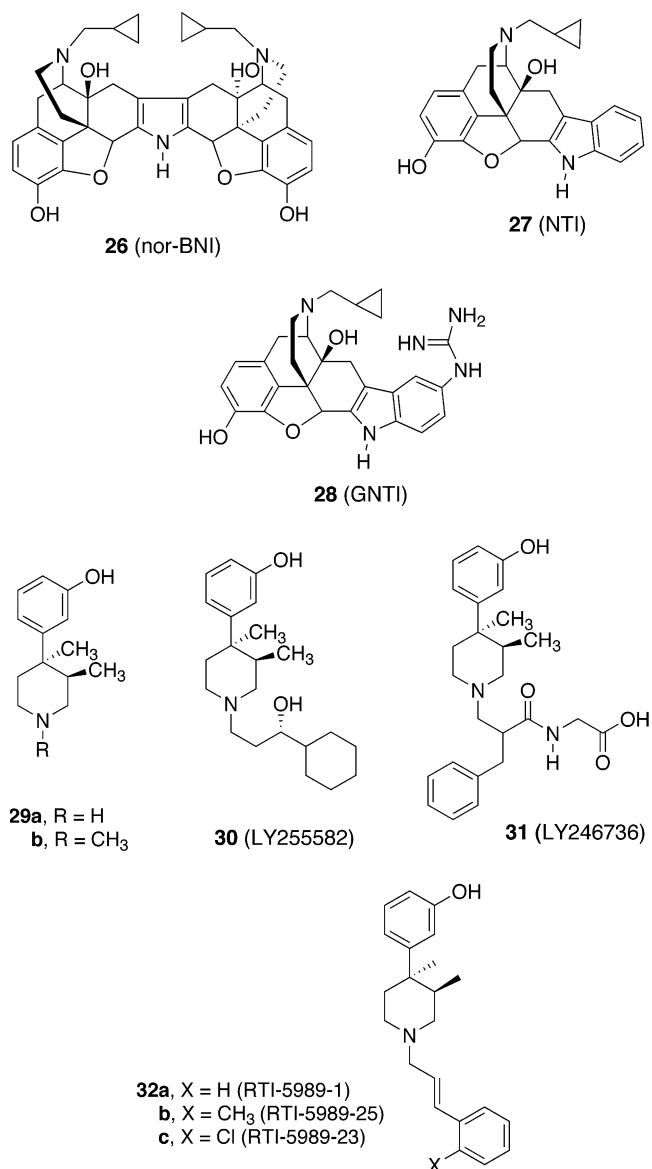
for treating drug addiction.¹³⁶ In contrast, much less effort has been directed toward the development of pure opioid antagonists. Consequently, there are only a few structural types that show potent pure opioid antagonist activity.^{136,137} In general, *N*-methyl-substituted opiates such as morphine (**17a**) and many types of structurally diverse *N*-methyl- or *N*-phenylethyl-substituted opioids are associated with agonist opioid activity, while *N*-allyl and *N*-cyclopropylmethyl groups confer antagonist activity.¹³⁶ For example, both naloxone (**24a**) and naltrexone (**24b**) are competitive antagonists at μ , δ , and κ opioid receptors. They have been extensively used as pharmacological tools to identify and characterize opioid systems and are also used as approved drugs to treat heroin overdose and to reverse the respiratory depression caused by morphine.³ A resurgence in heroin use in recent years, coupled with the demonstrated effectiveness of naltrexone for the treatment of alcohol abuse, has spurred new interest in the development of novel subtype selective opioid antagonists.^{3,138} The interest in a pure subtype selective opioid receptor antagonist is increased by reports that these types of compounds may have use in the treatment of obesity, psychosis, depression, and irritable bowel syndrome.^{139–142}

Receptor selectivity can be determined for opioid receptor ligands using displacement of opioid receptor subtype selective radioligands from receptor sites in membrane preparations prepared from animal brain tissue. More recently, cloned μ , δ , and κ receptors have been used to determine selectivity. [³⁵S]GTP γ S binding assays using either brain tissue or cloned opioid receptors are used for in vitro determination of antagonist or agonist functional potency and selectivity. Various antinociceptive tests in mice, rats, and monkeys are used to assess agonist and antagonist potency and selectivity in vivo. The more selective the compound employed, the firmer the conclusions are from a study. Ideally, a compound should display high selectivity in a variety of in vitro and in vivo assays. Sometimes the degree of selectivity of a particular compound can vary dramatically from one assay to another.

The few receptor-selective opioid binding ligands that display pure antagonist activity have served as useful tools in the study of both the structure and physiological function of the highly complex opioid receptor system, but the structural diversity of these antagonists is limited. One of the goals of our opioid program has been the discovery of structurally diverse, reversibly binding subtype selective ligands that display pure antagonist activity for use not only as molecular probes but also as potential drug candidates for the treatment of substance abuse and other CNS disorders.

The first selective κ opioid receptor antagonist was the β -naltrexamine derivative **25** (TENA).¹⁴³ This was followed by the discovery of the more potent and selective norbinaltorphamine (**26**, nor-BNI, Chart 5). However, nor-BNI showed only limited κ selectivity relative to μ when a short predose time was used.¹³⁷ Portoghese has explained the selectivity of opioid ligands in terms of the “message–address” concept of Schwyzler.¹⁴⁴ The “message” component of the ligand specifies primary receptor recognition, and the “address” portion confers selectivity by specific recognition at a particular

Chart 5. Compounds **26**–**32**



receptor subsite.^{145,146} In this design, the Tyr¹ residue of the enkephalins comprises the “message” component, and the sequence starting with Phe⁴ constitutes part of the “address” with Gly²–Gly³ serving as a spacer to connect the two components. Using this concept, Portoghese developed the δ -selective antagonist naltrindole (**27**, NTI) and the κ selective antagonists nor-BNI and 5'-guanidinonaltrindole (**28**, GNTI) from the nonselective opioid antagonist naltrexone. The phenyl ring moiety of the indole in NTI acts as the δ “address” and confers δ selectivity. The presence of a second positively charged group in nor-BNI and GNTI was shown to provide the κ selectivity. Since NTI, nor-BNI, and GNTI are all derived from naltrexone, they are dependent on the *N*-cyclopropylmethyl group for their antagonist properties.

(2.2) 3,4-Dimethyl-4-(3-hydroxyphenyl)piperidine Class of Opioid Antagonist. In 1978, Zimmerman and co-workers reported the discovery of a structurally unique series of opioid receptor pure antagonists based on *N*-substituted analogues of 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**29a**, LY272922).¹⁴⁷ These compounds were novel opioid receptor antagonists

Table 6. Radioligand Binding Results for RTI-5989-1, -23, and -25, JPP6, LY255582, and Naltrexone Using Guinea Pig Brain Membranes

compd	K_i , nM		
	[³ H]DAMGO	[³ H]DADLE	[³ H]U69,593
30 (LY255582) ^a	0.32	198	28.0
32a (RTI-5989-1) ^a	0.74	322	122
32b (RTI-5989-25) ^a	0.86	142	38.9
32c (RTI-5989-23) ^a	1.12	168	35.8
34 (JPP6) ^b	393	>5700	6.91
24b (naltrexone)	1.39	94.9	4.71

^a Taken from ref 160. ^b Taken from ref 165.

Table 7. Comparison of the Inhibition of [³⁵S]GTPγS Binding in Guinea Pig Caudate Stimulated by DAMGO (μ), SNC-80 (δ), and U69,593 (κ) Selective Opioid Agonists by N-Substituted *trans*-3,4-Dimethyl-(3-hydroxyphenyl)piperidine and 4β-Methyl- and 9β-Methyl-5-(3-hydroxyphenyl)morphan Analogues

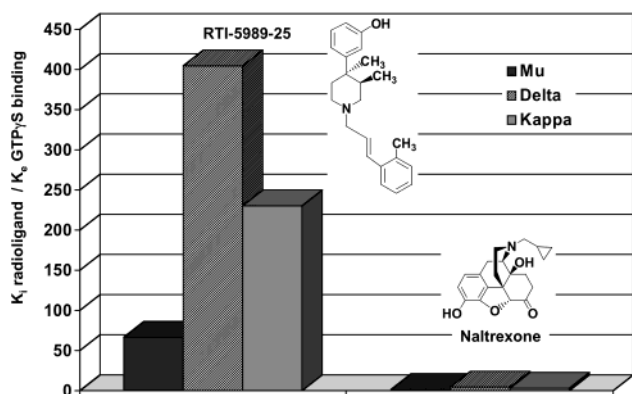
compd	K_e , nM		
	μ (DAMGO)	δ (SNC-80)	κ (U69,593)
24a (naltrexone)	0.930	19.3	2.06
26 (nor-BNI) ^b	16.75	10.14	0.038
30 (LY255582) ^a	0.021	0.312	0.330
32a (RTI-5989-1) ^a	0.039	1.48	1.04
32b (RTI-5989-25) ^a	0.013	0.355	0.170
32c (RTI-5989-23) ^a	0.026	1.07	0.567
34 (JPP6) ^b	7.25	450	4.70
38 (RTI-5989-56) ^c	25.2	76.3	76.4
39a (RTI-5989-30) ^d	21.2	750	105
39b (RTI-5989-31) ^d	0.338	12.6	1.34

^a Taken from ref 160. ^b Taken from ref 165. ^c Unpublished. ^d Taken from ref 172.

because their intrinsic antagonist activity was not mediated by the structure of the N-substituent. In the case of the 4-(3-hydroxyphenyl)piperidines, the *trans*-3,4-dimethyl orientation is required for pure opioid antagonist activity. The (3*R*,4*R*)-enantiomer is the most potent; however, in contrast to opiate antagonists, both enantiomers were opioid antagonists.^{137,148–154} Surprisingly, all N-substituted analogues, including the *N*-methyl analogue **29b**, were pure antagonists.¹⁴⁷ The nature of the N-substituents affected only the potency of the analogues.^{152,153,155}

On the basis of the report that naloxone decreased food and fluid intake in rats,¹⁵⁶ Zimmerman and co-workers examined the ability of a number of N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogues to antagonize bremazocine-induced diuresis and their effects in food consumption tests using obese rats.^{157,158} The most potent analogues possessed an aromatic or cyclohexyl ring separated from the piperidine nitrogen by three atoms. The most interesting analogue was (+)-*N*-[(3*S*)-3-hydroxy-3-cyclohexylpropyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**30**, LY255582), which possessed potent anorectant-type activity in rats.^{149,150} In addition, another analogue **31** (LY246736) has been developed for treatment of gastrointestinal motility disorders.^{142,151,159}

Even though numerous N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogues were studied,¹⁵² none of the compounds showed selectivity for the κ or δ opioid receptor subtypes. To gain a more thorough understanding of the importance of the conformational flexibility of the N-substituents, we studied the effects of the incorporation of rigid carbon-carbon double bonds and cyclic N-substituent linkers on the (+)-(3*R*,4*R*)-

**Figure 1.** Comparison of radioligand binding and functional assays for RTI-5989-25 and naltrexone.

dimethyl-4-(3-hydroxyphenyl)piperidine.¹⁶⁰ Radioligand binding inhibition studies showed that **32a–c** (RTI-5989-1, -23, and -25) bearing the *trans*-4'-aryl-2'-butenyl N-substituent displayed affinity and selectivity for the μ opioid receptor very similar to that of LY255582 (Table 6). RTI-5989-1, -23, and -25 and LY255582 all showed higher affinity and greater μ selectivity than naltrexone (see Table 6). RTI-5989-1 with K_i values of 0.74, 322, and 122 nM at μ, δ, and κ opioid receptors, respectively, displayed a combination of high affinity and μ receptor subtype selectivity superior to other alkaloid antagonists. In the [³⁵S]GTPγS functional assay, RTI-5989-1, -23, and -25 did not stimulate GTP binding at concentrations up to 10 μM.¹⁶⁰ Thus, each compound was a highly potent pure opioid antagonist (Table 7). With a potency 72 times greater than that of naltrexone (13 vs 930 pM), RTI-5989-25 is slightly more potent than LY255582 and is, to our knowledge, the most potent μ antagonist yet reported.

In general, these N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogues were more potent as inhibitors of agonist-stimulated [³⁵S]GTPγS binding than as inhibitors of radioligand binding. Figure 1 shows the ratio of K_i values in the binding assay to the K_e values in the [³⁵S]GTPγS assay for RTI-5989-25 and for naltrexone. The most pronounced differences were seen in the δ receptors where RTI-5989-25 was more than 400-fold more potent in the [³⁵S]GTPγS assay than the inhibition of radioligand binding assay. For comparison, naltrexone showed little variation from near unity (Figure 1).

It is now widely accepted that GPCRs can exist in equilibrium between an active (R*) and an inactive (R) state.^{161,162} Even in the absence of agonist, these receptors can maintain a conformation that can activate the G protein and thus display constitutive activity. Compounds that preferentially stabilize the R form of the receptor abolish this agonist-independent activity and are termed inverse agonists. We identified RTI-5989-1, -23, and -25 as a new class of small-molecule inverse agonists.¹⁶³ RTI-5989-25 was 27 times more potent as an inverse agonist at the δ receptor than the previously described peptide inverse agonist ICI-174864 (*N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu).¹⁶³ These compounds or future analogues will help determine potential functions of δ receptor constitutive activity in vivo. Recent reports suggest that in addition to modulating pain and gut transit, δ receptors may also regulate mood.¹⁶⁴

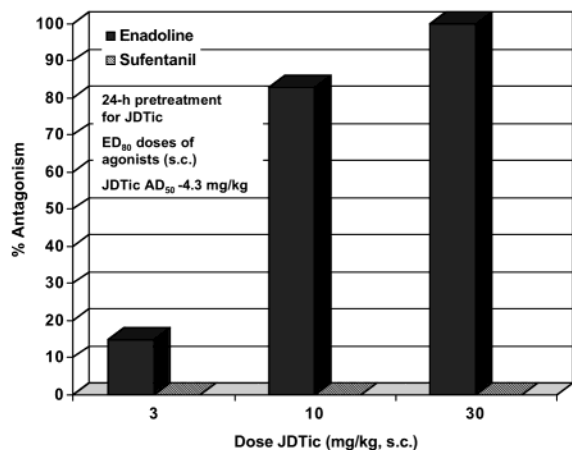


Figure 2. Antagonism of analgesia in tail-flick test by JD TIC.

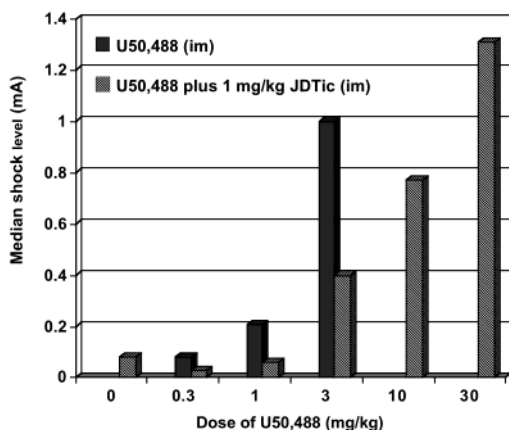


Figure 3. Reversal of U50,488-induced analgesia in tail shock titration in squirrel monkey by JD TIC.

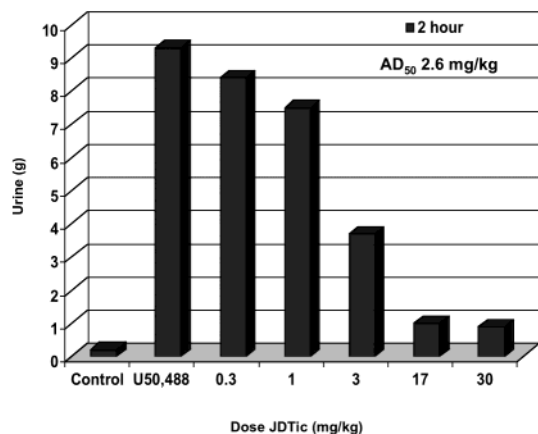


Figure 4. Antagonist of U50,488-induced urine output by various doses (mg/kg) of JD TIC.

the *trans*-3,4-dimethyl substituents on the piperidine ring and unlike the oxymorphanes naloxone and naltraxone is independent of the nature of the N-substituent. This contrasting structure–activity behavior was used to postulate that these two classes of opioid antagonists bind in different modes that force the nitrogen substituents to interact with different sites on the opioid receptor (see Figure 5). As depicted in Figure 5, opioid ligands could interact with the phenolic binding sites (“T”) and with an anionic site (“A”) in either of the phenyl-axial or phenyl-equatorial binding mode.^{143,167,168} The phenyl-axial mode allows the entire piperidine ring,

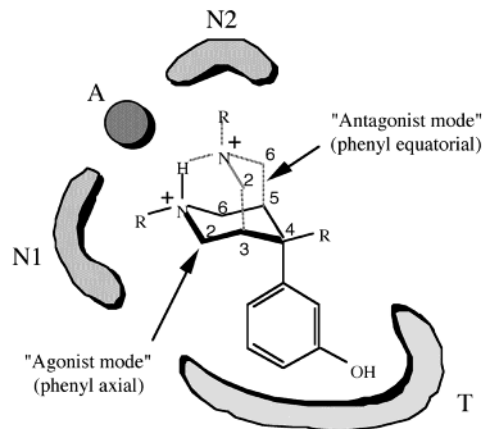


Figure 5. Schematic diagram of the hypothetical features of an opioid receptor model showing agonist and antagonist binding modes of a 4-phenylpiperidine ligand.

the ammonium proton, the N-substituent, and the phenolic ring to be superimposed on the position of the analogous atoms of the morphine molecule. This mode of binding is associated with agonist and N-substituent-mediated antagonist activity. In the phenyl-equatorial mode of binding, the phenolic ring, ammonium proton, and the 3, 4, and 5 atoms of the piperidine ring are again superimposable on the corresponding morphine atoms. However, the 2 and 6 carbon atoms, as well as the nitrogen atom of the piperidine chair, are in a unique location, and the nitrogen substituent is therefore necessarily projected into a different location (N2) than in the case of the phenyl-axial binding model (N1).

With opioid antagonists such as naloxone and naltraxone, the 3-hydroxyphenyl ring is fixed in an axial orientation relative to the piperidine ring by the rigid framework of the structure. The 3-hydroxyphenyl ring in the N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)-piperidine class of compounds can be in either the axial or equatorial position (Figure 6). Studies utilizing a variety of di- and trimethyl-substituted 4-(3-hydroxyphenyl)piperidines combined with NMR studies led to the hypothesis that a 3-hydroxyphenyl equatorial piperidine chair conformation mediated the antagonist properties of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)-2-piperidine class of opioid antagonist.^{169,170} However, access to the 3-hydroxyphenyl equatorial “antagonist” mode of binding to the opioid receptor does not appear to be sufficient to explain the pure antagonist activity of N-substituted *trans*-3,4-dimethyl-(3-hydroxyphenyl)piperidine class of antagonist. This is evident from the activity of *N*-methyl-5-(3-hydroxyphenyl) morphan (**37**) (see Figure 7), which can be viewed as a bridged analogue of *N*-methyl-4-(3-hydroxyphenyl)piperidine (**29b**). The morphan **37** is locked into an equatorial 3-hydroxyphenyl conformation but is an opioid agonist.¹⁷¹ This was used to formulate additional pharmacophoric requirements in determining the antagonist activity of *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of compounds. The additional pharmacophoric requirement can be satisfied by a substituent in the 3-axial position. In addition, since both optical isomers of the *trans*-3,5-dimethyl-4-(3-hydroxyphenyl)piperidines are antagonist, a pseudo 5-axial substituent would also satisfy the pharmacophoric requirement. This is illustrated in Figure 8 by overlaying N-substi-

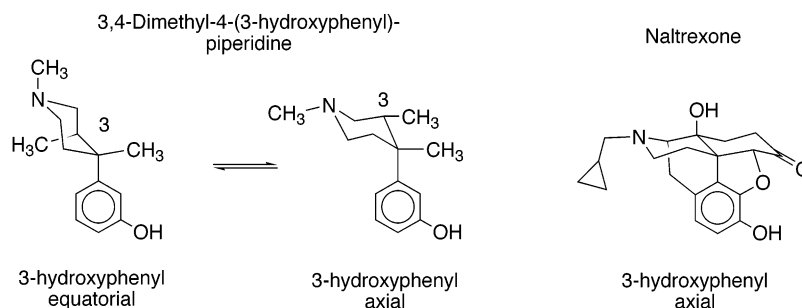


Figure 6. Comparison of the structures of the axial and equatorial conformers of *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine to naltrexone.

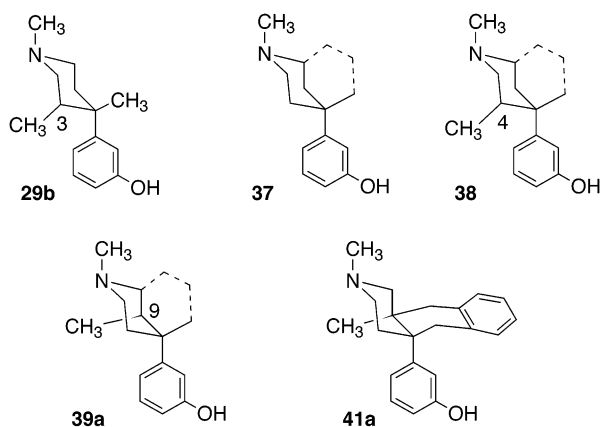


Figure 7. Comparison of the structures of *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (equatorial conformer) to that of 4 β -methyl- and 9 β -methyl-5-(3-hydroxyphenyl)morphan and octahydro-4 α -(3-hydroxyphenyl)-10 α -methylbenzo[glisoquinolines.

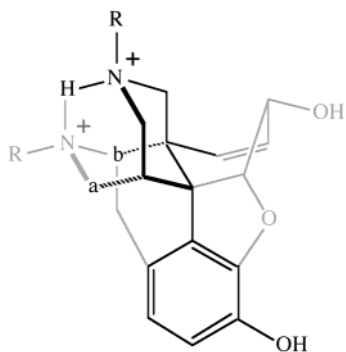


Figure 8. Overlay of 4-(3-hydroxyphenyl)piperidine (light gray) in the phenyl equatorial antagonist binding mode and morphine (black).

tuted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine on the structure of morphine. Note that the substituents that produced antagonist activity in the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine correspond to morphine ring positions "a" and "b".

(2.4) 4 β -Methyl- and 9 β -Methyl-5-(3-hydroxyphenyl)morphans. The 5-(3-hydroxyphenyl)morphans are a class of compounds that has produced both agonists and antagonists. As pointed out in the previous section, these compounds are structurally similar to the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonists. They differ only by the bridging ring between the 2 and 4 positions (see Figure 7). In the Introduction section, it was pointed out that *N*-methyl-substituted opioids are commonly agonists. Thus, it was not surprising to find that *N*-methyl-5-(3-hydroxyphenyl)morphans are agonists.¹⁷¹ The opioid antagonist model developed in the previous section would predict that agonist activity would be hindered by 4 β -methyl and 9 β -methyl groups in the 5-(3-hydroxyphenyl)morphans (Figure 7). Thus, the model predicted that *N*-methyl-4 β -methyl-5-(3-hydroxyphenyl)morphans (**38**) and *N*-methyl-9 β -methyl-5-(3-hydroxyphenyl)morphans (**39a**) would be pure opioid antagonists. Methods were developed to synthesize **38** and **39**, and evaluation in the [³⁵S]-GTP γ S assay showed that both **38** and **39a** were indeed nonselective pure opioid antagonists (Table 7). Since both the *N*-methyl- and *N*-phenylethyl analogues, **39a** and **39b**, respectively, were pure opioid antagonists, the antagonist properties are not dependent on the *N*-substituent, which is the same behavior seen in the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of antagonists. Since **38** and **39a** differ from the agonist *N*-methyl-5-(3-hydroxyphenyl)morphans (**37**) by the presence of the 4 β -methyl and 9 β -methyl groups, respectively, it is apparent that these β -methyl substituents are essential for pure opioid antagonist activity. The difference in SAR requirements for the *N*-substituents of the oxymorphan class of antagonists, which possess an axial 3-hydroxyphenyl group, and the 5-(3-hydroxyphenyl)morphans class, which has an equatorial 3-hydroxyphenyl group, can be accounted for by an extension of the model used by Portoghese to explain the *N*-substituent requirement differences between the benzomorphan (axial 3-hydroxyphenyl) and morphans (3-hydroxyphenyl equatorial) classes of opioid agonist.^{123,172} Viewed from this point, the very different behaviors associated with *N*-substituents of the 3-hydroxyphenyl equatorial antagonist are easily explained. The *N*-substituent structure in the phenyl axial series is the trigger for antagonistism, whereas the *N*-substituent structure in the phenyl equatorial series only governs potency. Thus, it is the axial 3-methyl substituent in the 4-(3-hydroxyphenyl)piperidines or the 4 β - and 9 β -methyl group in the 5-(3-hydroxyphenyl)morphans that triggers antagonist behavior.

Using simultaneous solution-phase synthetic methodology with *N*-phenylpropyl-4 β -methyl-5-(3-hydroxyphenyl)morphans as the core structure, we showed that the addition of a 7 α -3-(1-piperidinyl)propanamido group to the 4 β -methyl-5-(3-hydroxyphenyl)morphans structure provides (-)-**40** [(-)-KAA1], which possesses K_e values of 0.24, 32.7, and 41.7 nM at the κ , μ , and δ receptors, respectively, and thus is a potent and selective antagonist for the opioid κ receptor (Table 9).¹⁷³ To our knowledge, this is the first demonstration of κ -selective antagonist activity in the 5-phenylmorphans series.

Table 9. Inhibition of Agonist-Stimulated [³⁵S]GTPγS Binding by (–)-KAA1 in Cloned Human μ, δ, and κ Opioid Receptors^a

compd	K _e , nM			μ/κ	δ/κ
	μ (DAMGO)	δ (DPDPE)	κ (U69,593)		
(–)- 40 [(–)-KAA1]	41.7	32.9	0.24	175	137
nor-BNI	19	4.6	0.04	475	115

^a Data supplied by NIDA.

The discovery of κ-selective antagonist activity in (–)-KAA1 appears to be consistent with the concepts of “message” and “address” substructures described for previously discovered κ antagonists. In this case, the N-phenylpropyl-4β-methyl-5-(3-hydroxyphenyl)-morphan provides the opioid “message” while the 3-(1-piperidinyl) group is the κ “address” moiety.

(2.5) N-Substituted Octahydro-4α-(3-hydroxyphenyl)-10α-methylbenzo[*g*]isoquinolines. The N-substituted octahydro-4α-(3-hydroxyphenyl)-10α-methylbenzo[*g*]isoquinoline (**41**) is another fused ring system, which locks the piperidine substructure with the 3-hydroxyphenyl ring in the equatorial conformation. Comparison of the structure of **41a** to the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**29b**) and the 4β-methyl- and 9β-methyl-5-(3-hydroxyphenyl)morphans **37** and **38** is shown in Figure 7. The opioid antagonist model depicted in Figure 6 predicts that N-substituted octahydro-4α-(3-hydroxyphenyl)-10α-methylbenzo[*g*]isoquinolines (**41**) would be opioid pure antagonists independent of the N-substituent. Evaluation of **41a** and **41b** in the [³⁵S]GTPγS assay shows that both compounds are pure opioid antagonists with a profile similar to that of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines.¹⁷⁴

(3) Summary and Conclusions

(3.1) Monoamine Transporters. During the past several years, we conducted classical SAR studies combined with QSAR and CoMFA molecular modeling studies to characterize the cocaine binding site on the DAT, 5-HTT, and NET using WIN 35,065-2 as a lead structure. A large library of analogues was synthesized and evaluated. Our studies showed that binding affinity and transporter selectivity are both dependent on the type of substituents on the 3β-phenyl ring. In addition, a 2β-substituent was required for the highest affinity (Figure 9). For the 2β-position, we found that variously substituted esters (CO₂R), reverse esters (CH₂OCO₂R), amides (CO₂NR'R''), alcohols (CH₂OH), and several types of heterocyclic and aromatic rings all possessed high affinity for the DAT. In addition, we found that appropriate modifications of these groups provided compounds with selectivity for the DAT relative to the 5-HTT and NET. We also showed that nortropane analogues (*N*-methyl removed) showed high affinity for the DAT but had increased affinity at the 5-HTT and NET (Figure 9). Other investigators and we have made extensive use of the nonselective monoamine transporter ligands RTI-31, -32, -51, -55, -111, and -112 for various studies of the monoamine transporters. Through various modifications of the WIN 35,065-2 lead structure, we have been able to develop analogues selective for the DAT, the 5-HTT, and the NET. DAT-selective 3-phenyltropane analogues were obtained by changing the 2β-carbomethoxy group of WIN 35,065-2 to a larger ester group, an *N,N*-disubstituted carboxamide, various heterocyclic groups, or a phenyl ring. The DAT-selective

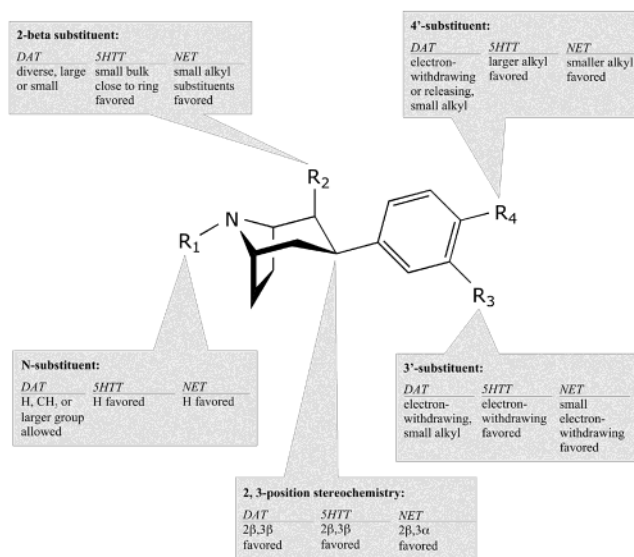


Figure 9. Summary of Structural Features That Favor Inhibition of Radioligand Binding at the DAT, 5-HTT, and NET.

ligands RTI-113, -117, -177, and -229 have proven to be highly useful for studying the DAT. We showed that selectivity for the 5-HTT could be achieved by removing the *N*-methyl groups and by combining a larger 4'-alkyl or alkenyl group with an electron-withdrawing 3'-substituent on the 3β-phenyl group (Figure 9). RTI-353 has thus far been the most widely used 3-phenyltropane analogue for studying the 5-HTT. The NET selective 3-phenyltropane analogue RTI-539 resulted from the combined effects of the 2β,3α-stereochemistry, the nortropane ring, and the 3'-fluoro-4'-methyl substituents (Figure 9).

Our studies have provided a number of new radioligands for studying the monoamine transporters. Compounds [¹²⁵I]RTI-121 and [¹²⁵I]RTI-229 are potent and selective radioligands for the dopamine transporter, and [¹²⁵I]RTI-55 is useful for biochemical studies of both the dopamine and serotonin transporters. Both RTI-55 and RTI-121 have been labeled with iodine-123 and carbon-11 and have been used in SPECT and positron emission tomography (PET) imaging studies in rats, baboons, and humans. [¹²³I]RTI-55 (DOPASCAN) is a useful diagnostic agent for Parkinson's disease. Phase 2 clinical studies with [¹²³I]RTI-55 have been completed, and phase 3 clinical trials are ongoing. [¹²⁵I]RTI-353 has proven to be highly useful for studying the 5-HTT.

RTI-76, an irreversible binding ligand, was developed and used to study the behavioral effects of blocking the DAT in rats to determine the turnover kinetics of the DAT and 5-HTT in rats and to determine the effects of dopamine agonists and antagonists upon DAT kinetics. The photoaffinity ligand [¹²⁵I]RTI-82 and the fluorescent ligand RTI-233 have proven to be useful in the characterization of the cocaine binding site on the DAT and 5-HTT, respectively.

Several of the 3-phenyltropane analogues developed from the SAR studies were evaluated in locomotor activity, drug discrimination, and self-administration studies to establish the pharmacological profile needed for a substitute pharmacotherapy for cocaine addiction. These studies combined with favorable toxicity profiles led to the selection of the nonselective monoamine transporter ligands RTI-112 and the DAT-selective ligands RTI-336 as clinical candidates for treatment of cocaine addiction.

(3.2) Opioid Receptors. Even though receptor-selective opioid binding ligands that display pure antagonist activity have served as useful tools in the study of both the structure and physiological function of the highly complex opioid receptor system, very few of these molecular probes are available. A goal of our opioid program has been the discovery of reversibly binding subtype selective ligands that display pure antagonist activity for use not only as molecular probes but also as potential drug candidates for treatment of substance abuse as well as other CNS disorders. To achieve this goal, we directed our studies toward the N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonist.

To gain a more thorough understanding of the importance of the conformational flexibility of the N-substituents, we first studied the effects of the incorporation of rigid carbon-carbon double bonds and cyclic N-substituent linkers on the (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine. These studies led to the discovery of RTI-5989-1, -23, and -25 as new, potent, pure antagonists for the opioid receptor. The superior potencies and μ selectivity demonstrated by RTI-5989-1, -23, and -25 in both binding and functional assays support the hypothesis that the active conformation of compounds such as LY255582 has the connecting chain and the appended ring extended away from the piperidino nitrogen in a manner consistent with the *trans*-cinnamyl skeleton. Additional studies identified RTI-5989-1, -23, and -25 as a new class of potent, small-molecule, δ inverse agonists. These compounds or future analogues will help determine potential functions of δ receptor constitutive activity in vivo.

The discovery of the potent and selective κ opioid receptor antagonist JD*Tic* (**36**) represents a significant advancement in the development of the *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonist. To our knowledge, JD*Tic* is the only potent and selective κ opioid receptor antagonist not derived from the opiate class of compounds. The potent and pure opioid antagonist activity results from the (3*R*,4*R*)-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine core structure. The κ opioid receptor selectivity results from a combination of (a) the isoquinoline amino group and 7-hydroxy group held in a rigid orientation by the 1,2,3,4-tetrahydroisoquinoline structure and its 3*R* attachment to the amide carboxyl, (b) an (*S*)-configuration of the 2-methylpropyl group in the spacer, and (c) the lack of a substituent on the amide nitrogen. The unique structure of JD*Tic* provides an additional pharmacological tool to further characterize the κ opioid receptor. Using site mutagenesis studies, Portoghese et al. have shown that Glu-297 in the κ receptor is critical to the κ selectivity shown by nor-BNI and GNTI.¹⁷⁵ It will be particularly

interesting to determine if Glu-297 is also critical for the κ selectivity of JD*Tic*. In addition, since the 7-hydroxy (phenolic) group is important to the κ selectivity of JD*Tic*, it will be of interest to see if site mutagenesis studies can identify the amino acid residue in the κ receptor responsible for this interaction.

The 4 β - and 9 β -methyl-2-alkyl-5-(3-hydroxyphenyl)morphans, as well as octahydro-4a-(3-hydroxyphenyl)-10a-methylbenzo[*g*]isoquinolines, can be viewed as conformationally rigid analogues of the important N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonists. We demonstrated that 4 β - and 9 β -methyl-5-(3-hydroxyphenyl)morphans such as **38** and **39**, as well as the octahydro-10a-methylbenzoisoquinoline (**41**), were three new structural types of pure opioid antagonists. Our studies also supported the proposed 4-(3-hydroxyphenyl) equatorial piperidine chair mode of interaction for the *trans*-3,4-dimethyl-3-(4-hydroxyphenyl)piperidine class of opioid antagonists. Using simultaneous solution-phase synthetic methodology, we identified (-)-KAA1 as the first potent and selective κ opioid receptor antagonist from the 5-phenylmorphans class of opioids.

In the case of JD*Tic*, the 7-hydroxy and aminomethylene group in the 1,2,3,4-tetrahydroisoquinoline are important to its potent and κ opioid receptor antagonist properties. The 7 α -3-(1-piperidinyl)propanamido group attached to the morphan core was needed for its κ selectivity. The κ selectivity of both JD*Tic* and (-)-KAA1 can be rationalized using the "message-address" concept.

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Biography

F. Ivy Carroll. After receiving a B.S. in chemistry from Auburn University (1957) and a Ph.D. degree in chemistry from The University of North Carolina at Chapel Hill (1961), Dr. Carroll joined the Research Triangle Institute, serving as Director of Organic and Medicinal Chemistry, Vice President of Chemistry and Life Sciences, and Distinguished Fellow. Dr. Carroll directs most of his research toward solving drug abuse problems. Over 359 scientific publications have resulted from his research, and he has trained 79 postdoctoral fellows. Dr. Carroll was awarded the Distinguished Lecturer Award from the American Chemical Society North Carolina Section (1993), Herty Medal (2001), and Southern Chemist Award (2000). The National Institute on Drug Abuse honored Dr. Carroll with the 1993 Pacesetter Award and 1996 MERIT Award for his research on the biochemical mechanisms of the action of cocaine.

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