Expedited Articles

Discovery of a Novel Dopamine Transporter Inhibitor, 4-Hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-Methylphenyl Ketone, as a Potential Cocaine Antagonist through 3D-Database Pharmacophore Searching. Molecular Modeling, Structure−Activity Relationships, and Behavioral Pharmacological Studies

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A novel, fairly potent dopamine transporter (DAT) inhibitor, 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone (**3**, K_i values of 492 and 360 nM in binding affinity and inhibition of dopamine reuptake, respectively), with significant functional antagonism against cocaine and a different in vitro pharmacological profile from cocaine at the three transporter sites (dopamine, serotonin, and norepinephrine) was discovered through 3D-database pharmacophore searching. Through structure−activity relationships and molecular modeling studies, we found that hydrophobicity and conformational preference are two additional important parameters that determine affinity at the DAT site. Chemical modifications of the lead compound (**3**) led to a high affinity analogue (**6**, K_i values of 11 and 55 nM in binding affinity and inhibition of dopamine reuptake, respectively). In behavioral pharmacological testing, **6** mimics partially the effect of cocaine in increasing locomotor activity in mice but lacks cocaine-like discriminative stimulus effect in rats. Taken together, these data suggest that **6** represents a promising lead for further evaluations as potential therapy for the treatment of cocaine abuse.

Introduction

Cocaine abuse is one of the greatest concerns of the American public today, and has therefore become a focus of medical, social, and political leaders. Cocaine is one of the most addictive substances known, and addicts may lose their ability to function at work or in interpersonal situations. Although cocaine potently inhibits the reuptake of both norepinephrine (NE) and serotonin (5-HT), many lines of evidence indicate that its ability to act as a reinforcer stems from its ability to inhibit the reuptake of dopamine (DA) into dopaminergic neurons.[1−8] Cocaine exerts this effect via specific interaction with DA transporter (DAT) proteins (cocaine receptor) located on DA nerve terminals.[1−5] This increase of dopaminergic transmission in the reward mediating brain mesolimbic system is the essence of the dopamine hypothesis of reinforcement advanced by Wise[9] and later elaborated for cocaine by Kuhar.[3]

In pursuit of the discovery of a cocaine antagonist as a potential therapy for the treatment of cocaine abuse, substantial effort has been focused on modifications of cocaine itself; this has led to a wealth of information about the structure−activity relationships (SARs) of cocaine analogues.[2,10−24] Despite intense research efforts in this area, very few compounds with significant cocaine antagonist activity have been reported and no effective therapy for the treatment of cocaine abuse is currently available clinically. Therefore, we believe that the discovery of novel lead compounds capable of antagonizing all or some of cocaine's actions represents a critical step.

Recent advances in molecular biology have identified the amino acid sequences of the DAT,[25,26] but no experimental 3D structures have been obtained for the DAT. The lack of the experimental structures makes it difficult to employ a structure-based design strategy for the discovery of novel DAT inhibitors as cocaine antagonists. Alternatively, a wealth of SAR data on cocaine analogues and other classes of dopamine transporter inhibitors makes it feasible to derive "putative 3D pharmacophore models", defined as the representation of crucial chemical structural features and their 3D geometric relationships that are important for the

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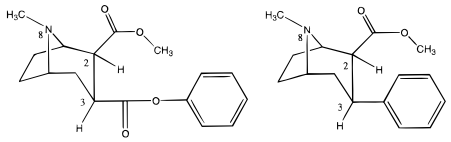
biological activity of interest. With the pharmacophore models, one can search large chemical databases to discover compounds whose 3D structures meet the pharmacophore requirements. In recent years, it has been shown that 3D-database pharmacophore searching is a very effective approach to discover novel lead compounds for drug discovery.[27−31]

Using this approach, we have discovered a large number of novel DA inhibitors, some of which display promising cocaine antagonism in our functional assay. In this paper, we report the discovery of one such lead compound, its structure−activity relationships and our molecular modeling studies, as well as behavioral pharmacological studies in mice and rats of the most potent compound in this class (K_i = 11 nM in binding affinity at the DAT site).

Results and Discussion

Previous extensive structure−activity relationship (SAR) studies on cocaine and its analogues[2,10−24] showed that three binding elements play important roles in the binding and reuptake activities of cocaine and its analogues: (1) the aromatic system at the 3β-position of the tropane ring; (2) the 2β ester group or a small hydrophobic group at this position; and (3) the nitrogen at position 8, although recent studies showed that this basic nitrogen may be replaced by an oxygen.[32,33] In the past, a number of molecular modeling studies were performed in an attempt to define 3D pharmacophores for DAT inhibitors or to seek quantitative structure−activity relationships (QSAR) for DAT inhibitors.[34−38] For example, Froimowitz et al. have elegantly shown that a common pharmacophore model exists between 2β-carbomethoxyl-3β-(4-flurophenyl)trophane (CFT) and methylphenidate,[36] demonstrating that despite the different 3D chemical structures, these DAT inhibitors may indeed share one or more common 3D pharmacophore models.

It is essential to determine the 3D geometric relationships of these crucial binding elements in cocaine and its analogues for the development of pharmacophore models. For this purpose, conformational analysis was performed on cocaine (**1**) and **2** (WIN-35065-2), starting from the X-ray crystal structure of cocaine.[39] The initial structure of **2** was built by replacing the benzoyloxy group with a phenyl group using the QUANTA molec-



1 (cocaine) **2** (WIN-35065-2)

ular modeling package.[40] The structures of both compounds were minimized, and a systematic conformational search was performed, using the QUANTA program.[40] The crucial binding components that were included in the first pharmacophore model (Figure 1) are the nitrogen atom, the carbonyl group, and the aromatic ring center, which represents the aromatic ring in cocaine and its analogues. Three distance parameters (Figure 1) were used to define the 3D geometric relationships of these three crucial binding elements, which

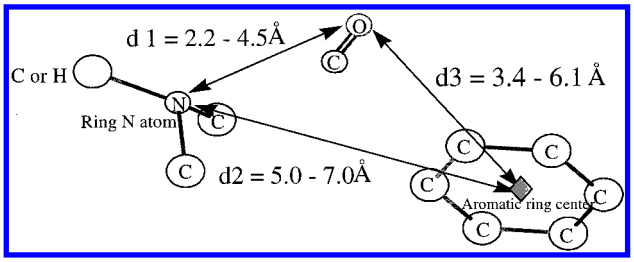


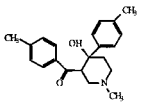
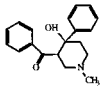
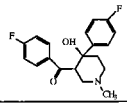
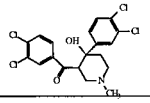
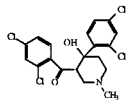
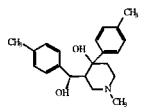
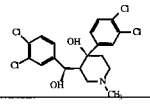
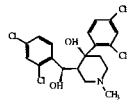
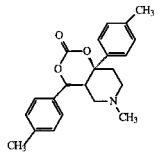
Figure 1. A 3D pharmacophore model derived from cocaine and its analogues used in 3D-database pharmacophore search.

were derived through examining the 3D geometric relationships between these three binding elements in both **1** and **2** in their low energy conformations, as well as distance ranges in all possible conformations. It should be emphasized that more than one common pharmacophore may exist between the different classes of DAT inhibitors such as between cocaine analogues and GBR compounds[41] and different pharmacophore models may need to be developed for DAT inhibitors that do not share a single common pharmacophore. As can be seen from Figure 1, the distance between the nitrogen and the carbonyl oxygen (d1) was chosen to be from 2.2 to 4.5 Å. This essentially covers the possible distance span between these atoms in **1** and **2**. Some margin was allowed for both the lowest distance value (2.6 Å) and largest distance value (4.2 Å). The distance between the nitrogen atom and the aromatic ring (d2) is set from 5.0 to 7.0 Å, assuming the value of 5.6 Å in **2** is probably the optimum for effective binding to the DAT since **2** is a high affinity DAT inhibitor.[2] The distance between the carbonyl oxygen and the aromatic ring center (d3) was set to be from 3.4 to 6.1 Å. It is of note that we have used fairly large distance tolerances in this pharmacophore model, for our purpose is to discover truly novel DAT inhibitors with broad structural diversity.

Using the pharmacophore model shown in Figure 1, we searched the NCI 3D-database of 206 876 "open" compounds,[42] whose structures can be accessed by the public, with the program Chem-X.[42] During the search process, a compound was first examined for the required chemical groups, i.e., a secondary or a tertiary nitrogen, a carbonyl group, and an aromatic ring system. If the chemical structural requirements were met, the program then investigated whether the compound had a conformation that meets the 3D geometric parameters of the pharmacophore model. If the program found that the compound indeed had at least one conformation that met the 3D geometric requirements, the compound would be considered as a "hit". Up to 3 000 000 conformations were examined for each compound. Using the pharmacophore model shown in Figure 1, a total of 4094 compounds, i.e., 2% of 206 876, were identified as "hits".

In selecting compounds for testing in [^{3}H]mazindol binding and [^{3}H]DA reuptake assays, we considered a number of additional criteria. Since our primary goal is to discover novel lead compounds, it is desirable that compounds have simple chemical structures and low molecular weight (MW), which would greatly facilitate subsequent chemical modifications. We therefore eliminated all compounds with MW greater than 1000. We also found among the 4094 "hits" that the pharmacophore nitrogen atom in a number of compounds was not

Table 1. Chemical Structures of DAT Inhibitors and Their Activities at Three Transporter Sites

		K_i (nM)			
		Binding	Uptake		
		([^{3}H]-Mazindol)	([^{3}H]-DA)	([^{3}H]-5-HT)	([^{3}H]-NE)
1 (cocaine)		231± 22[a]	274 ± 20	155 ± 0.4	108 ± 4
Lead Compound **3**		492 ± 34	360 ± 25	1630 ± 150	3860 ± 70
4		4420 ± 620	2590 ± 230		
5		5700 ± 360	4200 ± 70		
6		10.9 ± 1.4	51 ± 8	2380±140	177 ± 49
7		3650 ± 170	5850 ± 1080		
8		10650 ± 650	15800 ± 650		
9		4140 ± 60	4220 ± 20		
10		4350 ± 80	7520 ± 90		
11		8190 ± 30	9480 ± 740		

[a] Standard deviation was obtained by three experiments.

capable of functioning as a hydrogen bond acceptor, due to its local chemical environment, and these compounds were therefore eliminated from further consideration. Structural diversity was another important criterion, i.e., we sought to discover a large number of structurally diverse DAT inhibitors. However, in case one compound showed interesting pharmacological properties, we often identified its analogues by 2D-structure searching and subsequently tested these compounds to establish initial structure−activity relationships. A total of 385 compounds were finally selected for testing in [^{3}H]mazindol and [^{3}H]DA reuptake assays.

In the first batch of screening, 70 compounds out of the 385 selected candidates were evaluated in the [^{3}H]-mazindol binding assay. Thirteen compounds displayed more than 50% inhibition at 10 μM in the [^{3}H]mazindol binding assay (IC_{50} less than 10 μM). An additional 23 compounds showed an inhibitory activity of 30% to 50% at 10 μM and eight more compounds had an inhibitory activity of 20% to 30% at 10 μM in the [^{3}H]mazindol binding assay. Overall, 63% of 70 (44/70) compounds showed good activity at 10 μM in the [^{3}H]mazindol assay. These results suggested that the pharmacophore model used in our 3D pharmacophore search is effective in identifying compounds with diverse chemical structures that can effectively compete with [^{3}H]mazindol binding to the cocaine site on the DAT.

These 13 novel DAT inhibitors with an IC_{50} less than 10 μM were further evaluated for their ability to antagonize cocaine's inhibition of [^{3}H]DA uptake. Four classes of compounds were found to display significant functional antagonism. One such compound (**3**) was found to have good potency in DAT binding and uptake assays with K_i values of 492 nM and 360 nM, respectively, slightly less potent than cocaine. Importantly, this compound has a simple chemical structure, which

Table 2. Functional Antagonism of **3** against Cocaine

Drugs	IC_{50} (nM) [^{3}H]-Dopamine Uptake (Experimental)	IC_{50} (nM) [^{3}H]-Dopamine Uptake (Theoretical, assuming one binding site for cocaine and the drug)
Cocaine alone	297 ± 22[a]	
Cocaine + **3** (50 nM)	470 ± 25	331 ± 11
Cocaine + **3** (200 nM)	717 ± 49	438 ± 22
Cocaine + **3** (500 nM)	1161 ± 100	652 ± 24

[a] Standard error was based on three experiments.

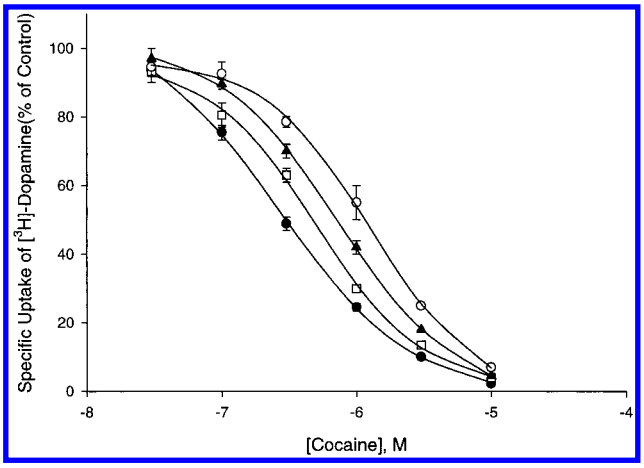
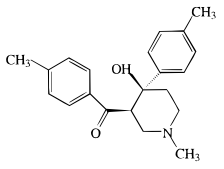


Figure 2. Effect of cocaine on [^{3}H]-dopamine uptake in the presence of lead compound **3** (solid circles, cocaine alone; open squares, 50 nM of **3** + cocaine; solid triangles, 200 nM of **3** + cocaine; open circles, 500 nM of **3** + cocaine).

greatly facilitates our subsequent chemical modifications and structure−activity relationship studies. It is of note that a class of structurally related compounds, piperidine-based analogues of cocaine, were found to be potent PAT inhibitors.[54]

We have evaluated the functional antagonism of **3** in terms of its effects on the inhibition of [^{3}H]dopamine uptake by cocaine (Table 2 and Figure 2). The IC_{50} values of cocaine in the presence of **3** were determined and compared to the IC_{50} value of cocaine alone. If significant differences in IC_{50} values were found, they were then compared to theoretical IC_{50} values expected from models of "same site" antagonism.[43−45] As can be seen from Table 2 and Figure 2, at each of the three



Lead Compound (**3**)

concentrations of **3** used in our test (50, 200, and 500 nM), the IC_{50} value obtained for cocaine in the presence of **3** was significantly greater than that expected from "same site" antagonism. Therefore, **3** was considered to have significant functional antagonism against cocaine, though it is important to point out that at these concentrations **3** significantly inhibited the DA reuptake alone and that the data are normalized to 100% for the analysis.

Since cocaine is a potent inhibitor not only of DA uptake but also of serotonin (5-HT) and norepinephrine (NE) uptake (Table 1), we have further evaluated **3** as inhibitor of these transporters. The results are shown in Table 1. As can be seen, **3** is more selective for DAT than is cocaine. The selectivity of **3** is 4.5-fold between DA and 5-HT transporter sites and is 10.7-fold between DA and NE transporter sites. Furthermore, we have evaluated **3** for its ability to stimulate the locomotor activity in mice and found that **3** failed to stimulate the locomotor activity at concentrations from 1.0 mg/kg to 30 mg/kg.

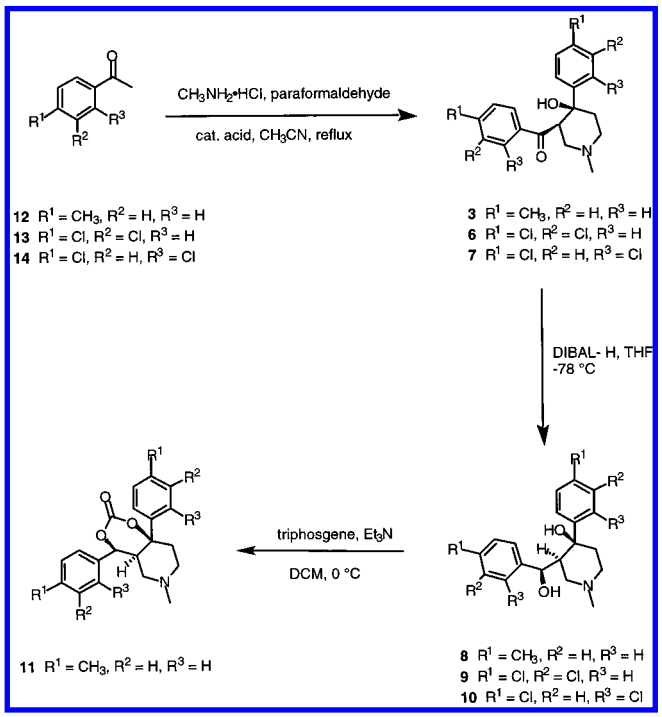
Taken together, on the basis of its fairly potent activities in binding and uptake, its simple chemical structure, its different pharmacological profile from cocaine at the three different transporter sites, its functional antagonism, and its inability to stimulate the locomotor activity in mice, **3** appears to represent a promising lead compound for further development and evaluations.

On the basis of the lead compound, we have synthesized and tested a series of analogues to investigate its structure−activity relationships. The results are summarized in Table 1.

The general synthesis of the analogues is illustrated in Scheme 1. Briefly, reaction of methylamine hydrochloride with an excess of aryl methyl ketone and paraformaldehyde in the presence of a catalytic amount of acid led to compounds **3**, **6**, and **7**.[46] The 1,3-dihydroxy compounds (**8**, **9**, and **10**) were synthesized by reducing the appropriate ketones with DIBAL-H in tetrahydrofuran (THF) at −78 °C.[47] Under these conditions, the reduction led to the cis-stereoisomers **8**, **9**, and **10**. The cyclic carbonate **11** was prepared by the treatment ofcompound **8** with triphosgene in dichloromethane (DCM). Compounds **4** and **5** were purchased from commercial suppliers.

As can be seen, analogue **4** without the methyl group on each aromatic ring has binding and uptake activities reduced by 9- and 7-fold, respectively, as compared to the lead compound **3**. Replacement of the methyl group on each aromatic ring by a fluorine atom (**5**) reduces the binding and uptake activities each by 12-fold. Our molecular modeling studies showed that **4**, **5**, and the lead compound (**3**) have essentially the same conformational profiles. This suggests that the activity difference between these two compounds may be primarily due to the difference in their hydrophobicity. Accordingly, compounds **6** and **7** were synthesized. As expected, analogue **6** bearing 3,4-dichloro substitutions has a much-improved activity in both binding and uptake. Its

Scheme 1



binding affinity to the DAT was increased by 45-fold, and its uptake activity at the DA site was increased by 7-fold. It is of note that **6** is more selective for the DA site relative to the 5-HT and NE sites. While **6** has a relatively potent activity at the NE site, it has a very weak activity at the 5-HT site.

In sharp contrast, analogue **7** with 2,4-dicholoro substitutions has a much-reduced activity in binding and uptake. Its binding affinity to the DAT was reduced by 7-fold, while its uptake activity at the DA site was reduced by 16-fold. Thus, despite the similar hydrophobicity of **6** and **7**, their binding affinities to the DAT differ by 335-fold and their uptake activities at the DA site differ by 115-fold. These data showed that, in addition to hydrophobicity, other factors, such as the conformational differences between **6** and **7** may play a role in their binding and uptake activities.

On the basis of pharmacophore model (Figure 1), the carbonyl group in this class of compounds mimics the carbonyl group of the ester group in cocaine and WIN compounds. To investigate the importance of this carbonyl group, we have reduced this group to a hydroxyl group in the lead compound (**3**), **6**, and **7**, which resulted in compounds **8**, **9**, **10**. As compared to the lead compound **3**, the binding affinity for **8** was reduced by 22-fold and the uptake activity was reduced by 44-fold, highlighting the importance of the carbonyl group. The binding and uptake activities for **9** were reduced even more, by 380- and 83-fold, respectively, as compared to the corresponding compound **6**. There is only a slight reduction of the activities for **10** as compared to **7**, probably because both compounds have only weak activities. Our data therefore suggest that the carbonyl group in this class of compounds is important for their activity. Because both carbonyl and hydroxyl groups can function as hydrogen bonding acceptor, the diminished activity in the corresponding hydroxyl compounds (**8**, **9**, and **10**) may be primarily due to the change in conformational profiles of these compounds. Constrained compound **11** was synthesized to investigate further the active conformation for this class of compounds. In comparison to the lead compound (**3**), the binding affinity and the inhibition of DA reuptake of **11** were reduced by 17-fold and 26-fold, respectively.

Each of these compounds in Table 1 contains the pharmacophore as shown in Figure 1, yet they display significantly different activities. The most potent compound **6** has a binding affinity of 11 nM, while the least potent compound **8** has a binding affinity of 11 μM, 3 orders of magnitude difference. Therefore, it is clear that in addition to the pharmacophore, other factors also play a role in the binding and uptake activities to the DAT for this class of compounds. To gain a better understanding on the binding of this class of compounds to the DAT, we have carried out extensive conformational analysis.

Comparison of the low energy conformational clusters of lead compound (**3**), **4**, **5**, and **6** showed that these compounds have virtually identical conformational profiles (Figure 3A), suggesting that the differences in their binding and uptake activity are not due to their conformational difference. In fact, their binding and uptake activities have a good correlation with their hydrophobicity, i.e., the most hydrophobic compound **6** has the most potent binding and uptake activities while the least hydrophobic compound **4** is the least potent. However, it is clear that the hydrophobicity is not the only factor important for the binding and uptake activities of this class of compounds. Compound **7** is more than 300-fold less potent than **6** in their binding affinity, despite their similar hydrophobicity. Conformational analysis showed that **7** has a different conformational profile as compared to the lead compound **3** and analogue **6**. Although the piperidinyl ring and the phenyl ring at position 4 adopt identical orientation in the lowest energy conformations and other low energy conformations of **3**, **6**, and **7**, the phenyl ring connected to the carbonyl group in the lowest energy conformation of **7** deviates by 39° from the phenyl ring in the lowest energy conformations of **3**, **4**, and **6** (Figure 3B). To achieve the same orientation, **7** would have to pay an energy penalty of 6 kcal/mol. These data suggest that in addition to their hydrophobicity, conformational preference of these compounds plays a role for their binding and uptake activities. The importance of the conformational preference is highlighted by **9**. Despite its similar hydrophobicity to **6**, **9** is 380-fold less potent than **6** in its binding affinity. Indeed, conformational analysis showed that the lowest energy conformation of **9** is significantly different from that of **3** and **6** (Figure 3C). To adopt the same conformation as the lowest energy conformation of **6**, **9** would have to pay an energy penalty of as much as 8 kcal/mol. Rigid analogue **11** is 751-fold less potent than **6** upon comparison of their binding affinities. Conformational analysis showed that the lowest energy conformation of **11** is significantly different from that of **6**. In fact, the corresponding phenyl rings at position 3 deviate as much as 94° (Figure 3D) and no low energy conformations of **11** are similar to the lowest energy conformation of **6**. In summary, our molecular modeling studies showed that hydrophobicity and conformational preference of these compounds play a role in determining their binding and

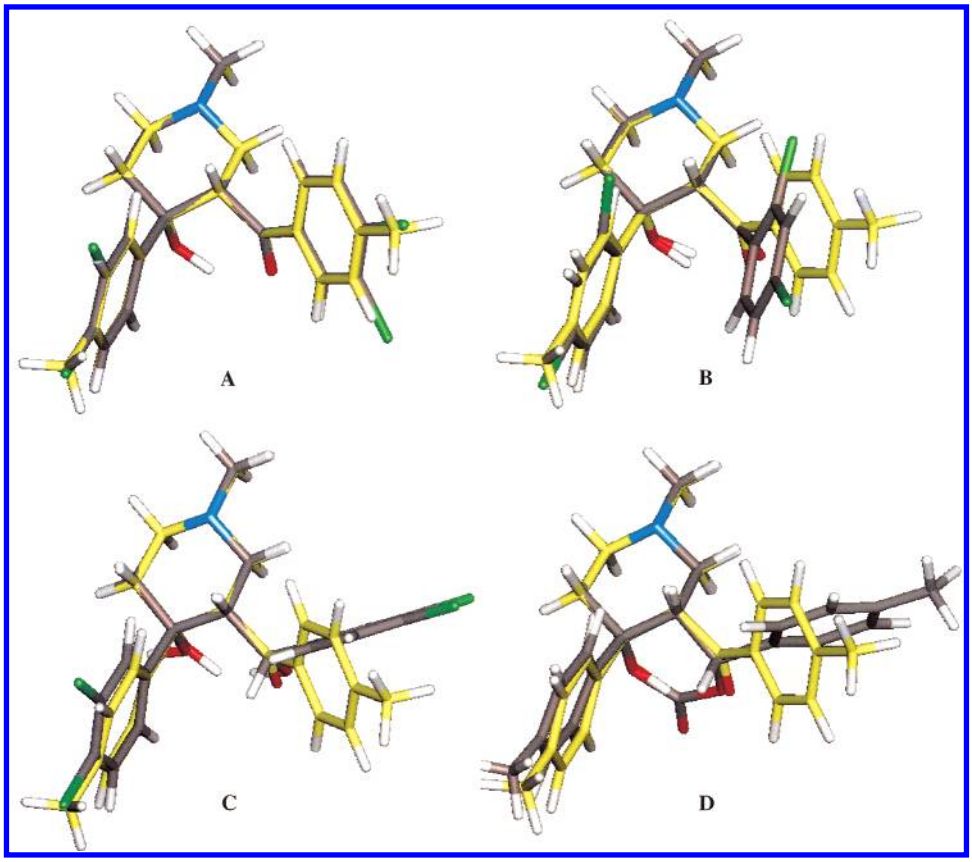


Figure 3. Superposition of the lowest energy conformations: (A) lead compound **3** (yellow) on analogue **6** (gray); (B) lead compound **3** (yellow) on analogue **7** (gray); (C) lead compound **3** (yellow) on analogue **9** (gray); (D) lead compound **3** (yellow) on analogue **11** (gray).

uptake activities and suggested that the lowest energy conformation of **3**, **4**, **5**, and **6** may represent the biologically active conformation for this class of compounds in binding to the DAT.

Our chemical modifications of the lead compound led to a very potent DAT inhibitor (**6**, K_i values of 11 nM and 51 nM in binding and reuptake, respectively). To further assess the therapeutic potential of this class of compounds, we have evaluated the antagonism effect of **6** against cocaine in our functional assay and more importantly its behavioral pharmacological profile in mice and rats. It was found that despite its much higher affinities in binding and uptake than the lead compound (**3**), **6** only showed a marginal functional antagonism, significantly less than that of the lead compound **3** (data not shown). But the reason for its diminished functional antagonism of **6** is not clear. Locomotor activity studies in mice showed both cocaine (3−30 mg/kg) and **6** (10−156 mg/kg) produced dose-dependent enhancements in the distance traveled and stereotypic movements (Figure 4A and 4B). Further increase in the doses of both cocaine and **6** produced convulsions and death, suggesting that both cocaine and **6** enter the brain. However, cocaine is about 11.8- (95% confidence limits: 8.8−16.3) and 17.3- (95% confidence limits: 10.5−35.1) fold more potent (by parallel lines bioassay test) than **6** in increasing the distance traveled and the stereotypic movements, respectively. Cocaine (30 mg/kg) is also significantly more efficacious than **6** (156 mg/kg) in increasing the distance traveled ($P = 0.016$) and the stereotypic movements ($P = 0.001$) at the maximal tolerated doses. In drug discrimination testing in rats trained to discriminate 10 mg/kg ip cocaine from saline, cocaine (3−10 mg/kg) produced dose-dependent and full substitution, while **6** (1.56−30 mg/kg) produced little or no substitution for cocaine (Figure 4C). Furthermore, **6** did not alter the response rates (Figure 4D). Higher doses of **6** were not tested in a drug discrimination test due to our observation of delayed toxicity in mice treated with higher doses (100 and 156 mg/kg) of this drug. Taken together, our behavioral data indicate that although **6** is less potent and efficacious than cocaine in increasing the locomotor activity in mice, it does partially mimic the effect of cocaine. However, **6** lacks the cocaine-like discriminative stimulus effect in rats trained to discriminate cocaine from saline in the dose range tested. It has been suggested that one potential therapeutic approach for the treatment of cocaine abuse is to have a compound that has some of the behavioral effects of cocaine but is not self-administered.[15] The fact that **6**, a high affinity DAT inhibitor (21- and 5-times more potent than cocaine in DAT binding affinity and inhibition of reuptake of dopamine, respectively), mimics partially the effect of cocaine in increasing locomotor activity in mice but lacks cocaine-like discriminative stimulus effect in rats warrants further investigation of this compound or other analogues as a potential therapy for the treatment of cocaine abuse.

Experimental Section

Molecular Modeling. Conformational analysis was performed using the conformational analysis module in the QUANTA program.[40] Generally, if a compound has fewer than five rotatable single bonds, the grid scan conformational search

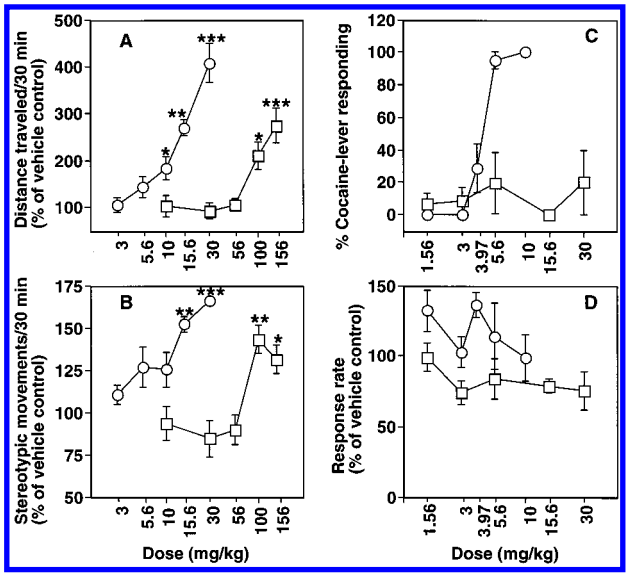


Figure 4. Behavioral effects of cocaine (circles) and compound **6** (squares). Both compound **6** ($F_{5,61} = 11.29$, $P < 0.001$) and cocaine ($F_{5,74} = 30.5$, $P < 0.001$) produced significant and dose-dependent increases in the distance traveled (A) in male Swiss−Webster mice. Similarly, compound **6** ($F_{5,61} = 6.89$, $P < 0.001$) and cocaine ($F_{5,74} = 8.31$, $P < 0.001$) significantly increased stereotypic movements (B). The distance traveled and the stereotypic movement responses in the saline control group were 3517 ± 325.6 cm and 1299 ± 71, respectively. The corresponding numbers for 10% DMSO vehicle control group were 3402 ± 363 cm and 1159 ± 80. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ as compared to the responses in the corresponding vehicle control group by Tukey's post hoc test. The results of the discriminative stimulus testing in rats ($n = 5-7$) are shown in panels C and D. The data points in the figure represent the mean ± SEM.

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 within QUANTA. If a compound has 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 a vacuum). It is noted, however, that the lowest energy conformation may not be the bioactive conformation, as was shown previously.[48] 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 bioactive conformations for a compound.

3D-Database Search. The Chem-X program (version July 96),[42] running on a Silicon Graphics Indigo2 R10000, was used to carry out 3D-database pharmacophore searching. This program has been used to build the NCI-3D database[42] and was successfully used to carry out 3D-database pharmacophore searching in a number of other studies.[30,31] 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 3D-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[42] that the best way to handle this is 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 for 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 current version of the NCI 3D database 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.[42] Employing the Chem-X program, it is straightforward to search the NCI 3D-database of 206 876 "open" compounds for structures that meet the requirements specified in the pharmacophore models. The defined pharmacophore model was built into a query, which included all the specifications as described in the pharmacophore models, such as substructural requirements, distance, and distance ranges between these crucial pharmacophore components. The Chem-X program first checks if the compound has a carbonyl group, an aromatic ring, and a nitrogen attached to at least two carbon atoms and one more carbon or hydrogen. After a compound passed this substructural check, it was subjected to a conformational analysis. In this step, conformations were generated and evaluated with regard to geometric requirements specified in the pharmacophore query. Compounds, which have at least one conformation satisfying the geometric requirements, were considered as "hits". It is of note that no conformational energy calculation was performed during the search. "Hits" are only considered as potential candidates for biological testing. 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 molecule, nonpeptidic, and chemical structure diversity.

Chemistry. General Methods. THF was freshly distilled under nitrogen from sodium benzophenone. ^{1}H NMR 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 throughout the Chemistry Experimental Section: THF = tetrahydrofuran; DCM = dichloromethane; CH_3CN = acetonitrile; ether = diethyl ether.

General Procedure for the Synthesis of Compounds 3, 6, and 7. To an equimolar mixture of aryl methyl ketone and paraformaldehyde in acetonitrile (15 mL/g of ketone) was added methylamine hydrochloride (0.25 equiv), and the mixture was refluxed for 20 h in the presence of a catalytic amount of hydrochloric acid (37% w/v, 0.02 mL/1 g of ketone). The reaction mixture was cooled to room temperature, and volatiles were removed under reduced pressure. The resulting mass was dissolved in DCM, washed with aqueous $NaHCO_3$ solution,

water, and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude compounds were purified by column chromatography using triethylamine/diethyl ether as eluent to afford the following compounds:

4-Hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-Methylphenyl Ketone (3). White solid; yield 70%; mp 143−145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78−1.88 (1H, m), 1.98−2.15 (1H, m), 2.25 (3H, s), 2.40 (6H, s), 2.60−2.85 (3H, m), 2.95 (1H, dd, J = 10.5 Hz, 2.9 Hz), 4.20 (1H, dd, J = 11.5 Hz, 3.7 Hz), 5.22 (1H, d, J = 2.5 Hz), 7.07 (2H, d, J = 8.1 Hz), 7.25 (2H, d, J = 8.1 Hz), 7.40 (2H, d, J = 8.3 Hz), 7.82 (2H, d, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 20.8, 21.7, 40.2, 45.9, 50.3, 51.5, 54.8, 72.4, 124.4, 128.5, 128.9, 129.5, 133.4, 136.2, 144.3, 144.9, 203.8. Anal. ($C_{21}H_{25}NO_2$) C, H, N.

3,4-Dichlorophenyl 4-(3,4-Dichlorophenyl)-4-hydroxy-1-methyl-3-piperidyl Ketone (6). White solid; yield 71%; mp 120 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80 (1H, br d, J = 13.9 Hz), 1.95−2.06 (1H, m), 2.42 (3H, s), 2.63−2.72 (2H, m), 2.80 (1H, br d, J = 9.1 Hz), 2.90 (1H, dd, J = 11.2 Hz, 8.0 Hz), 4.22 (1H, dd, J = 11.5 Hz, 7.8 Hz), 4.98 (1H, d, J = 2.4 Hz), 7.24 (1H, dd, J = 8.5 Hz, 6.6 Hz), 7.32 (2H, t, J = 8.2 Hz), 7.55 (1H, d, J = 8.3 Hz), 7.64 (1H, d, J = 1.7 Hz), 7.72 (1H, dd, J = 8.3 Hz, 6.5 Hz), 7.96 (1H, d, J = 1.4 Hz); ^{13}C NMR ($CDCl_3$) δ 39.6, 45.7, 50.5, 50.9, 54.2, 72.1, 123.6, 127.1, 130.0, 130.1, 130.8, 130.9, 132.5, 133.6, 134.9, 138.8, 147.3, 201.2. Anal. ($C_{19}H_{17}Cl_4NO_2$) C, H, N.

2,4-Dichlorophenyl 4-(2,4-Dichlorophenyl)-4-hydroxy-1-methyl-3-piperidyl Ketone (7). Yellow solid; yield 51%; mp 101−103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (1H, br d, J = 13.7 Hz), 2.42 (3H, s), 2.68−2.84 (4H, m), 3.00 (1H, dd, J = 11.2 Hz, 7.8 Hz), 4.55 (1H, s), 5.14 (1H, dd, J = 11.5 Hz, 7.6 Hz), 7.00 (1H, d, J = 8.3 Hz), 7.15 (1H, d, J = 1.8 Hz), 7.18 (1H, d, J = 2.0 Hz), 7.25 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 2.0 Hz) 7.89 (1H, d, J = 2.5 Hz); ^{13}C NMR ($CDCl_3$) δ 34.0, 46.1, 50.8, 51.4, 53.2, 73.1, 127.1, 127.3, 129.9, 130.1, 130.5, 130.8, 132.8, 133.9, 136.3, 138.3, 140.9, 205.7. Anal. ($C_{19}H_{17}Cl_4NO_2$) C, H, N.

General Procedure for the Synthesis of Compounds 8, 9, and 10. To a solution of the appropriate ketone in THF (10 mL/g) was added dropwise 1 M DIBAL-H in hexane (2.5 equiv) at −78 °C under nitrogen. After being stirred for 2.5 h at the same temperature, the reaction mixture was quenched with aqueous NH_4Cl and extracted with DCM, and the organic phase was washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting crude compound was purified by column chromatography using Et_3N/ether as eluent to afford the following compounds:

3-[Hydroxy(4-methylphenyl)methyl]-1-methyl-4-(4-methylphenyl)piperidin-4-ol (8). White solid; yield 64%; mp 178−180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (1H, br d, J = 14.2 Hz), 1.94−2.06 (1H, m), 2.19−2.43 (11H, m), 2.59−2.71 (3H, m), 4.64 (1H, d, J = 4.8 Hz), 6.91 (2H, d, J = 8.0 Hz), 6.96 (2H, d, J = 8.2 Hz), 7.03 (2H, d, J = 8.1 Hz), 7.24 (2H, d, J = 9.3 Hz); ^{13}C NMR ($CDCl_3$) δ 20.9, 21.0, 41.8, 46.2, 50.3, 51.5, 55.1, 73.3, 74.9, 124.6, 126.1, 128.7, 128.8, 135.9, 136.7, 139.7, 144.5. Anal. ($C_{21}H_{27}NO_2$) C, H, N.

4-(3,4-Dichlorophenyl)-3-[(3,4-dichlorophenyl)hydroxymethyl]-1-methylpiperidin-4-ol (9). Light yellow solid; yield 79%; mp >220 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (1H, br d, J = 13.9 Hz), 1.87−1.98 (1H, m), 2.45 (3H, s), 2.54−2.61 (2H, m), 2.74−2.92 (3H, m), 4.75 (1H, br s), 6.96 (1H, br d, J = 8.5 Hz), 7.05 (1H, s), 7.19−7.28 (4H, m); ^{13}C NMR ($CDCl_3$) δ 39.9, 44.5, 48.0, 48.3, 51.5, 56.0, 73.5, 74.7, 126.3, 126.6, 128.8, 128.9, 130.8, 130.9, 131.5, 131.9, 132.9, 133.1, 144.8, 147.5. Anal. ($C_{19}H_{19}Cl_4NO_2$) C, H, N.

4-(2,4-Dichlorophenyl)-3-[(2,4-dichlorophenyl)hydroxymethyl]-1-methylpiperidin-4-ol (10). White solid; yield 76%; mp 215−217 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (1H, d, J = 7.3 Hz), 2.43 (3H, s), 2.67−2.78 (3H, m), 2.93−3.05 (2H, m), 3.33−3.40 (1H, m), 3.68−3.73 (1H, m), 6.71 (2H, s), 6.85 (1H, s), 7.29 (2H, d, J = 11.2 Hz), 7.95 (1H, t, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 37.1, 41.9, 46.4, 51.8, 57.8, 75.1, 75.9, 127.0, 128.1, 129.4, 130.0, 131.4, 131.5, 133.0, 133.7, 134.4, 140.3, 144.3. Anal. ($C_{19}H_{19}Cl_4NO_2$) C, H, N.

8-Aza-1,5-bis(4-methylphenyl)-8-methyl-2,4-dioxabicyclo[4.4.0]decan-3-one (11). To a mixture of compound **8** (75 mg, 0.23 mmol) and Et_3N (0.035 mL, 25 mmol) in dry DCM (3 mL) at 0 °C was added triphosgene (82 mg, 28 mmol). The reaction mixture was stirred for 2 h, during which time it was allowed to return to room temperature, then diluted with DCM (10 mL). The solution was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting crude compound was purified by column chromatography using Et_3N/ether as eluent to give the title compound as a white solid (35 mg, 44%); mp 195−197 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16−2.37 (11H, m), 2.44−2.53 (2H, m), 2.62−2.70 (2H, m), 2.88−2.94 (1H, m), 5.55 (1H, d, J = 4.9 Hz), 6.92 (2H, d, J = 8.1 Hz), 6.99 (4H, d, J = 8.6 Hz), 7.12 (2H, d, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 20.8, 21.0, 30.3, 37.5, 42.8, 45.7, 52.0, 55.2, 81.5, 82.8, 125.0, 125.2, 128.7, 128.9, 134.8, 137.4, 137.6, 137.8, 149.2. Anal. ($C_{22}H_{25}NO_3$) C, H, N.

Pharmacology. [^{3}H]Mazindol Binding. For binding assays, caudate nuclei were homogenized using a polytron in 0.32 M sucrose and centrifuged for 10 min at 1000*g*. The supernatant was resuspended in cold sucrose and centrifuged at 17 500*g* for 20 min. The 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)[52] and centrifuged again. Finally, the pellet was resuspended in 30 volumes of buffer, pelleted at 15 000*g* 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]Dopamine. The effect of candidate compounds in antagonizing dopamine high affinity uptake was determined using a method previously employed.[49] For [^{3}H]DA uptake, 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.[49] An aliquot of the synaptosomal suspension was preincubated with the buffer and drug for 30 min at 37 °C, and uptake was initiated by the addition of [^{3}H]dopamine (5 nM, final concentration). 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. [^{3}H]5-HT and [^{3}H]NE uptake were measured in an entirely analogous fashion using synaptosomes prepared from rat midbrain and parietal/occipital cortex, respectively. Also, specific uptake of [^{3}H]5-HT and [^{3}H]NE were defined in the presence of 10 μM fluoxetine and 1 μM desipramine, respectively.

Functional Antagonism. First, the effects of approximate IC_{10} to IC_{50} concentrations of candidate compounds on the inhibition of [^{3}H]dopamine uptake by cocaine were determined. The IC_{50} value of cocaine in the presence of antagonist was then compared to the IC_{50} value of cocaine alone. Significant differences in IC_{50} values will be compared to theoretical IC_{50}

values expected from models of "same site" antagonism.[51,52] IC_{50} values greater than those expected for "same site" antagonism will be taken as evidence of functional antagonism. Compounds demonstrating antagonism were tested at lower concentrations to determine their minimum effective concentration. This test was performed under the preincubation conditions described above to allow slowly equilibrating compounds to reach equilibrium. Further, any artifactual differences in K_i due to differences in temperature, buffer, etc. were negated in this assay as binding of cocaine and the putative antagonists to both the cocaine binding site and the transporter occurred under identical conditions. This ensures that a right shift in the cocaine inhibition curve beyond what is expected for two drugs acting at the same site is a true measure of functional antagonism.

Behavioral Pharmacology. Locomotor Activity. 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 **6**, 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 was converted to 30 min totals. The maximal 30 min activity that occurred within the 2 h session following a given test drug injection, was determined for each dose level, and was expressed as the percent of its corresponding vehicle control response for plotting the dose−response curves.

Drug Discrimination. The drug discrimination study was conducted using male Sprague−Dawley rats according to the procedure described elsewhere.[53] 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.

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Supporting Information Available: Elemental analysis of compounds **3**, **6**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science*, **1987**, *237*, 1219.
(2) Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Cocaine receptor: Biochemical characterization and structure−activity relationships of cocaine analogues at the dopamine transporter. *J. Med. Chem*. **1992**, *35*, 969.
(3) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* **1991**, *14*, 299−302.
(4) Sharkey, J.; Ritz, M. C.; Schenden, J. A.; Hanson, R. C.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 1048.
(5) Woolverton, W. L.; Johnson, K. M. Neurobiology of cocaine abuse. *Trends Pharmacol. Sci.*, **1992**, *13*, 193−200.
(6) Woolverton, W. L.; Kleven, M. S. Mechanisms of Cocaine Abuse and Toxicity. *NIDA Res. Monogr.* **1988**, *88*, 160−184.
(7) Spealman, R. D.; Madras, B. K.; Bergman, J. Effects of Cocaine and Related Drugs in Nonhuman Primates. II. Stimulant Effects on Schedule-Controlled Behavior. *Pharmacol. Exp. Ther.* **1989**, *251*, 142.
(8) Carroll, F. I.; Lewin, A. H.; Biswas, J. Chemical approaches to the treatment of cocaine abuse. *Pharm. News* **1994**, *1*, 11−16.
(9) Wise, R. A. Catecholamine theories and reward: A critical review. *Brain Res.* **1978**, *152*, 215−247.
(10) Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Cocaine and 3β-(4′-substituted phenyl)tropane-2β-carboxylic acid ester and amide analogues. New high-affinity and selective compounds for the dopamine transporter. *J. Med. Chem*. **1995**, *38*, 379−388.
(11) Carroll, F. I.; Gray, J. L.; Abraham, P.; Kuzemko, M. A.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. 3-Aryl-2-(3′-substituted-1′,2′,4′-oxadiazol-5′-yl)tropane analogues of cocaine: Affinities at the cocaine binding site at the dopamine, serotonin, and norepinephrine transporters. *J. Med. Chem*. **1993**, *36*, 2886−2890.
(12) Holmquist, C. R.; Keverline-Frantz, K. I.; Abraham, P.; Boja, J. W.; Kumar, M. J.; Carroll, F. I. 3α-(4′-Substituted phenyl)-tropane-2β-carboxylic acid methyl esters: Novel ligands with high affinity and selectivity at the dopamine transporter, *J. Med. Chem*. **1996**, *39*, 4139−4141.
(13) Meltzer, P. C.; Liang, A. Y.; Madras, B. K. The discovery of an unusually selective and novel cocaine analog: Difluoropine. Synthesis and inhibition of binding at cocaine recognition sites. *J. Med. Chem*. **1994**, *37*, 2001−2010.
(14) Newman, A. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L. Novel 3α-(diphenylmethoxy)tropane analogues: Potent dopamine uptake inhibitors without cocaine-like behavioral profiles. *J. Med. Chem*. **1994**, *37*, 2258−2261.
(15) Newman, A. H.; Kline, R. H.; Allen, A. C.; Izenwasser, S.; George, C.; Katz, J. L. Novel 4′-substituted and 4′,4″-distituted 3α-(diphenylmethoxy)tropane analogues as potent and selective dopamine uptake inhibitors. *J. Med. Chem*. **1995**, *38*, 3933−3940.
(16) Meltzer, P. C.; Liang, A. Y.; Brownell, A.-L.; Elmaleh, D. R.; Madras, B. K. Substituted 3-phenyltropane analogues of cocaine: Synthesis, inhibition of binding at cocaine recognition sites, and positron emission tomography imaging. *J. Med. Chem*. **1993**, *36*, 855−862.
(17) Kozikowski, A. P.; Roberti, M.; Xiang, L.; Bergmann, J. S.; Callahan, P. M.; Cunningham, K. A.; Johnson, K. M. SAR studies of cocaine: replacement of the C-2 ester group by vinyl argues against H-bonding and provides an esterase resistant, high affinity cocaine analogue. *J. Med. Chem*. **1992**, *35*, 4764−4766.
(18) Simoni, D.; Stoelwinder, J.; Kozikowski, A. P.; Johnson, K. M.; Bergmann, J. S.; Ball, R. G. Methoxylation of cocaine reduces binding affinity and produces compounds of differential binding and uptake activity: discovery of a weak cocaine "antagonist". *J. Med. Chem*. **1993**, *36*, 3975−3977.
(19) Kozikowski, A. P.; Saiah, M. K. E.; Bergmann, J. S.; Johnson, K. M. Structure−activity relationship studies of cocaine: activity of n-sulfonyl analogues possibly excludes cocaine binding by an ionic interaction. *J. Med. Chem*. **1994**, *37*, 3440.
(20) Kozikowski, A. P.; Saiah, M. K. E.; Johnson, K. M.; Bergmann, J. S. Chemistry and biology of 2β-alkyltropane 3β-phenyl analogues of cocaine provide subnanomolar affinity ligands and suggest a new pharmacophore model at the C-2 position. *J. Med. Chem*. **1995**, *38*, 3086−3093.
(21) Smith, M. P.; Johnson, K. M.; Zheng, M.; Flippen-Anderson, J. L.; Kozikowski, A. P. Tuning the selectivity of monoamine transporter inhibitors by the stereochemistry of the nitrogen lone pair. *J. Am. Chem. Soc.* **1998**, *120*, 9072−9073.
(22) Singh, S.; Basmadjian, G. P.; Avor, K. S.; Pouw, B.; Seale, T. W. Synthesis and ligand binding studies of 4′-iodobenzoyl esters of tropanes and piperidines at the dopamine transporter. *J. Med. Chem*. **1997**, *40*, 2474−2481.
(23) Lieske, S. F.; Yang, B.; Eldefrawi, M. E.; MacKerell, A. D., Jr.; Wright, J. (−)-3b-Substituted ecgonine methyl esters as inhibitors for cocaine binding and dopamine uptake. *J. Med. Chem*. **1998**, *41*, 864−876.
(24) Chen, Z.; Izenwasser, S.; Katz, J.L.; Klein, C. L.; Zhu, N.; Trudell, M. L. Synthesis and dopamine transporter affinity of 2-methoxylcarbonyl-9-methyl-3-phenyl-azibicyclo[3.3.1]-nonane derivatives. *J. Med. Chem*. **1996**, *39*, 4744−4749.

(25) Shimada, S.; Kitayama, S.; Lin, C.-L.; Patel, A.; Nanthakumar, E.; Gregor, P.; Kumar, M.; Uhl, G. Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* **1991**, *254*, 576−577.
(26) Kilty, J. E.; Lorang, D.; Amara, S. G. Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science* **1991**, *254*, 578−579.
(27) Martin, Y. C. 1992 3D database searching in drug design. *J. Med. Chem.* **35**, 2145−2154.
(28) Martin, Y. C.; Bures, M. G.; Willett, P. Searching databases of three-dimensional structures. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; 1990 VCH Publishers: New York, 1990; pp 213−263.
(29) Sheridan, R. P.; Rusinko, A., III; Nilakantan, R.; Venkataraghavan, R. Searching for pharmacophores in large coordinate databases and its use in drug design. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8165−8169.
(30) Wang, S.; Milne, G. W. A.; Zaharevitz, D.; Sharma, R.; Marquez, V. E.; Lewin, N. E.; Blumberg, P. M. The discovery of novel, structurally diverse PK-C agonists through computer 3D-database pharmacophore search. Molecular modeling studies. *J. Med. Chem.* **1994**, *37*, 4479−4489.
(31) Wang, S.; Milne, G. W. A.; Yan, X.; Posey, I.; Nicklaus, M. C.; Graham, L.; Rice, W. G. Discovery of potent, non-peptide HIV protease inhibitors through 3D-database pharmacophore searching. *J. Med. Chem.* **1996**, *39*, 2047−2054.
(32) Madras, B. K.; Pristupa, Z. B.; Niznik, H. B.; Liang, A. Y.; Blundell, P.; Gonzales, M. D.; Meltzer, P. C. Nitrogen-based drugs are not essential for blockade of monoamine transporters. *Synapse* **1996**, *24*, 340−348.
(33) Kozikowski, A. P.; Simoni, D.; Roberti, M.; Rondanin, R.; Wang, S.; Du, P.; Johnson, K. M. Synthesis of 8-oxa analogues of norcocaine endowed with interesting cocaine-like activity. *Bioorg. Med. Chem Lett.* **1999**, *9*, 1831−1836.
(34) Yang, B.; Wright, J.; Eldfrawi, M. E.; Pou, S.; MacKerell, A. D., Jr. Conformational, aqueous solvation, and pK_a contributions to the binding and activity of cocaine, WIN 32065-2, and the WIN vinyl analogue. *J. Am. Chem. Soc.* **1994**, *116*, 8722−8732.
(35) Froimowitz, M. Conformational analysis of cocaine, the potent analogue of 2β-carbomethoxy-3β-(4-flurophenyl)tropane (CFT), and other dopamine reuptake blockers. *J. Comput. Chem.* **1993**, *14*, 934−943.
(36) Froimowitz, M.; Patrick, K. S.; Cody, V. Conformational analysis of methylphenidate and its structural relationship to other dopamine reuptake blockers such as CFT. *Pharm. Res.* **1995**, *12*, 1430−1434.
(37) Srivastava, S.; Crippen, G. M. Analysis of cocaine receptor site ligand binding by three-dimensional Voronoi site modeling approach. *J. Med. Chem.* **1993**, *36*, 3572−3579.
(38) Carroll, F. I.; Gao, Y.; Rahman, Y. A.; Abraham, P.; Parham, K.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis, ligand binding, QSAR, and CoMFA study of 3β-(p-substituted phenyl)-tropane-2β-carboxylic acid methyl esters. *J. Med. Chem.* **1991**, *34*, 2719.
(39) Hrynchuk, R. J.; Barton, R.; Robertson, B. E. The crystal structure of free base cocaine, C17H21NO4. *Can. J. Chem.* **1981**, *61*, 481−487.
(40) QUANTA, a molecular modeling system, is supplied by Molecular Simulations Inc., 200 Fifth Avenue, Waltham, MA 01803-5279.
(41) Matecka, D.; Rothman, R. B.; Radesca, L.; de Costa, B. R.; Dersch, C. M.; Partilla, J. S.; Pert, A.; Glowa, J. R. Wojnicki, F. H. E.; Rice, K. C. Development of novel, potent, and selective dopamine inhibitors through alteration of the piperazine ring of 1-[2-diphenylmethoxy)ethyl]-and1-[2-[bis(4-flurophenyl)-methoxy]-4-(3-phenylpropyl)piperazines (GBR 12935 and GBR 12909). *J. Med. Chem.* **1996**, *39*, 4704−4716.
(42) Milne, G. W. A.; Nicklaus, M. C.; Driscoll, J. S.; Wang, S.; Zaharevitz, D. W. The NCI Drug Information System 3D Database. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 1219−1224.
(43) Chem-X is a product of Oxford Molecular Group PLC, the Medawar Centre, Oxford Science Park, Oxford OX4 4GA, U.K.
(44) Huang, L.-Y. M.; Ehrenstein, G. Local anesthetic QX 572 and benzocaine act at separate sites on the batrachotoxin-activated sodium channel. *J. Gen. Physiol.* **1981**, *77*, 37−153.
(45) Reith, M. E. A.; Selmeci, F. Radiolabeling of dopamine uptake sties in mouse striatum; comparison of binding sites for cocaine, mazindol and GBR 12935. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1992**, *345*, 309−318.
(46) Dimmock, J. R.; Vashishtha, S. C.; Wilson Quail, J.; Pugazhenthi, U.; Zimpel, Z.; Sudom, A. M.; Allen, T. M.; Kao, G. Y.; Balzarini, J.; De Clercq, E. 4-(β-Arylvinyl)-3-(β-arylvinylketo)-1-ethyl-4-piperidinols and related compounds: A novel class of cytotoxic and anticancer agents. *J. Med. Chem.* **1998**, *41*, 4012.
(47) Kiyooka, S.-i.; Kuroda, H.; Shimasaki, Y. 1,3-Syn diastereoselective reduction of β-hydroxyketones with diisobutylaluminum hydride and tributyltin hydride. *Tetrahedron Lett.* **1986**, *27*, 3009.
(48) Nicklaus, M. C.; Wang, S.; Driscoll, J.; Milne, G. W. A. Conformational changes of small molecules binding to proteins. *Bioorg. Med. Chem.* **1995**, *3*, 411−428.
(49) Yi, S.-J.; Johnson, K. M. Effects of acute and chronic cocaine on [^{3}H]-dopamine uptake, compartmentalization and release. *Neuropharmacology* **1990**, *29*, 475−486.
(50) Huang, L.-Y. M.; Ehrenstein, G. Local anesthetic QX 572 and benzocaine act at separate sites on the batrachotoxin-activated sodium channel. *J. Gen. Physiol.* **1981**, *77*, 37−153.
(51) Reith, M. E. A.; Selmeci, F. Radiolabeling of dopamine uptake sties in mouse striatum; comparison of binding sites for cocaine, mazindol and GBR 12935. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1992**, *345*, 309−318.
(52) Slusher, B. S.; Tiffany, C. W.; Olkowski, J. L.; Jackson, P. F. Use of identical assay conditions for cocaine analogue binding and dopamine uptake to identify potential cocaine antagonists. *Drug Alcohol Depend.* **1997**, *48*, 43−50.
(53) Yasar, S.; Schindler, C. W.; Thorndike, E. B.; Szelenyi, I.; Goldberg, S. R. Evaluation of the stereoisomers of deprenyl for amphetamine-like discriminative stimulus effects in rats. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 1−6.
(54) Kozikowski, A. P.; Araldi, G. L.; Boja, J.; Meil, M. W.; Johnson, K. M.; Flippen-Anderson, J.; George, G.; Saiah, E. Chemistry and pharmacology of the piperidine-based analogues of cocaine. Identification of potent DAT inhibitors lacking the tropane skeleton. *J. Med. Chem.* **1998**, *41*, 1962−1969.

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