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2,3-Disubstituted Quinuclidines as a Novel Class of Dopamine Transporter Inhibitors

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Abstract—There is considerable interest in developing dopamine transporter (DAT) inhibitors as potential therapies for the treatment of cocaine abuse. We report herein our pharmacophore-based discovery and molecular modeling-assisted rational design of 2,3-disubstituted quinuclidines as potent DAT inhibitors with a novel chemical scaffold. Through 3-D-database pharmacophore searching, compound **12** was identified as a very weak DAT inhibitor with K_i values of 7.3 and 8.9 μM in [^{3}H]mazindol binding and in inhibition of dopamine reuptake, respectively. Molecular modeling-assisted rational design and chemical modifications led to identification of potent analogues (−)-**29** and **34** with K_i values of 14 and 32 nM for both compounds in binding affinity and inhibition of dopamine reuptake, respectively. Behavioral pharmacological evaluations in rodents showed that **34** has a profile very different from cocaine. While **34** is substantially more potent than cocaine as a DAT inhibitor, it is approximately four times less potent than cocaine in mimicking the discriminative stimulus properties of cocaine in rat. On the other hand, **34** (3–30 mg/kg) lacks either the locomotor stimulant or stereotypic properties of cocaine in mice. Importantly, **34** blocks locomotor stimulant activity induced by 20 mg/kg cocaine in mice, with an estimated ED_{50} of 19 mg/kg. Taken together, our data suggest that **34** represents a class of potent DAT inhibitors with a novel chemical scaffold and a behavioral pharmacological profile different from that of cocaine in rodents. Thus, **34** may serve as a novel lead compound in the ultimate development of therapeutic entities for cocaine abuse and/or addiction.

Introduction

Cocaine (**1**) abuse is one of the greatest concerns of the American public today and therefore has become a focus of medical, social, and political leaders. Despite intensive research efforts, no effective pharmacotherapy for the treatment of cocaine abuse is currently available.[1]

Although cocaine (**1**) potently inhibits the reuptake of both norepinephrine (NE) and serotonin (SERT), many lines of evidence indicate that its reinforcing property stems from its ability to inhibit the reuptake of dopamine (DA) into dopaminergic neurons.[2–6] Cocaine's ability to inhibit DA reuptake and to consequently increase dopaminergic transmission in the reward mediating mesolimbic system of the brain is the essence of the dopamine hypothesis of reinforcement advanced by Wise[6] and later elaborated for cocaine by Kuhar.[4]

There is considerable interest in the development of DAT inhibitors as potential therapies for the treatment of cocaine abuse.[1,7] Two DAT inhibitors, RTI 113 (**2**)[8] and GBR 12909 (**3**),[9] were shown to effectively decrease cocaine self-administration behavior in monkeys, and

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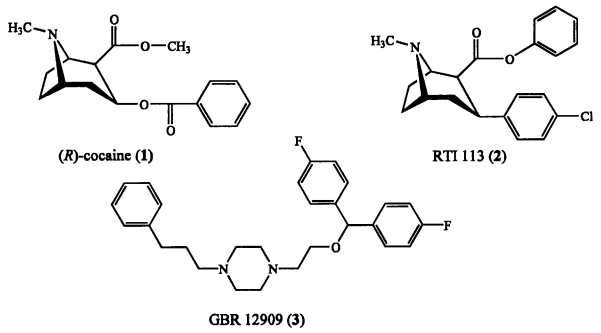
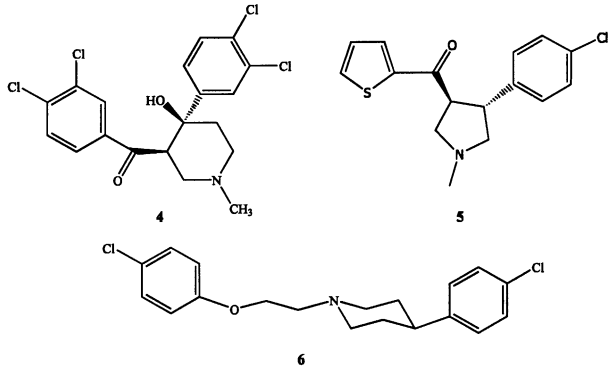


are currently being considered for clinical development for the treatment of cocaine abuse by the National Institute on Drug Abuse. In view of the urgency and complexity in the development of an effective cocaine therapy, we believe that discovery of DAT inhibitors that have truly novel chemical scaffolds would have considerable value. Novel DAT inhibitors may have pharmacological and behavioral profiles different from cocaine and other known DAT inhibitors. DAT inhibitors with truly novel chemical scaffolds will also provide new insights into the 3-D structure of DAT and its interactions with cocaine and dopamine.



The approach we have been employing in our laboratory for the discovery of potent DAT inhibitors with novel chemical scaffolds is to first identify potential DAT inhibitors through 3-D-pharmacophore searching of large chemical databases, followed by confirmation in DAT binding and DA uptake assays. To date, using this approach, we have discovered several classes of novel DAT inhibitors (**4**, **5**, and **6**).[10–15] Interestingly, some of these novel DAT inhibitors have a significant functional antagonism against cocaine in vitro.[10–12] It was found that a potent DAT inhibitor, 3,4-dichlorophenyl 4-(3,4-dichlorophenyl)-4-hydroxy-1-methyl-3-piperidyl ketone (**4**), mimics some of cocaine's effects in tests of locomotor activity and drug discrimination in rodents.[10] Further behavioral pharmacological evaluations showed that **4** attenuates locomotor activity induced by 20 mg/kg cocaine in mice and appears to be a mild stimulant with a slow onset and long duration of action.[16] Collectively, our results support our approach that potent DAT inhibitors with novel chemical scaffolds may have the therapeutic potential for the treatment of cocaine abuse, functioning either as cocaine antagonists or 'partial agonists'.

One major advantage of 3-D-pharmacophore database searching is its ability to identify many structurally diverse 'lead' compounds, thus providing great chemical diversity to the design of novel DAT inhibitors.[10] However, many such compounds only have moderate or weak activity, typically with a K_i value in the micromolar range. Inhibitors with weak activity are not useful pharmacological agents or potential therapeutic agents unless their activity can be significantly improved. A big challenge we face is how to effectively optimize compounds with weak activity. Herein, we wish to report the discovery, molecular modeling-assisted design and rapid optimization of 2,3-disubstituted quinuclidines as a novel class of dopamine transporter inhibitors. Behavioral pharmacological evaluation of analogue **34** suggests that this compound is distinct from cocaine and other classes of known DAT inhibitors and that it may be useful in the ultimate development of therapeutic entities for cocaine abuse and/or addiction.

Results and Discussion

Identification of novel DAT inhibitors through 3-D-database pharmacophore searching

Based upon the extensive structure–activity relationships (SARs) of cocaine (**1**) and its analogues, and molecular modeling studies, a simple pharmacophore model was proposed.[10] This pharmacophore model includes three chemical groups that are important for binding to the DAT and their three-dimensional (3-D) geometrical parameters (Fig. 1).[10] A 3-D-database pharmacophore searching was then performed to identify potential inhibitors of dopamine transporter (DAT), whose 3-D structures meet the requirements specified in the model. To date, testing of compounds identified from this approach has led to the discovery of several classes of novel DAT inhibitors, some of which display fairly potent activity in binding and uptake assays.[10–16] In addition to those DAT inhibitors reported in our studies,[10–15] several other compounds showed moderate activity. The chemical structures and the K_i values of these compounds as inhibitors of DA uptake are provided in Chart 1. Because of their moderate potency, the DAT inhibitors shown in Chart 1 probably would not be useful therapeutic agents unless their potency can be significantly improved. Although each of these compounds could be considered as a potential lead, we first carried out optimization of compound **12**.

Molecular modeling-assisted design

Compound **12** can be classified as a 2,3-disubstituted quinuclidine. This compound has a moderate potency with K_i values of 7.3 and 8.9 μM in inhibition of [^{3}H]-mazindol binding and DA reuptake, respectively (Table 1). Despite its very weak activity, this compound has a structural scaffold different from other classes of known DAT inhibitors. Thus, we proposed that **12** may represent a 'lead' compound for a novel class of DAT inhibitors if its potency could be significantly improved.

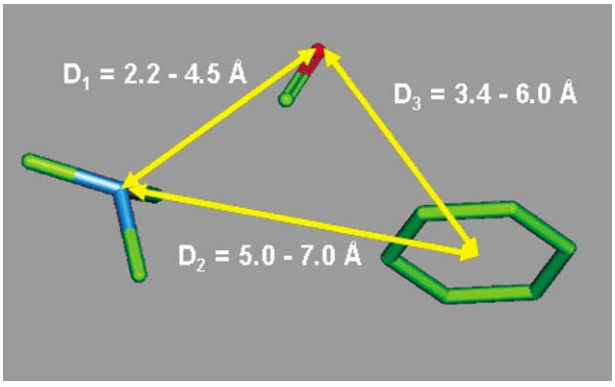


Figure 1. The pharmacophore model used in 3-D-database pharmacophore searching, which led to the identification of the initial compound **12**.

Toward this end, we carried out molecular modeling-assisted design and chemical modifications of **12**.

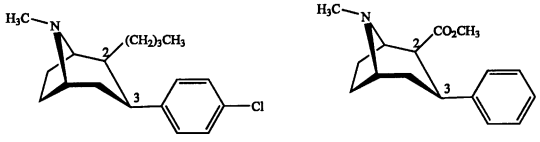
Compound **12** has two basic nitrogen atoms, one carbonyl group and two equivalent phenyl groups. Thus, two different overlaps are possible between **12** and cocaine using the three pharmacophore elements defined in Figure 1 (i.e., a tertiary nitrogen, a carbonyl group, and a phenyl ring as the reference points). It was found that lead compound **12** has a fairly good overlap with cocaine with respect to the three crucial pharmacophore elements. The lowest root-mean-square deviation (RMSD) values in these two different overlaps (Fig. 2A and B) between low energy conformations of **12** and the X-ray structure of cocaine (**1**) are 1.12 and 0.95 Å, respectively, using the three reference points (a nitrogen atom, a carbonyl group and an aromatic ring center).

Although the lead compound **12** and cocaine have fairly good overlap with respect to the pharmacophore elements defined in Figure 1, close examination of the two overlaps between **12** and cocaine (Fig. 2) showed that there is a large exclusion volume between these two molecules. While **12** and cocaine have an overlapping volume of 159 and 174 $Å^3$ with the superposition shown in Fig. 2A and B, respectively, they have an exclusion volume of 212 and 198 $Å^3$, respectively. Van der Waals (steric) interaction is perhaps the single most important factor in determining the binding mode of a drug molecule to its receptor. Thus, two compounds binding to the same binding site with similar binding modes often have a minimal exclusion volume especially if the binding site is not on the receptor surface. Mutagenesis analysis showed that the binding site of cocaine at the DAT is not located on a surface.[17,18] Therefore, the two overlaps shown in Figure 2 may not represent the 'true' binding mode of the compound **12** in comparison to that of cocaine.

Table 1. Binding and DA reuptake activity at the DAT site

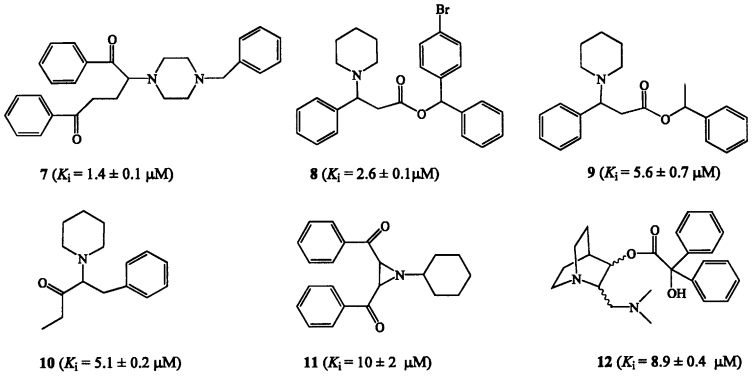
Compd	K_i (nM)[a] Mazindol binding	DA reuptake
R-Cocaine (**1**)	274±22	274±20
12	7.3±0.4 (μM)	8.9±0.4 (μM)
17	n.t.	>10 (μM)
18	n.t.	31 3 (μM)
23	260±4	461±18
(±)-**28**	210±25	237±7
(±)-**29**	20±1	49±1
(+)-**29**	343±16	354±1
(−)-**29**	14±2	32±2
30	155±21	186±16
31	30±1	57±4
32	n.t.	119±10
33	n.t.	198±10
34	14±1	32±5

n.t., Not tested.
[a]Mean±standard error or range of 2–3 experiments, each conducted using six concentrations of drug in triplicate.



13 **14 (WIN 35065-2)**

Therefore, we investigated whether there was an alternative overlap between the compound **12** and cocaine. It was shown that replacement of the ester group in cocaine at position 2 with small alkyl groups resulted in very potent DAT inhibitors. For example, compound **13** with a butyl group at the 2β position and a *p*-Cl-phenyl group at the 3β position is a highly potent DAT inhibitor with a low nanomolar potency in binding affinity and inhibition of DA reuptake.[19] This suggested that the carbonyl group defined in the pharmacophore model in Figure 1 can be modified to



7 (K_i = 1.4 ± 0.1 μM) **8** (K_i = 2.6 ± 0.1μM) **9** (K_i = 5.6 ± 0.7 μM)

10 (K_i = 5.1 ± 0.2 μM) **11** (K_i = 10 ± 2 μM) **12** (K_i = 8.9 ± 0.4 μM)

Chart 1. Novel DAT inhibitors discovered through 3-D-database pharmacophore searching that have not been reported previously.

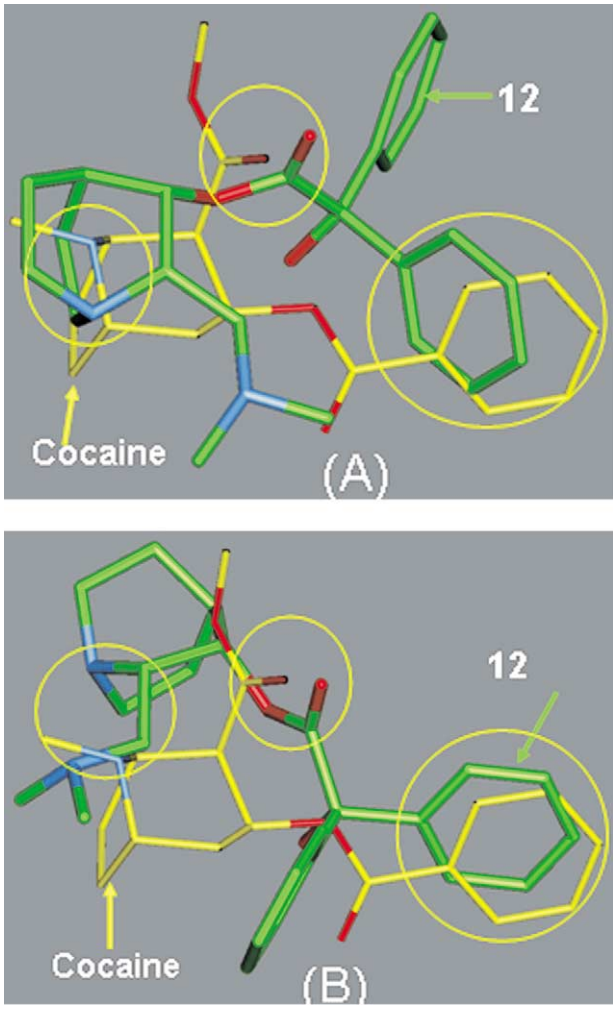


Figure 2. Two possible overlaps between the compound **12** (green) and cocaine (**1**, yellow) using the three pharmacophore elements defined in Figure 1.

include simple alkyl groups. With this modified pharmacophore model, it is possible that the small *N*,*N*-dimethylmethylamino group of **12** may mimic the ester group at the 2β position of cocaine and the 2-hydroxyl-2,2-diphenylacetate group at position 3 may mimic the benzoate group at the 3β position of cocaine. The lowest RMSD value obtained between the low energy conformations of **12** and the X-ray structure of cocaine is 0.50 Å, using the five reference points shown in Figure 3. As can be seen, a good overlap was found between these two molecules (Fig. 3). The 2-hydroxy-2,2-diphenylacetate group at position 3 of the quinuclidine ring locates in the same region as the phenyl ester group at the 3β position of cocaine, and the *N*,*N*-dimethylamino group at position 2 of the quinuclidine ring overlaps nicely with the methyl ester group at the 2β position of cocaine. However, the 2-hydroxyl-2,2-diphenylacetate group at position 3 of the quinuclidine ring appeared to be too bulky for achieving optimal potency based upon the structure–activity relationships (SARs) of cocaine and its analogues. Molecular volume calculations showed that with the overlap depicted in Fig. 3, compound **12** and cocaine have an overlapping volume of 179 $Å^3$ and an exclusion volume of 194 $Å^3$. Although

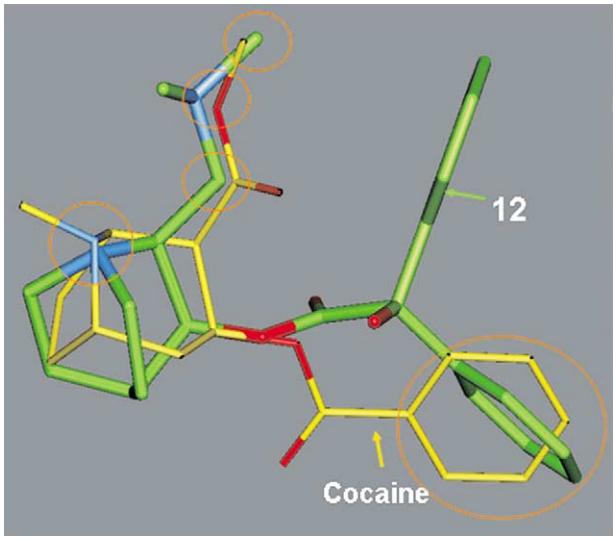
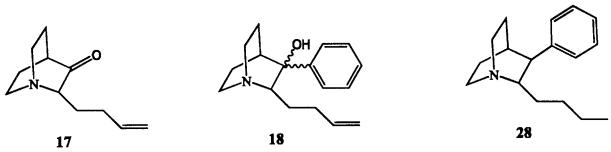


Figure 3. An alternative overlap between the compound **12** (green) and cocaine (**1**, yellow) using an augmented pharmacophore model.

the exclusion volume is only slightly better than that shown in Figure 2, it was found that the bulky 2-hydroxyl-2,2-diphenylacetate group accounts for much of this exclusion volume. It was shown that in cocaine, replacement of its benzoate group at the 3β position with a phenyl group resulted in compound **14** (WIN 35065-2) with a binding affinity 4 times better than cocaine at the DAT site.[20] Thus, the bulky 2-hydroxy-2,2-diphenylacetate group at position 3 of the quinuclidine ring in **12** may be replaced with a simple phenyl group to improve the overlapping volume and consequently the activity. Since a small ester or a simple alkyl group at the 2β position of cocaine is desirable for high affinity at the DAT site,[19,20,23] the *N*,*N*-dimethylmethylamino group at position 2 of the quinuclidine ring in **12** may be replaced with a simple alkyl group for achieving potent activity at the DAT site. The two substituents at positions 2 and 3 of the quinuclidine ring can be in either *trans* or *cis* configurations. Molecular modeling showed that compounds with *cis*-configuration have a better overlap with cocaine (**1**) and WIN 35065-2 (**14**).



17 **18** **28**

Based upon these molecular modeling analyses, compound **28** was designed, which has a simple butyl group at position 2 and a phenyl group at position 3 with a *cis*-configuration between them. A fairly good overlap was found between **28** and cocaine as depicted in Figure 4A and the lowest RMSD value was 1.07 Å using the 5 reference points shown in Figure 4A with the low energy conformations of **28** and the X-ray structure of cocaine. But an excellent overlap was found between **28** and WIN 35065-2 (**14**), an analogue more potent than cocaine (Fig. 4B). The lowest RMSD value was 0.30 Å between their low energy conformations using the 5 reference points shown in Figure 4B for superposition.

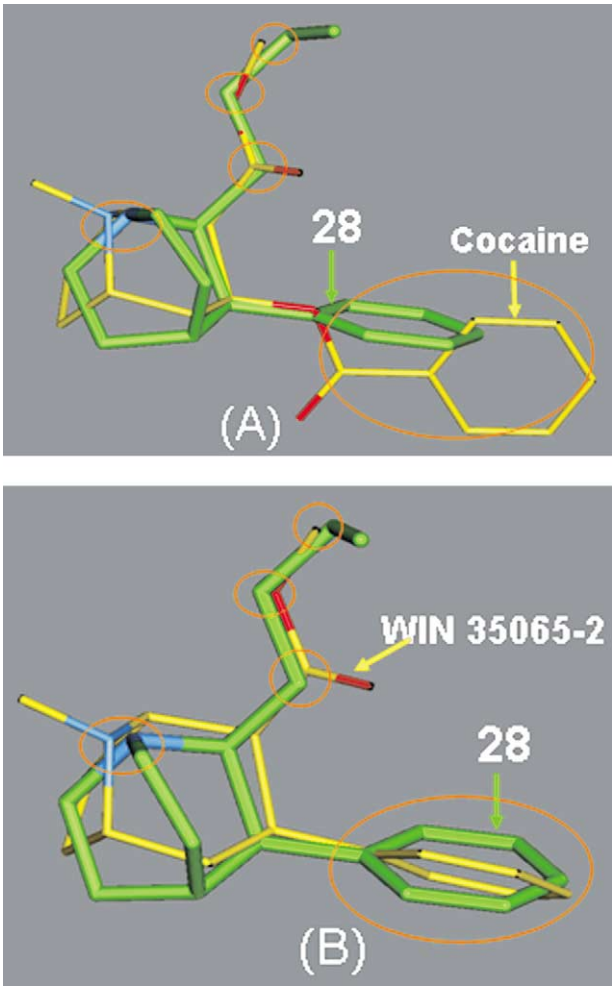


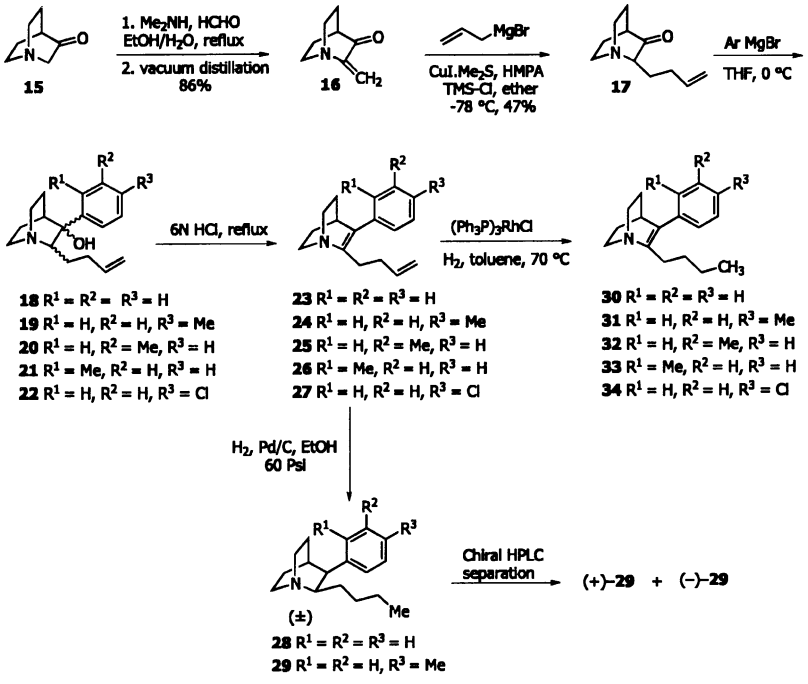
Figure 4. Overlaps between the designed analogue **28** (green) and cocaine (**1**, yellow) (A), and between **28** (green) and WIN 35065-2 (**14**, yellow) (B).

Compound **28** and WIN 35065-2 (**14**) have an overlapping volume of 179 Å^3 and an exclusion volume of 54 Å^3, indicating an excellent overlap in terms of their overall shape. It is of interest to note that although the locations of the nitrogen atom in **28** and WIN 35065-2 (**14**) (Fig. 4B) are within 0.1 Å, the orientations of the nitrogen lone pair in these two compounds differ by approximately 60°. Taken together, our molecular modeling results suggested that **28** should have a much improved potency as compared to **12**.

Compound **28** was synthesized (Scheme 1) and evaluated as a DAT inhibitor. The K_i values of **28** in [^{3}H]-mazindol binding and inhibition of DA reuptake are 210 and 237 nM (Table 1), respectively. Thus, **28** is more than 30 times more potent than **12** both in binding and in inhibition of DA reuptake and is as potent as cocaine. These data provide unambiguous confirmation of our molecular modeling-assisted design strategy. Two intermediates **17** and **18** were also tested to obtain additional information about the SARs of this class of compounds. Compound **17** did not show any measurable activity at 10 μM in inhibition of DA reuptake (Table 1), suggesting an important role of the phenyl group and/or a detrimental effect of the ketone group at position 3. Compound **18** had a K_i value of 31 μM in inhibition of DA reuptake (Table 1), 131-fold less potent than **28**. Since solvation effect plays a major role for the binding of DAT inhibitors, the weak binding affinity of **18** is probably due to its unfavorable solvation energy as compared to **28**.[37]

Chemistry

Synthesis of **28** and other 2,3-disubstituted quinuclidines in racemic form was accomplished using a synthetic procedure as shown in Scheme 1.[15] Briefly, starting from



Scheme 1. Synthesis of 2,3-disubtituted quinuclidines.

3-quinuclidinone (**15**), 2-methylene-3-quinuclidinone (**16**) was prepared by using Mannich reaction.[21] Reaction of **15** with aq dimethylamine and aq formaldehyde in ethanol–water mixture at 70 °C gave the Mannich base, which on deamination under distillation gave compound **16** in 86% yield. Reaction of **16** with allylmagnesium bromide in the presence of CuI·Me_2S and Me_3SiCl at −78 °C furnished the conjugate addition product **17** in 47% yield along with the 1,2-addition product in 12% yield (structure not shown). Aryl Grignard addition was carried out using arylmagnesium bromide in THF at 0 °C to give compounds **18**–**22** in good yield. Dehydration using a 1:1 mixture of EtOH and 6 N HCl under reflux conditions gave the dehydrated compounds **23**–**27**. Reduction of the double bonds in compounds **23** and **24** was carried out using standard hydrogenation conditions (Pd/C, H_2, EtOH, 60 psi) to provide compounds **28** and **29** in near quantitative yield. Selective reduction of terminal double bond in compounds **23**–**27** using Wilkinson catalyst under hydrogen atmosphere in toluene at 70 °C overnight gave compounds **30**–**34** in good yield.

The enantiomers (+)-**29** and (−)-**29** were obtained using a semi-preparative chiral HPLC column (Chirex 3018), in which chiral stationary phase (CPS) consists of (*S*)-proline and (*R*)-1-α-naphthylethylamine covalently bound to a γ-aminopropyl silanized silica gel, and hexane/CH_2Cl_2/EtOH–TFA (20:1) in 83:15:2 ratio as the eluent.[22] The optical rotation of (+)-**29** was found to be $[\alpha]_D = +104°$ (*c* 0.5, acetone) and that of (−)-**29** was $[\alpha]_D = -104°$ (*c* 0.5, acetone).

Structure–activity relationship studies

To further improve the potency of **28**, we have carried out focused structure–activity relationship studies. Previous studies have shown that an additional substitution to the phenyl ring such as a *p*-methyl group may further improve the potency.[23] Thus, compound **29** with an additional *p*-methyl group should have an improved activity if it can adopt the similar low energy conformation of **28** as shown in Figure 4. Molecular modeling analysis confirmed that **29** has an excellent overlap with WIN 35065-2 (**14**) and **28**. Compound **29** was synthesized in racemic form using the same procedure as for **28** (shown in Scheme 1) and evaluated as a DAT inhibitor. It was found that **29** has K_i values of 20 and 49 nM in binding affinity and inhibition of DA reuptake, respectively. Thus, **29** is 11 and 5 times more potent than **28** in its binding and uptake activities, respectively.

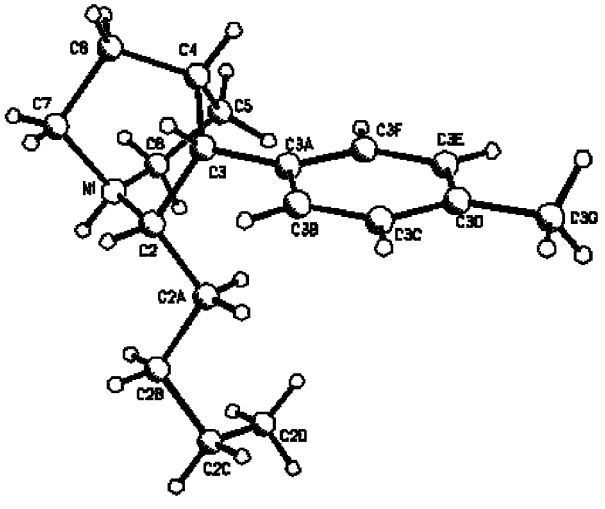
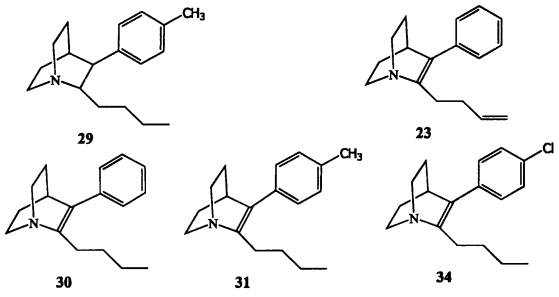


Figure 5. The structure of compound **29** drawn using the experimentally determined X-ray coordinates with arbitrary thermal parameters.



To confirm the *cis*-configuration between substituents at positions 2 and 3 in **29** and our molecular modeling results, the X-ray structure of **29** was obtained (Fig. 5). As can be seen, the butyl group at position 2 and the *p*-methylphenyl at position 3 indeed have the desired *cis*-configuration. Since the binding of cocaine to DAT is stereospecific, it was thus interesting to investigate the stereospecificity of compound **29** in binding to the DAT. It was found that (−)-**29** has K_i values of 14 and 32 nM, while (+)-**29** has K_i values of 343 and 354 nM in binding affinity and inhibition of DA reuptake, respectively. Hence, (−)-**29** is approximately 2-fold more potent than (±)-**29** and is 11-fold more potent than its enantiomer (+)-**29**.

To overcome the tedious separation of the enantiomers using chiral HPLC, we tested the hypothesis that compounds with a double bond at the C2–C3 position, such as **23**, have reasonable activity. Molecular modeling analysis showed that **23** has an excellent overlap with **28**, suggesting that **23** may be a potent DAT inhibitor. Indeed, **23** was found to have K_i values of 260 and 461 nM in binding affinity and inhibition of DA reuptake, only slightly less potent than **28**. Reduction of the terminal double bond in **23** gave compound **30**. Compound **30** was found to have K_i values of 155 and 186 nM in binding and inhibition of DA reuptake, respectively. Thus, **30** is as potent as **28**. Encouraged by the good potency of **30**, compound **31** with a *para*-methylphenyl substitution at the C3 position was synthesized. It was found that compound **31** has K_i values of 30 and 57 nM in binding and inhibition of DA reuptake, respectively. Thus, **31** is as potent as (±)-**29** and is only 2-fold less potent than its active enantiomer (−)-**29**.

We further investigated the effect of the aromatic substituent. First, compounds **32** and **33** with a *meta*- or an *ortho*-methyl group in the phenyl ring were synthesized and tested. While **32** with a methyl group at *meta*-position is 2-fold less potent than **31**, **33** with a methyl group at *ortho*-position is approximately 4-fold less potent than **31** in inhibition of DA reuptake. These results

clearly suggest that a methyl substitution at the *para*-position is more favorable than that at either the *ortho*- or the *meta*-position for the DAT activity.

In the WIN analogues, it is well known that replacing hydrogen with a chlorine at *para*-position yielded more potent DAT inhibitors than the unsubtituted WIN compound.[20,23] Accordingly, compound **34** with a *para*-chloro substituent was synthesized and tested. Compound **34** was found to have K_i values of 14 and 32 nM in binding and inhibition of DA reuptake, respectively. Thus, **34** is 2 times more potent than **31** and is as potent as (−)-**29** in both binding and inhibition of DA reuptake. Although **34** is not the most potent DAT inhibitor reported in the literature, this compound is approximately 16- and 8-fold more potent than cocaine in binding and inhibition of DA reuptake, respectively (Table 1), representing a promising lead compound for further design and structure–activity relationship studies. Compound **34** is also potent enough for carrying out behavioral pharmacological evaluations.

Behavioral pharmacological studies

Several observations have demonstrated that potent DAT inhibitors may have promise as potential medications for cocaine addiction. First, although many potent DAT inhibitors indeed behave exactly like cocaine in behavioral pharmacological models, some DAT inhibitors such as RTI-113 (**2**) and GBR 12909 (**3**) as well as compound **4** are milder stimulants that are slower in onset and have a longer duration of action than cocaine.[8,9] Second, some potent DAT inhibitors including several benzotropine analogues[24,25] and compound **4** were found to mimic some of cocaine's effects in behavioral models.[10] Third, recent studies showed that potent DAT inhibitors antagonize locomotor stimulant effects induced by cocaine in mice, while having no significant effect on locomotor activity when tested alone.[26] Because of its novel chemical scaffold and its good potency, we decided to scale up the synthesis of compound **34** and evaluate it in behavioral pharmacological models in rodents.

In rats trained to discriminate 10 mg/kg cocaine (ip) from saline, both cocaine (1.56–10 mg/kg) and **34** (0.3–30 mg/kg) produced dose-dependent, full substitution (Fig. 6a). The doses of cocaine and **34** needed to produce 50% generalization (ED_{50} value) to training dose of cocaine (10 mg/kg) are 3 and 12 mg/kg, respectively. Thus, **34** is about 4-fold less potent than cocaine, although **34** is 16 and 8 times more potent than cocaine in mazindol binding and DA reuptake assays, respectively. Neither cocaine nor **34** altered the response rates (Fig. 6b).

In locomotor activity testing in mice, cocaine (3–30 mg/kg) produced dose-dependent enhancements in the distance traveled and stereotypic movements (Fig. 7a and b); **34** produced no significant increase in either locomotor activity or stereotypic behavior in mice in the dose range (3–30 mg/kg) tested. It is of note that at 56 and 100 mg/kg, **34** produced convulsions in 50% (4 out of 8 animals) and 100% (8 out of 8 animals) of the animals tested, respectively.

Because of its lack of locomotor stimulant activity, we decided to determine whether **34** was capable of blocking the cocaine-induced locomotor stimulant activity. As can be seen in Figure 8a, **34** significantly attenuated cocaine-induced locomotor activity as measured by distance traveled. At 30 mg/kg, **34** blocked the cocaine-induced locomotor stimulant activity by 80% and has an estimated ED_{50} value of 19 mg/kg. But **34** had no significant effect on cocaine-induced stereotypic movement (Fig. 8b).

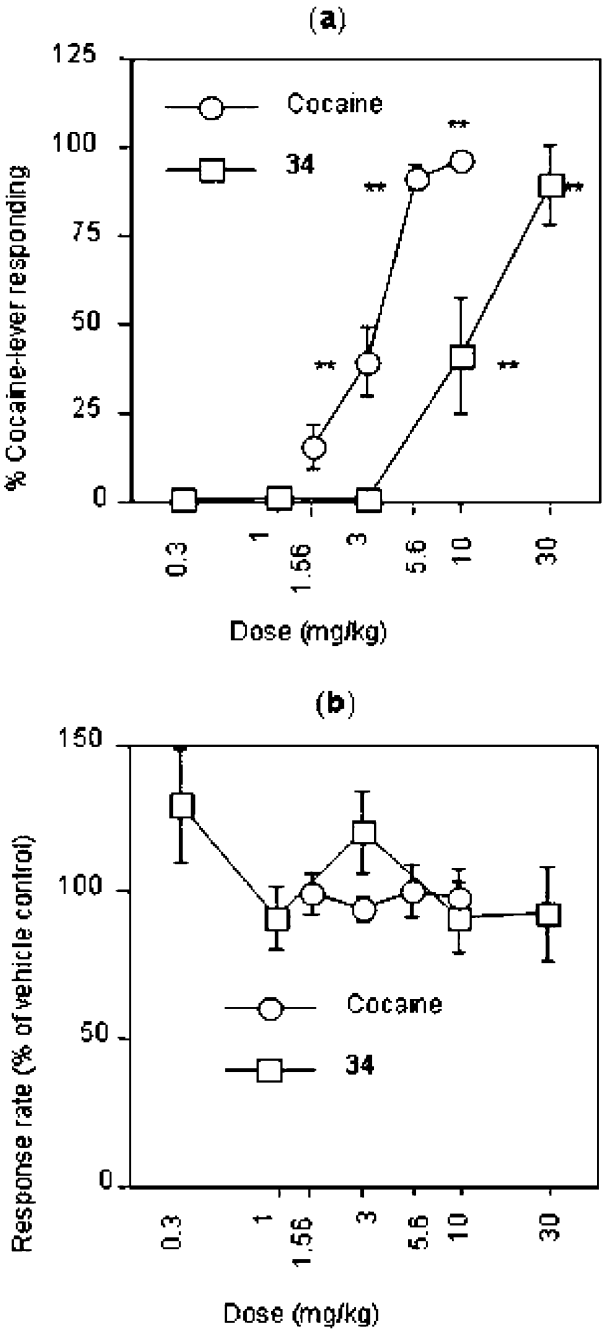


Figure 6. Discriminative stimulus effects of cocaine (circles) and **34** (squares) in Sprague–Dawley rats. Both cocaine and **34** produced dose-dependent generalization to cocaine but **34** is less efficacious than cocaine. Both drugs were administered ip 10 min prior to the testing. The response requirement for food reinforcement was a fixed-ratio 10 (FR10). A maximum of 20 pellets/session was allowed. (a) Percent cocaine-lever response. (b) Response rate as percentage of vehicle control. $^{**}P < 0.001$ as compared to the saline control response.

The generalization to cocaine by **34** in drug discrimination experiments is consistent with the fact that **34** is a potent DAT inhibitor. However, the differential correlation between the discriminative stimulus effects and the inhibition of DAT function for cocaine and **34** suggest that these molecules differ radically from each other in either pharmacokinetic properties and/or in basic biologic mechanisms of action. The complete lack of locomotor stimulant activity of **34** in mice is surprising since a strong correlation was reported between the relative dopamine transporter binding affinities of cocaine analogues versus their locomotor stimulant potencies.[27–29] As evident from its ability to antagonize the stimulant effect of cocaine, **34** is accessible to the central nervous system in mice. Thus, the lack of locomotor stimulant activity of **34** cannot be attributed to its possible inability to pass through the blood–brain barrier.

Since cocaine functions as an indirect dopamine receptor agonist, we investigated the possibility that the lack

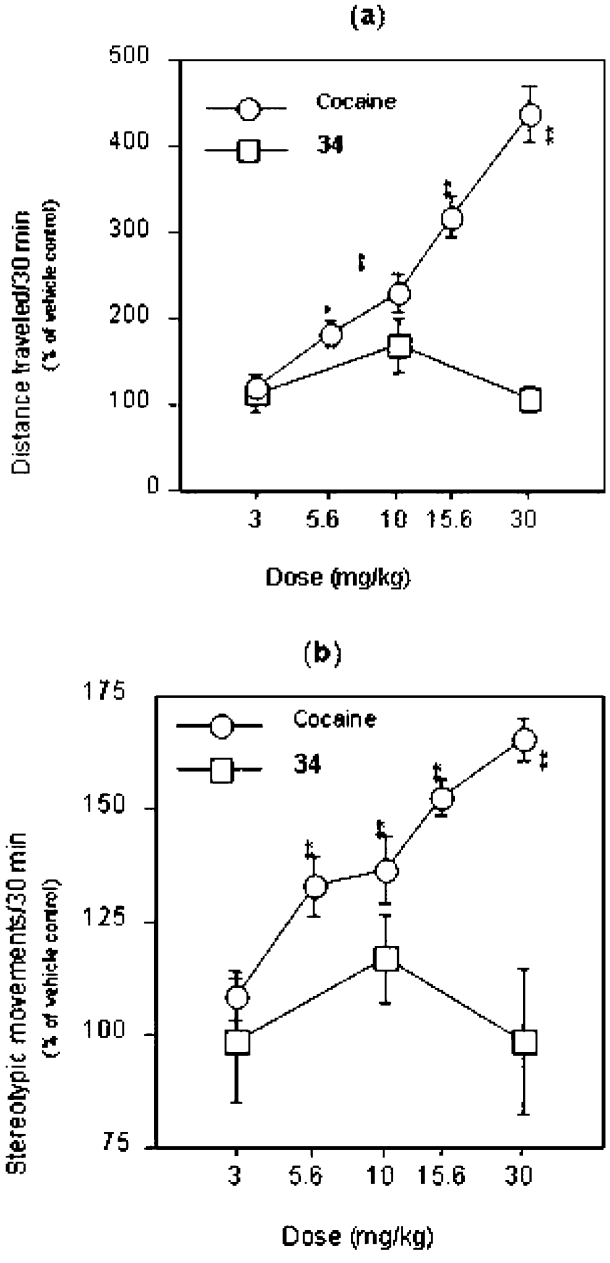


Figure 7. Locomotor stimulant effects of cocaine (circles) and compound **34** (squares) in Swiss Webster mice. The locomotor activity was recorded using Truscan activity monitors (Coulbourn Instruments, Columbus, Ohio). Following 1 h of habituation to test environment, cocaine, **34** or their vehicles were administered ip. The data were recorded for the next 2 h. The original 12 blocks (10-min bins) of 2-h data were converted to 30-min totals. The maximal 30-min total for each dose was identified and presented as the response. Cocaine produced significant and dose-dependent locomotor activation. Unlike cocaine, **34** did not produce locomotor stimulation. (a) Distance traveled. (b) Stereotypic movement. $^{*}P<0.05$; $^{**}P<0.001$ as compared to the saline control response.

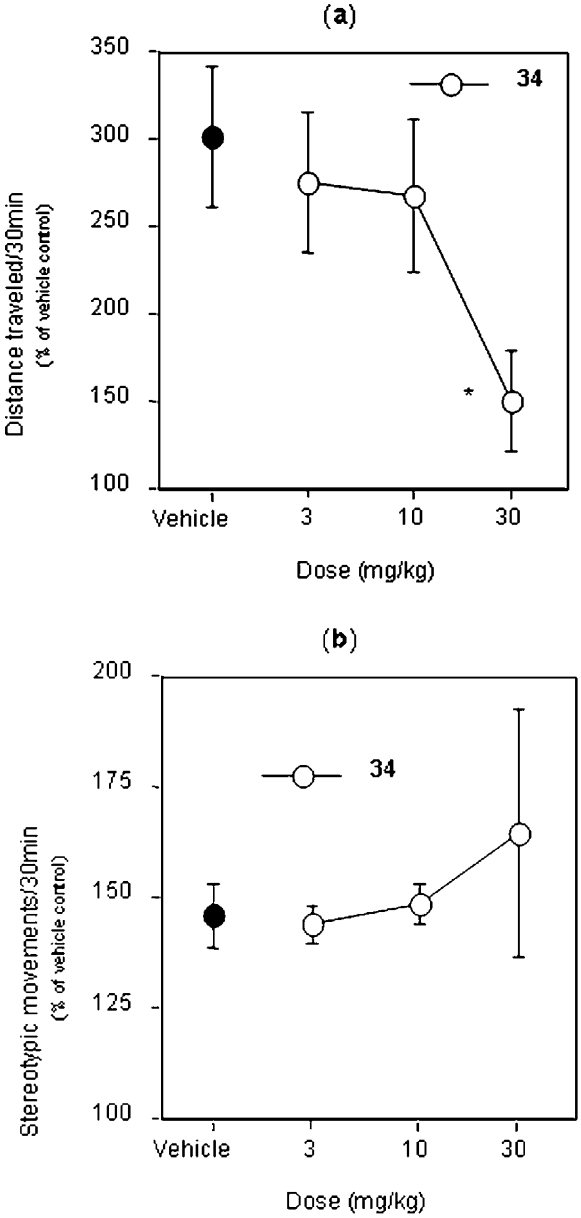


Figure 8. Effect of **34** on locomotor activity induced by cocaine in mice (pretreatment experiments). A group of 9–10 male Swiss Webster mice were injected with **34**, 20 min before the ip injection of 20 mg/kg cocaine. The data were recorded for 2 h. The first 30-min period was selected for analysis of dose–response data using standard analysis of variance. (a) Distance traveled. (b) Stereotypic movement. $^{*}P<0.05$ as compared to the group pretreated with vehicle.

of locomotor stimulant activity of **34** when tested alone and its ability to antagonize cocaine-induced locomotor activity could be due to its interaction with dopamine receptors. Compound **34** was tested for its binding to the D_1, D_2, and D_3 receptors by the Medication Development Division at the National Institute on Drug Abuse (NIDA). No significant inhibition of ligand binding to either D_1, D_2, or D_3 receptors was found for **34** at 10 μM (data not shown). We also evaluated **34** for its binding to serotonin receptors (5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}). Compound **34** has no significant inhibition of ligand binding to 5-HT_{1A} and 5-HT_{2C} receptors at 10 μM and only has a weak activity to 5-HT_{2A} receptor ($K_i = 3.5$ μM), 100 times less potent than its activity at the DAT site. Therefore, the lack of locomotor stimulant activity of **34** is not due to its possible interaction with the D_1, D_2 and D_3 receptors and three serotonin receptors (5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}).

Consistent with the dopamine hypothesis, most potent DAT inhibitors were found to stimulate locomotor activity and mimic cocaine's discriminative stimulus. However, there are some exceptions to this rule. For example, Newman's group found that some novel benzotropine analogues produce locomotor stimulation, but lack cocaine-like discriminative stimulus effects.[24] Previously, we found that a potent DAT inhibitor (**4**) has a behavioral pharmacological profile similar to that of the novel benzotropine analogues reported by Newman.[10] However, **34** lacks locomotor stimulant activity but fully generalizes to cocaine in drug discrimination experiments. Furthermore, compound **34** dose-dependently antagonizes locomotor activity induced by cocaine in mice. Taken together, these data show that **34** has a behavioral pharmacological profile that is very different from that of cocaine in rodents.

Summary

Novel DAT inhibitors have promise as effective therapies for the treatment of cocaine abuse. Toward this end, we have employed a 3-D-database pharmacophore searching approach. This approach has identified many classes of novel DAT inhibitors and has provided a considerable chemical diversity to the design of DAT inhibitors. A major challenge is how to effectively optimize the DAT inhibitors with weak activity discovered by this approach.

In the current study, we reported our molecular modeling-assisted design and chemical modifications toward optimization of one class of DAT inhibitors, 2,3-disubstituted quinuclidines. The initial compound (**12**) has K_i values of 7.2 and 8.9 μM in binding and inhibition of dopamine reuptake, respectively. Through molecular modeling-assisted design and chemical modifications, potent analogues such as (−)-**29** and **34** with much improved potency were quickly identified. It is of note that our design and optimization efforts have benefited enormously from previous extensive SAR studies on cocaine and WIN analogues, pioneered by Carroll's group.[5,20]

We have chosen one potent analogue **34** to carry out behavioral pharmacological evaluations in rodents. Our results showed that **34** has a behavioral pharmacological profile different from cocaine. While **34** lacks cocaine's locomotor stimulant activity in mice, it dose-dependently generalizes to cocaine in drug discrimination test in rats. Importantly, **34** was found to antagonize cocaine-induced locomotor activity induced with an estimated ED_{50} of 19 mg/kg. Taken together, our data suggest that **34** represents a class of potent DAT inhibitors with a novel chemical scaffold and a behavioral pharmacological profile different from that of cocaine in rodents. Thus, **34** may serve as a novel lead compound in the ultimate development of therapeutic entities for cocaine abuse and/or addiction. The ability of **34** to antagonize cocaine-induced locomotor activity by a mechanism that is incompletely understood also emphasizes the need to carry out additional neuropharmacological studies on this class of DAT inhibitors.

Experimental

Molecular modeling

Conformational analysis was performed using the conformational analysis module in the QUANTA program.[30] Generally, if a compound had fewer than five rotatable single bonds, the grid scan conformational search protocol was employed. In this protocol, each rotatable bond was systematically rotated to generate a starting conformation, which was subsequently minimized using the CHARMm program[31] within QUANTA. If a compound had more than five rotatable bonds, a random sampling protocol was used to generate conformations. Up to 5000 conformations were generated and minimized. Energy minimization of each conformation was computed with 5000 iterations or until convergence, defined as an energy gradient of 0.001 kcal mol^{-1} $Å^{-1}$ or less. An Adopted Basis Newton–Raphson algorithm, implemented in the CHARMm program, was used for energy minimization. A constant dielectric constant (equal to 1) was used throughout all the calculations. Upon the completion of conformation generation and energy minimization, the most stable conformation was identified (the global minimum in vacuum). It is noted, however, that the lowest energy conformation may not be the bio-active conformation, as was shown previously.[32] For this reason, other low energy conformations, typically within 5 kcal/mol of the global minimum, were identified. Cluster analysis was performed to determine the number of truly unique conformations (clusters), using the cluster analysis module available in the QUANTA program. These low energy conformational clusters together are likely to include the bio-active conformations for a compound.

3-D-Database search

The Chem-X program (version July 96),[33] running on a Silicon Graphics Indigo2 R10000, was used to carry out 3-D-database pharmacophore searching. The primary

reason for choosing this program was its ability to generate and search multiple conformations for flexible compounds in the database. The problem of multiple conformations for flexible compounds was found to be important in building and searching a 3-D-database because flexible compounds may be able to adopt a number of different conformations depending on their environment. It is often difficult to know precisely which conformation is the biologically active one if a compound can adopt multiple conformations with little energy difference. The biologically active conformations may be different for the same compound when it binds to different receptors. Therefore, it was decided that the best way to handle this was to generate and search multiple conformations for flexible compounds. The ability of the Chem-X program to generate and search a large number of conformations for flexible compounds was found to be one key factor to our success in identifying a large number of structurally novel, diverse lead compounds in several projects carried out so far. We have found that if only single conformations for flexible compounds are searched, many identified lead compounds would be missed. Therefore, multiple conformations for flexible compounds are necessary. However, for a flexible compound with more than 10 single bonds, using a step size of 60° in generating conformations, the total number of possible conformations will exceed 60 million. In practice, we set 3 million conformations as the maximum number to be examined for any single compound.

The initial compound (**12**) was identified through the search of the NCI 3-D-database. The version of the NCI 3-D-database[34] used in our 3-D-database search was built using the July 94 version of the Chem-X program. It consists of 206,876 'open' compounds, whose structures and related biological data can be accessed by the public.[34] Employing the Chem-X program, a total of 4094 compounds were identified as 'hits', that is, compounds that met the requirements specified in the pharmacophore model (Fig. 1). A number of additional criteria were used in the selection of compounds for biological evaluation in order to achieve maximum efficiency in the discovery of lead compounds. These criteria include simple chemical structure, small molecular weight, non-peptidic and chemical structure diversity.

Chemistry

General methods. THF was freshly distilled under nitrogen from sodium benzophenone. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity Inova instrument at 300 and 75.46 MHz, respectively. ^{1}H chemical shifts (δ) are reported in ppm downfield from internal TMS. ^{13}C chemical shifts are referenced to $CDCl_3$ (central peak, δ = 77.0 ppm).

Melting points were determined in Pyrex capillaries with a Thomas-Hoover Unimelt apparatus and are uncorrected. Mass spectra were measured in the EI mode at an ionization potential of 70 eV. TLC was performed on Merck silica gel $60F_{254}$ glass plates; column chromatography was performed using Merck silica gel (60–200 mesh). The following abbreviations are used: THF = tetrahydrofuran; DCM = dichloromethane; ether = diethyl ether.

2-Methylene-3-quinuclidinone (16). A solution of 3-quinuclidinone (**15**, 6.0 g, 48.0 mmol), 40% aqueous dimethylamine (10.0 mL, 72.0 mmol), 37% aqueous formaldehyde (6.0 mL, 72.0 mmol), 20.0 mL of ethanol and 8.0 mL of water was stirred at reflux for 1 h, then at 70 °C for 17 h and allowed to cool to room temperature. The solvents and excess reagents were evaporated in vacuo and the oily residue fractionally distilled to provide 5.7 g (86%) of title compound as a light yellow oil, bp 91–92°/7 mm. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90–1.98 (4H, m), 2.51–2.55 (1H, narrow m), 2.87–2.98 (2H, m), 3.03–3.13 (2H, m), 5.18 (1H, s), 5.78 (1H, s); ^{13}C NMR ($CDCl_3$) δ 24.9, 40.3, 48.3, 113.3, 152.3, 204.1. Anal. ($C_8H_{11}NO$) C, H, N.

2-But-3-enylquinuclidin-3-one (17). To a solution of $CuI{\cdot}Me_2S$ complex [prepared by the addition of Me_2S (0.8 mL, 10.9 mmol) to CuI (1.4 g, 7.3 mmol) at 0 °C] in THF at −78 °C was added 1 M solution of allylmagnesium bromide (9.5 mL) and HMPA (2.5 mL, 15.6 mmol) stirred for 20 min. To this, a mixture 2-methylene-3-quinuclidinone (**16**, 1.0 g, 7.3 mmol) and TMS-Cl (1.02 mL, 8.0 mmol) in THF was slowly added and stirred at the same temperature for 2 h, quenched with aq NH_4Cl solution. The organic layer was separated and the aqueous layer extracted with ethyl acetate, and the combined organic layers were dried over Na_2SO_4 and evaporated to get the crude compound. This was purified by column chromatography using ether:-acetone: triethylamine in 85:10:5 ratio to afford the title compound as a colorless oil (610 mg, 47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46–1.59 (1H, m), 1.79–1.93 (5H, m), 2.05–2.23 (2H, m), 2.30–2.35 (1H, m), 2.71–3.11 (5H, two m), 4.90–5.02 (2H, m), 5.68–5.82 (1H, m); ^{13}C NMR ($CDCl_3$) δ 25.3, 26.0, 27.1, 30.4, 39.8, 40.6, 48.5, 68.8, 115.1, 137.5, 221.7; MS *m*/*z* (%) 179 (6), 110 (100). Anal. ($C_{11}H_{17}NO{\cdot}HCl$) C, H, N.

General procedure for the aryl Grignard addition. To the ketone in dry THF at 0 °C was added the appropriate Grignard reagent (1.1 equiv). The mixture was stirred at the same temperature for 30 min, quenched with aq NH_4Cl, and extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The resulting crude compound was purified by column chromatography using ether/acetone/triethylamine as eluent to afford the following compounds:

2-But-3-enyl-3-phenylquinuclidin-3-ol (18). Colorless thick syrup; yield 70%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34–1.46 (3H, m), 1.48–1.58 (1H, m), 1.81–1.94 (2H, m), 2.05–2.33 (4H, m), 2.64–2.75 (1H, m), 2.87 (2H, broad t, J = 8.3 Hz), 3.10–3.20 (1H, m), 3.35–3.42 (1H, m), 4.98–5.07 (2H, m), 5.79–5.93 (1H, m), 7.30 (1H, d, J = 7.3 Hz), 7.39 (2H, t, J = 7.1 Hz), 7.58 (1H, d, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 21.8, 23.2, 26.1, 31.4, 35.6, 41.1, 48.8, 61.8, 75.1, 114.8, 126.0, 127.2, 128.2, 138.7, 146.2; MS *m*/*z* (%) 257 (12), 124 (100). Anal. ($C_{17}H_{23}NO{\cdot}HCl$) C, H, N.

2-But-3-enyl-3-(4-methylphenyl)quinuclidin-3-ol (19). Colorless syrup; yield 74%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32–1.54 (4H, m), 1.78–1.89 (2H, m), 2.01–2.28 (4H, two m), 2.34 (3H, s), 2.62–2.71 (1H, m), 2.80–2.86 (2H, m), 3.06–3.17 (1H, m), 3.29–3.34 (1H, m), 4.94–5.05 (2H, m), 5.76–5.89 (1H, m), 7.16 (2H, d, J = 8.6 Hz), 7.42 (2H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 21.0, 22.0, 23.5, 26.2, 31.6, 35.8, 41.3, 49.0, 62.1, 75.1, 114.9, 126.1, 129.1, 136.9, 139.0, 143.5. Anal. ($C_{18}H_{25}NO$·HCl) C, H, N.

2-But-3-enyl-3-(3-methylphenyl)quinuclidin-3-ol (20). Colorless syrup; yield 72%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34–1.58 (4H, m), 1.80–1.93 (2H, m), 2.06–2.31 (4H, m), 2.40 (3H, s), 2.66–2.75 (1H, m), 2.84–2.93 (2H, m), 3.10–3.20 (1H, m), 3.38 (1H, dd, J = 4.1, 9.8 Hz), 4.98–5.09 (2H, m), 5.80–5.94 (1H, m), 7.12 (1H, d, J = 7.3 Hz), 7.24–7.40 (3H, m); ^{13}C NMR ($CDCl_3$) δ 21.6, 21.8, 23.2, 26.0, 31.4, 35.6, 41.1, 48.8, 61.8, 75.1, 114.7, 122.9, 126.9, 127.9, 128.1, 137.9, 138.8, 146.2. Anal. ($C_{18}H_{25}NO$·HCl) C, H, N.

2-But-3-enyl-3-(2-methylphenyl)quinuclidin-3-ol (21). Colorless syrup; yield 68%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.43 (4H, m), 1.72–1.87 (2H, m), 2.11–2.23 (2H, m), 2.27–2.44 (2H, m), 2.62 (3H, s), 2.65–2.93 (2H, m), 3.10–3.20 (1H, m), 3.49 (1H, dd, J = 2.9, 10.2 Hz), 4.99–5.10 (2H, m), 5.80–5.93 (1H, m), 7.10–7.31 (4H, m); ^{13}C NMR ($CDCl_3$) δ 21.5, 22.5, 22.9, 25.4, 31.3, 32.3, 41.8, 48.9, 60.4, 76.6, 114.9, 125.0, 125.8, 127.2, 133.8, 138.6, 138.8, 141.8. Anal. ($C_{18}H_{25}NO$·HCl) C, H, N.

2-But-3-enyl-3-(4-chlorophenyl)quinuclidin-3-ol (22). Light-yellow syrup, yield 70%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32–1.54 (4H, m), 1.77–2.26 (5H, m), 2.64–2.73 (1H, m), 2.85 (2H, t, J = 7.9 Hz), 3.06–3.16 (1H, m), 3.27 (1H, dd, J = 4.4, 9.5 Hz), 4.96–5.04 (2H, m), 5.75–5.89 (1H, m), 7.34 (2H, d, J = 6.6 Hz), 7.47 (2H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 21.6, 23.3, 26.2, 31.5, 35.9, 41.2, 48.9, 62.1, 75.0, 115.2, 127.8, 128.6, 133.2, 138.8, 145.1. Anal. ($C_{17}H_{22}ClNO$·HCl) C, H, N.

General procedure for the dehydration. To a solution of hydroxy compound in EtOH, 6 N HCl was added, refluxed overnight and cooled to room temperature. The reaction mixture was neutralized by slow addition of solid $NaHCO_3$ and extracted with ethyl acetate. The combined organic layers were washed with satd NaCl solution, dried (Na_2SO_4) and concentrated to get the crude compound, which was purified by passing through a silica gel column using acetone/ether as eluent.

2-But-3-enyl-2,3-didehydro-3-phenylquinuclidine (23). Colorless syrup; yield 61%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–1.79 (4H, m), 2.30–2.42 (4H, m), 2.64–2.73 (2H, m), 2.86–2.92 (1H, narrow m), 3.01–3.10 (2H, m), 4.95–5.08 (2H, m), 5.78–5.90 (1H, m), 7.24–7.29 (3H, m), 7.37 (2H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 29.1, 30.8, 32.2, 38.8, 48.9, 114.5, 126.4, 127.6, 128.1, 138.4, 139.5, 140.2, 146.9; MS m/z (%) 239 (22), 82 (100), Anal. ($C_{17}H_{21}N$·HCl) C, H, N.

2-But-3-enyl-2,3-didehydro-3-(4-methylphenyl)quinuclidine (24). Colorless syrup; yield 66%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56–1.75 (4H, m), 2.28–2.38 (7H, m), 2.60–2.70 (2H, m), 2.81–2.86 (1H, m), 2.96–3.05 (2H, m), 4.90–5.05 (2H, m), 5.75–5.88 (1H, m), 7.18 (4H, s); ^{13}C NMR ($CDCl_3$) δ 21.2, 29.4, 31.2, 32.4, 34.0, 49.2, 114.6, 127.6, 129.0, 136.1, 136.9, 138.7, 140.1, 147.0. Anal. ($C_{18}H_{23}N$·HCl) C, H, N.

2-But-3-enyl-2,3-didehydro-3-(3-methylphenyl)quinuclidine (25). Colorless syrup; yield 68%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.58–1.75 (4H, m), 2.71–2.39 (7H, m), 2.60–2.70 (2H, m), 2.83–2.87 (1H, m), 2.97–3.05 (2H, m), 4.91–5.06 (2H, m), 5.76–5.89 (1H, m), 6.79–7.05 (3H, m), 7.19–7.26 (1H, m); ^{13}C NMR ($CDCl_3$) δ 21.4, 29.3, 30.2, 31.0, 32.2, 33.8, 48.9, 114.5, 124.7, 127.1, 127.9, 128.3, 137.6, 138.6, 139.6, 140.1, 147.1. Anal. ($C_{18}H_{23}N$·HCl) C, H, N.

2-But-3-enyl-2,3-didehydro-3-(2-methylphenyl)quinuclidine (26). Colorless syrup; yield 62%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–1.75 (4H, m), 2.04–2.12 (2H, m), 2.20–2.27 (5H, m), 2.52–2.59 (1H, m), 2.62–2.75 (2H, m) 2.95–3.14 (2H, m), 4.85–5.01 (2H, m), 5.62–5.76 (1H, m), 7.02–7.21 (4H, m); ^{13}C NMR ($CDCl_3$) δ 20.4, 28.6, 29.9, 31.1, 31.6, 33.6, 48.9, 49.6, 114.3, 125.4, 126.7, 128.9, 129.9, 135.3, 138.6, 139.9, 140.3, 147.2. Anal. ($C_{18}H_{23}N$·HCl) C, H, N.

2-But-3-enyl-2, 3-didehydro-3-(4-chlorophenyl)quinuclidine (27). Colorless syrup; yield 63%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57–1.74 (5H, m), 1.91–2.01 (1H, m), 2.27–2.37 (5H, m), 2.61–2.71 (2H, m), 2.81–2.84 (1H, m), 2.99–3.08 (2H, m), 4.94–5.07 (2H, m), 5.75–5.87 (1H, m), 7.19 (2H, d, J = 6.4 Hz), 7.31 (2H, d, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 29.5, 31.2, 32.3, 34.0, 49.2, 114.9, 128.5, 129.1, 132.3, 138.4, 138.6, 139.4, 148.3. Anal. ($C_{17}H_{20}ClN$·HCl) C, H, N.

General procedure for the hydrogenation. A mixture of olefin and a catalytic amount of Pd/C in EtOH was hydrogenated under 60 psi of H_2 at 25 °C for 24 h. The catalyst was filtered off, and the filtrate was concentrated to give the crude compound as a yellow syrup, which on purification by column chromatography with ether/triethylamine afforded the saturated compound as a colorless thick syrup in quantitative yield.

2-Butyl-3-phenylquinuclidine (28). Colorless syrup; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (3H, t, J = 7.1 Hz), 1.07–1.37 (6H, two m), 1.46–1.54 (1H, m), 1.70–1.76 (2H, m), 2.01–2.10 (2H, m), 2.67–2.78 (1H, m), 2.96–3.05 (1H, m), 3.09–3.29 (4H, m), 7.19–7.34 (5H, m); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.3, 22.7, 26.8, 29.7, 30.2, 30.3, 40.7, 45.4, 49.4, 60.2, 125.5, 127.8, 128.9, 142.9; MS m/z (%) 243 (18), 42 (100). Anal. ($C_{17}H_{25}N$·HCl) C, H, N.

2-Butyl-3-(4-methylphenyl)quinuclidine (29). Colorless syrup; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77 (3H, t, J = 6.8 Hz), 1.02–1.32 (6H, two m), 1.40–1.49 (1H, m), 1.65–1.72 (2H, m), 1.96–2.06 (2H, m), 2.32 (3H, s), 2.64–2.74 (1H, m), 2.89–3.23 (5H, two m), 7.06–7.14 (4H, m); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.1, 22.5, 22.9, 27.2, 29.9, 30.4, 30.6, 40.9, 45.3, 49.7, 60.4, 128.8, 129.0, 135.1, 140.0; MS m/z (%) 257 (29), 42 (100). Anal. ($C_{18}H_{27}N$·HCl) C, H, N.

General procedure for the terminal double bond reduction. To a solution of olefin in toluene was added Chlorotris(triphenylphosphine)rhodium(1) and heated at 70 °C under hydrogen atmosphere for 20 h. Volatiles were removed under vacuum and passed through a small bed of silica gel using acetone/hexane as eluent.

2-Butyl-2,3-didehydro-3-phenylquinuclidine (30). Colorless liquid; yield 94%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, t, J = 7.3 Hz), 1.28–1.38 (2H, m), 1.54–1.78 (6H, two m), 2.36 (2H, broad d, J = 8.1 Hz), 2.63–2.72 (2H, m), 2.86–2.89 (1H, narrow m), 3.00–3.09 (2H, m), 7.24–7.28 (3H, m), 7.36 (2H, broad t, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 23.3, 29.3, 30.5, 31.5, 33.7, 49.0, 126.2, 127.6, 128.0, 139.2, 139.9, 148.4. Anal. ($C_{17}H_{23}N$·HCl) C, H, N.

2-Butyl-2,3-didehydro-3-(4-methylphenyl)quinuclidine (31). Colorless liquid; yield 96%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.4 Hz), 1.25–1.38 (2H, m), 1.51–1.75 (6H, m), 2.20 (2H, broad t, J = 8.0 Hz), 2.35 (3H, s), 2.59–2.69 (2H, m), 2.80–2.86 (1H, narrow m), 2.96–3.05 (2H, m), 7.10–7.17 (4H, m); ^{13}C NMR ($CDCl_3$) δ 14.0, 21.1, 23.0, 29.3, 30.5, 31.5, 33.7, 49.0, 127.5, 128.7, 135.8, 136.9, 139.0, 147.9; MS m/z (%) 255 (37), 213 (100). Anal. ($C_{18}H_{25}N$·HCl) C, H, N.

2-Butyl-2,3-didehydro-3-(3-methylphenyl)quinuclidine (32). Colorless liquid; yield 97%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.5 Hz), 1.26–1.36 (2H, m), 1.52–1.71 (6H, m), 2.20 (2H, t, J = 8.1 Hz), 2.36 (3H, s), 2.60–269 (2H, m), 2.80–2.86 (1H, m), 2.97–3.06 (2H, m), 7.01–7.06 (3H, m), 7.23 (1H, t, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.9, 21.5, 22.9, 29.4, 30.5, 31.5, 33.7, 49.1, 124.7, 127.0, 127.9, 128.3, 137.6, 139.3, 139.9, 148.2; MS m/z (%) 255 (24), 42 (100). Anal. ($C_{18}H_{25}N$·HCl) C, H, N.

2-Butyl-2,3-didehydro-3-(2-methylphenyl)quinuclidine (33). Colorless liquid; yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (3H, t, J = 7.4 Hz), 1.14–1.27 (2H, m), 1.43–1.49 (2H, m), 1.62–1.74 (4H, m), 1.94–2.01 (2H, m), 2.25 (3H, s), 2.55–2.60 (1H, m), 2.63–2.73 (2H, m), 2.98–3.12 (2H, m), 7.02–7.06 (1H, m), 7.11–7.26 (3H, m); ^{13}C NMR ($CDCl_3$) δ 13.9, 20.4, 22.7, 28.6, 29.7, 29.9, 31.6, 33.6, 49.1, 49.6, 125.4, 126.6, 128.9, 129.8, 135.4, 139.5, 140.2, 148.2; MS m/z (%) 255 (26), 56 (100). Anal. ($C_{18}H_{25}N$·HCl) C, H, N.

2-Butyl-2,3-didehydro-3-(4-chlorophenyl)quinuclidine (34). Colorless liquid; yield 89%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.3 Hz), 1.24–1.34 (2H, m), 1.50–1.75 (6H, m), 2.18 (2H, t, J = 8.0 Hz), 2.58–2.67 (2H, m), 2.78–2.82 (1H, m), 2.97–3.06 (2H, m), 7.15 (2H, d, J = 7.7 Hz), 7.30 (2H, d, J = 8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.2, 23.2, 29.5, 30.6, 31.7, 33.9, 49.2, 125.7, 127.8, 128.3, 128.4, 129.1, 132.2, 138.5, 149.3; MS m/z (%) 275 (18), 233 (100). Anal. ($C_{17}H_{22}ClN$·HCl) C, H, N.

HPLC separation of (±)-29. The chiral HPLC was performed on a Shimadzu SCL-10A-VP system at a flow rate of 5 mL/min at room temperature and UV detection at 254 and 280 nm. The sample for injection was prepared by dissolving racemic compound (5 mg/mL) in mobile phase and the separation was carried out by injecting 30 μL on a 250×10 mm chiral column.

X-ray crystallography. Crystal structure details: monoclinic crystal in space group $P2_1/c$, a = 18.059(9), b = 9.162(2), and c = 15.799(2) Å, β = 92.21(3)°, V = 2612(1) Å^3, Z = 4. Dx = 1.40 g cm^{-3}, μ = 1.62 mm^{-1}, $F(000)$ = 1160, T = 293 K. Final agreement factor was $R(F)$ = 0.099 for the 1623 observed data. The higher than normal R-factor was due in part to the extended alkane side chain being disordered over at least two different positions as well as to the possibility of some unresolved twinning. Atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre.[35]

Pharmacology

[^{3}H]Mazindol binding. Binding assays were conducted as previously described.[10] Briefly conventional P_2 membrane pellets were prepared by differential centrifugation from rat striatum. The P_2 pellet was resuspended in Krebs–Ringer–HEPES (KRH) buffer consisting of (in mM): NaCl (125), KCl (4.8), $MgSO_4$ (1.2), $CaCl_2$ (1.3), KH_2PO_4 (1.2), glucose (5.6), nialamide (0.01), and HEPES (25) (pH 7.4) and centrifuged again. Finally, the pellet was resuspended in 30 volumes of buffer, pelleted at 15,000g and frozen at −80 °C until used. The striatal homogenates were thawed by resuspension in the buffer described above at 75–125 μg protein/mL and incubated with [^{3}H]mazindol, with or without competing drugs, for 60 min in a 4 °C cold room. Nonspecific binding was determined with 30 μM cocaine. The bound and free [^{3}H]mazindol were separated by rapid vacuum filtration over Whatman GF/C filters, using a Brandel M24R cell harvester, followed by two washes with 5 mL of cold buffer. Radioactivity on the filters was then extracted by allowing to sit overnight with 5 mL of scintillant. The vials were vortexed and counted. IC_{50} values were determined using the computer program LIGAND.

Synaptosomal uptake of [^{3}H]DA. The effect of candidate compounds in antagonizing dopamine high-affinity uptake was determined using a method previously employed.[10] For [^{3}H]DA uptake, freshly dissected rat striata were homogenized with a Teflon–glass pestle in ice-cold 0.32 M sucrose and centrifuged for 10 min at 1000 g. The supernatant was centrifuged at 17,500 g for 20 min. This P_2 synaptosomal pellet was resuspended in 30 volumes of ice-cold modified KRH buffer. An aliquot of the synaptosomal suspension was preincubated with the buffer and drug for 30 min at 37 °C, and uptake initiated by the addition of [^{3}H]dopamine (5 nM, final concn). After 5 min, uptake was terminated by adding 5 mL of cold buffer containing glucosamine as a substitute for NaCl and then finally by rapid vacuum filtration over GF-C glass fiber filters, followed by washing with two 5 mL volumes of ice-cold, sodium-free buffer. Radioactivity retained on the filters was determined by liquid scintillation spectrometry. Specific uptake is defined as that which is sensitive to inhibition by 30 μM cocaine. It is identical to that calculated by subtracting the mean of identical tubes incubated at 0 °C.

IC_{50} values were determined by a computer-assisted, iterative fit to a four-parameter sigmoidal equation (ALLFIT). These values were then converted to K_i values according to the Cheng–Prusoff equation assuming classical competitive inhibition. Preincubation of the drug and synaptosomes at 37 °C for 30 min was used to approximate equilibrium conditions as necessary to satisfy the requirements of the Cheng–Prusoff equation.

Behavioral pharmacology

Drug discrimination. The drug discrimination study was conducted using male Sprague–Dawley rats according to the procedure described elsewhere.[36] Rats were trained to discriminate 10 mg/kg ip cocaine from saline. All drugs were administered ip in a volume of 1 mL/kg 10 min prior to the testing. The response rate on both keys and the percent cocaine lever-appropriate responding were calculated for each rat. The response rates following a given test drug injection were presented as the percent of its corresponding vehicle control response rates.

Locomotor activity

Drugs alone. Locomotor activity of male Swiss Webster mice was recorded using Truscan activity monitors (Coulbourn Instruments, Allentown, PA) and a computer. The activity monitors consist of acrylic chambers, which are placed inside the sensor ring. The sensor ring is equipped with light-sensitive detectors and the infrared light beams. The X–Y coordinates of the body center of the subject are sampled by scanning the beams and then the successive locations of coordinates are compared. The sum of distances between successive coordinates is measured as the distance traveled, while the total number of coordinate changes are recorded as the stereotypic movements. Following 1 h of habituation to test arenas, several groups of mice were injected intraperitoneally (ip) with different doses of cocaine, compound **34** or its corresponding vehicles, saline and 10% DMSO, in a volume of 10 mL/kg. Locomotor activity was recorded in 10-min bins for the next 2 h. The raw data were converted to 30-min totals. The maximal 30-min activity occurring within the 2-h session following a given test drug injection was determined for each dose level and expressed as the percent of its corresponding vehicle control response for plotting the dose–response curves.

Interactions between cocaine and drugs. Following 40 min of habituation to test environment, different doses of drug (**34** or saline) were injected ip in a volume of 1 mL/100 g body weight and immediately placed back in the activity monitors. Twenty min later, a 20-mg/kg dose of cocaine was injected ip to all groups of animals and immediately placed back in the activity monitors. The data were recorded for a minimum of 2 h. Each dose of a given drug was studied in a separate group of mice and each mouse was used only once. The 30-min period responses were computed from the 2-h data. The 30-min period during which the maximal responses would occur was used for plotting the dose–response function. Data were analyzed using standard analysis of variance (Table 2).

Table 2. Elemental analysis

Compd No.	Molecular formula	Calculated			Found		
		C	H	N	C	H	N
16	$C_8H_{11}NO$	70.04	8.08	10.21	70.06	8.19	10.20
17	$C_{11}H_{17}NO{\cdot}HCl$	61.25	8.41	6.49	61.18	8.52	6.44
18	$C_{17}H_{23}NO{\cdot}HCl$	69.49	8.23	4.77	69.41	8.17	4.69
19	$C_{18}H_{25}NO{\cdot}HCl$	70.22	8.51	4.55	70.34	8.42	4.59
20	$C_{18}H_{25}NO{\cdot}HCl$	70.22	8.51	4.55	70.09	8.61	4.48
21	$C_{18}H_{25}NO{\cdot}HCl$	70.22	8.51	4.55	69.98	8.46	4.43
22	$C_{17}H_{22}ClNO{\cdot}HCl$	62.20	7.06	4.27	62.11	6.97	4.18
23	$C_{17}H_{21}N{\cdot}HCl$	74.03	8.04	5.08	73.95	7.94	5.03
24	$C_{18}H_{23}N{\cdot}HCl$	74.59	8.35	4.83	74.52	8.26	4.79
25	$C_{18}H_{23}N{\cdot}HCl$	74.59	8.35	4.83	74.41	8.34	4.73
26	$C_{18}H_{23}N{\cdot}HCl$	74.59	8.35	4.83	74.72	8.48	4.70
27	$C_{17}H_{20}ClN{\cdot}HCl$	65.81	6.82	4.51	65.69	6.94	4.66
28	$C_{17}H_{25}N{\cdot}HCl$	72.96	9.36	5.01	73.06	9.27	4.99
29	$C_{18}H_{27}N{\cdot}HCl$	73.57	9.60	4.77	73.49	9.60	4.55
30	$C_{17}H_{23}N{\cdot}HCl$	73.49	8.71	5.04	73.22	8.62	4.97
31	$C_{18}H_{25}N{\cdot}HCl$	74.07	8.98	4.80	74.19	9.10	4.92
32	$C_{18}H_{25}N{\cdot}HCl$	74.07	8.98	4.80	73.97	8.89	4.76
33	$C_{18}H_{25}N{\cdot}HCl$	74.07	8.98	4.80	74.10	8.90	4.81
34	$C_{17}H_{22}ClN{\cdot}HCl$	65.38	7.42	4.49	65.34	7.38	4.40

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