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Pharmacophore-Based Discovery of 3,4-Disubstituted Pyrrolidines as a Novel Class of Monoamine Transporter Inhibitors

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Abstract—3,4-Disubstituted pyrrolidines were discovered as a novel class of monoamine transporter inhibitors through 3-D database pharmacophore searching using a new pharmacophore model. The most potent analogue **12** has K_i values of 0.084 μM in [^{3}H]mazindol binding, 0.20, 0.23, and 0.031 μM in inhibition of dopamine (DA), serotonin (SER), and norepinephrine (NE) reuptake, respectively. Functional antagonism testing in vitro showed that **11** and **12** are weak cocaine antagonists. © 2001 Elsevier Science Ltd. All rights reserved.

Cocaine (**1**) is one of the most addictive substances known.[1,2] The level of cocaine abuse has reached epidemic proportions in recent years and an effective pharmacotherapy is immediately needed for the treatment of cocaine abuse.[2,3] Dopamine transporter (DAT) inhibitors are potential therapeutic agents for cocaine abuse treatment.[2] Although there is no specific pharmacotherapy available in the clinic, two potent DAT inhibitors are now in clinical trials for the treatment of cocaine abuse.[8] Over the last 20 years, extensive chemical and pharmacological studies have been performed on several classes of DAT inhibitors, including tropanes (mainly in the WIN series),[5] benzotropanes,[6] piperazines (also called GBR series),[7] and, most recently, piperidines.[4]

We are interested in the discovery of novel DAT inhibitors that can be used as either cocaine antagonists or 'partial agonists'.[9] For this purpose, we have used a 3-D database pharmacophore searching approach to discover novel DAT inhibitors[9] that were subsequently evaluated as potential cocaine antagonists in an in vitro functional antagonism assay. Recently, we reported the discovery of 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketones as a novel class of dopamine transporter inhibitors through 3-D database pharmacophore searching.[9a] In addition to the lead compound, a number of more potent analogues were shown to have a functional antagonism against cocaine in vitro and/or partially mimic cocaine in tests of locomotor activity and drug discrimination in rodents.[9a,b] Furthermore, our structure–activity relationship studies of 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketones also suggested that a possible new pharmacophore model (Fig. 1) may be constructed from this class of compounds for binding to monoamine transporters. This pharmacophore model may be used for the design and discovery of additional novel inhibitors. Herein, we report our discovery of 3,4-disubstituted pyrrolidines as a new class of monoamine transporter inhibitors using the new pharmacophore model shown in Figure 1. It should be noted that more

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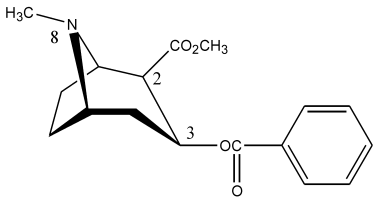
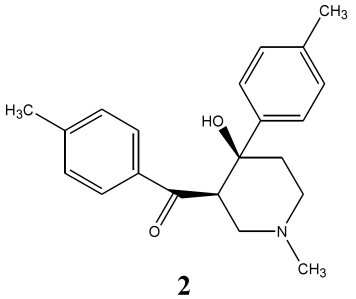


1 (cocaine)



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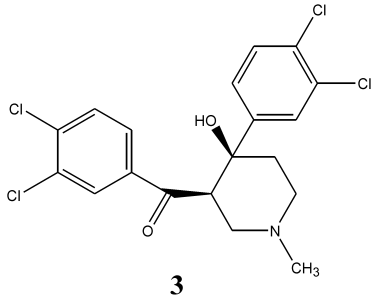

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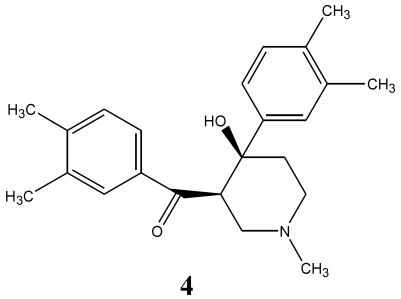

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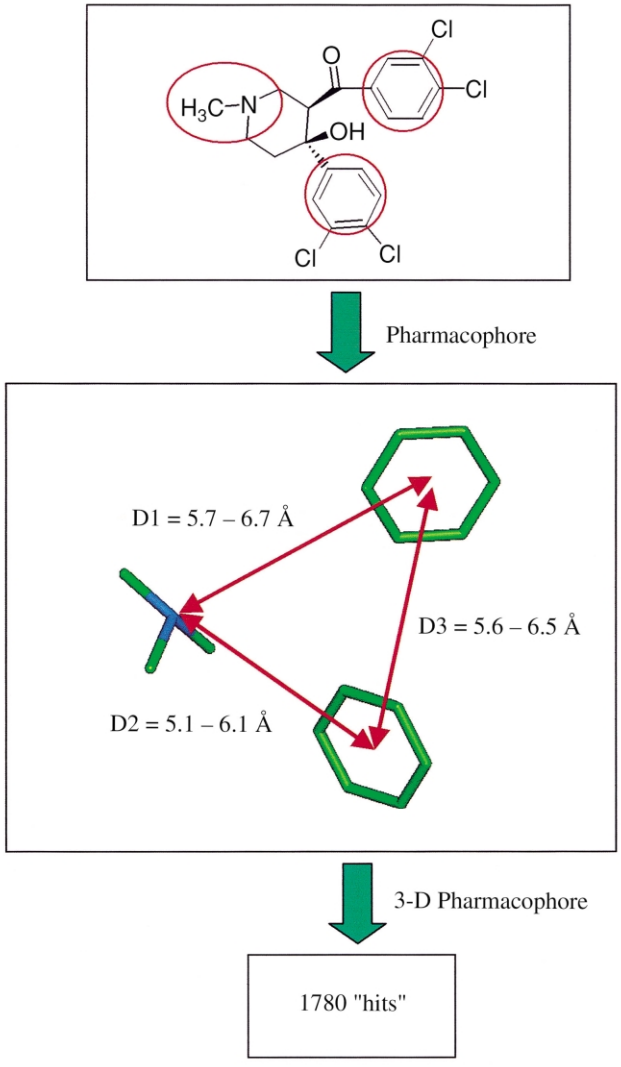


Figure 1. Derivation of a new pharmacophore model and searching the Available Chemical Database of 215,000 compounds using this new pharmacophore model.

than one pharmacophore model may be constructed for DAT inhibitors with diverse chemical structures, and using different pharmacophore models, compounds with unique binding modes can be discovered.[9]

Previous extensive SAR studies on cocaine and its analogues showed that the tertiary amine at position 8, the phenyl group at the 3β position and a small ester or a small hydrophobic group at the 2β position are crucial for their binding to the DAT. Based upon the SARs and our molecular modeling analysis, we constructed our first pharmacophore model, which was used in our previous 3-D database pharmacophore search and led to the identification of 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone (**2**) and several other classes of DAT inhibitors.[9a] The discovery of **2** as a new class of DAT inhibitors and our SAR studies on this class of compounds, as well as other studies, suggested that a phenyl group located at a position equivalent to the 2β position in cocaine, a tertiary amine located at a position equivalent to the position 8 in cocaine, and a second phenyl group located at a position equivalent to the 3β position in cocaine may constitute a new pharmacophore model. Conformational analysis of **2** and its more potent analogues **3** and **4** showed that the distance between two phenyl groups is between 5.6 and 6.5 Å, while the distances between the tertiary amine and the two phenyl groups are 4.5–5.6 and 5.0–6.2 Å, respectively. Accordingly, a new pharmacophore model was constructed (as shown in Fig. 1). It is of note that, although **2**, **3**, and **4** are more selective for DAT, they also display low micromolar to nanomolar potency in inhibition of the reuptake of SER and NE. Furthermore, extensive SAR studies on cocaine and other tropane analogues showed that the selectivity of inhibitors between these three monoamine transporters can be achieved through subtle structural modifications.[4] Accordingly, the pharmacophore model shown in Figure 1 can be used for searching new inhibitors not only for DAT but also for SER transporter (SERT) and

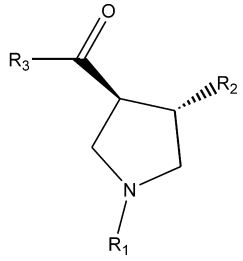
NE transporter (NET). It is of further note that a previous study has proposed a simple common pharmacophore model consisting of a phenyl ring and a nitrogen atom for central nervous system agents.[10] Thus, the second aromatic ring proposed in our pharmacophore model may provide a specific interaction site for the binding to monoamine transporters (Fig. 1).

Using the pharmacophore model in Figure 1, we searched the available chemical database (ACD)[11] of approximately 215,000 compounds using the Chem-X program.[12] A total of 1780 compounds or 0.8% were identified that met the pharmacophore requirements as specified in Figure 1. Since our purpose was to discover novel leads, in the first batch of compounds selected for testing we only chose compounds that are structurally simple, have novel chemical scaffolds, have molecular weight less than 600, and are nonpeptides. Fourteen compounds were selected as potential inhibitors of DA reuptake in the first batch to validate the new pharmacophore model shown in Figure 1. These compounds were evaluated for their ability to inhibit reuptake of DA into striatal nerve endings (synaptosomes) using an assay as described previously.[9a] Eight of those 14 compounds had an IC_{50} value less than 10 μM in inhibition of DA reuptake (data not shown). It is of note that, although some compounds have chemical structures similar to compounds **2**, **3**, and **4**, several new inhibitors have chemical structures that are very different from known DAT inhibitors. Thus, our results showed that the new pharmacophore model in Figure 1 is effective for discovery of novel DAT inhibitors. Out of those eight lead compounds identified, compound **5**, 1-methyl-4-phenylpyrrolidin-3-yl phenyl ketone, is of particular interest because of its structural simplicity. It is of note that the lead compound **5** and the piperidine compounds are structurally related to each other. Compound **5** has K_i values of 1.18 and 1.41 μM in mazindol binding and inhibition of DA reuptake, approximately 5 times less potent than cocaine in binding and inhibition of DA reuptake (Table 1). Conformational analysis using the QUANTA program[13] showed that **5** has a good overlap on **3** (Fig. 2).

To investigate its selectivity, **5** was evaluated as an inhibitor of NE and SER reuptake. It was found that **5** is a reasonably potent NET inhibitor, with a K_i value of 0.15 μM, as potent as cocaine and a moderately potent SERT inhibitor with a K_i value of 0.75 μM. While cocaine exhibits little selectivity among DAT, SERT and NET (Table 1), the lead compound **5** has a selectivity toward NET.

To gain further understanding of the SARs of 3,4-disubstituted pyrrolidines as monoamine transporter inhibitors, we have identified a number of analogues of compound **5**.[14] These compounds were evaluated as inhibitors of DA reuptake and the results are summarized in

Table 1. Structure–activity relationships of 3,4-disubstituted pyrrolidines



	R_1	R_2	R_3	K_i (μM)			
				[^{3}H]Mazindol binding	DAT	SERT	NET
Cocaine (**1**)				0.23±0.02[a]	0.27±0.02	0.16±0.01	0.19±0.01
5	CH_3	Phenyl	Phenyl	1.18±0.04	1.41±0.09	0.75±0.06	0.15±0.02
6	CH_3	*para*-F-Phenyl	*para*-F-Phenyl	3.55±0.12	3.96±0.70	0.38±0.05	0.80±0.02
7	CH_3	Phenyl	*para*-Cl-Phenyl	1.25±0.02	1.22±0.03	0.52±0.02	0.43±0.05
8	CH_3	*para*-Cl-Phenyl	para-Methoxyphenyl		1.16±0.07		
9	CH_3	*para*-Cl-Phenyl	*meta*-Trifluoromethylphenyl		3.17±0.10		
10	CH_3	*para*-Cl-Phenyl	*ortho*-Cl-Phenyl	0.34±0.01	0.61±0.03	0.54±0.03	0.069±0.001
11	CH_3	Phenyl	Pyridinyl	0.51±0.01	0.63±0.02	0.83±0.05	0.044±0.001
12	CH_3	*para*-Cl-Phenyl	Thienyl	0.084±0.015	0.20±0.01	0.23±0.01	0.031±0.004
13	CH_3	*para*-CH_3-Phenyl	*ortho*-F-Phenyl		1.93±0.20		
14	CH_3	2′,4′-diCl-Phenyl	*para*-Cl-Phenyl		1.26±0.04		
15	CH_3	*ortho*-F-Phenyl	*para*-Cl-Phenyl		1.28±0.01		
16	Phenyl	Phenyl	Pyridinyl	>10	>10	>10	>10
17	Phenyl	Phenyl	Thienyl	>10	>10	>10	>10
18	Phenyl	*para*-Cl-Phenyl	*ortho*-Methoxyphenyl		>10		
19	Phenyl	*para*-Cl-Phenyl	Phenyl		>10		
20	Phenyl	*para*-Methylphenyl	*para*-F-phenyl		>10		

[a]Range or standard error, based on two to four experiments.

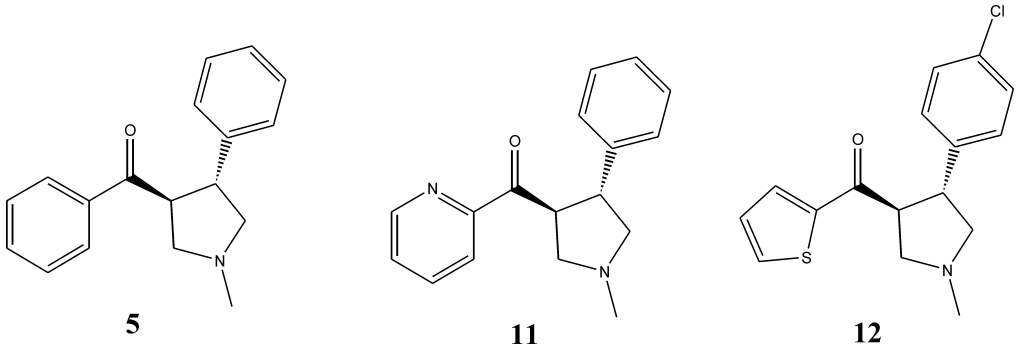


Table 1. Out of these analogues evaluated, five analogues (**7**, **8**, **13**, **14**, and **15**) showed a similar potency in inhibition of DA reuptake as compared to **5**. Three analogues (**10**, **11**, and **12**) are more potent inhibitors than **5**, with K_i values of 0.61, 0.63, and 0.20 μM in inhibition of DA reuptake, respectively. The potency of **12** in inhibition of DA reuptake is comparable to that of cocaine (0.20 μM vs 0.27 μM).

To assess the selectivity of these more potent analogues, they were also evaluated as inhibitors of NE and SER reuptake, using assays as described previously.[9a] It was found that a *para*-F substituent in both phenyl rings (**6**) decreases the potency by 3-fold at DAT and by 5-fold at NET, but increases the potency by 2-fold at SERT. A *para*-Cl substitution in the phenyl ring (**7**) at position 3 has no effect on its activity at DAT, slightly improves its activity at SERT but decreases its activity by 3-fold at NET. These data indicate that substituents in the two phenyl rings in **5** have different effects to its activity at the three transporters.

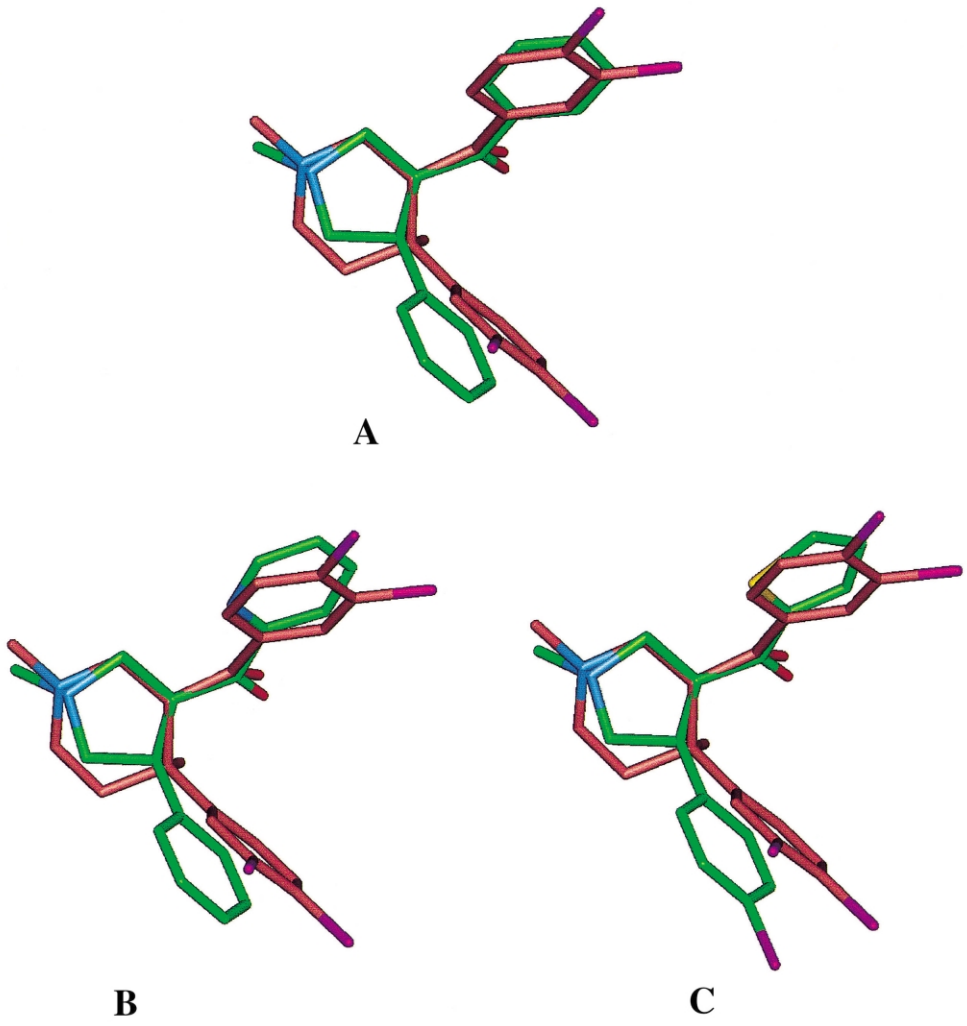


Figure 2. Overlay of pyrrolidines **5** (A), **11** (B) and **12** (C) in green on compound **3** in brown.

Analogues **10**, **11**, and **12** have improved activity at all the three transporters, as compared to the lead compound (**5**). Conformational analysis showed that **11** and **12** have good overlap on **3** (Fig. 2). A *para*-Cl substituted phenyl ring at position 3 and an *ortho*-Cl substituted phenyl ring at position 4 (**10**) resulted in a 2-fold improvement in the DAT and NET activities, but only a slight improvement in the SERT activity, as compared to **5**. A pyridinyl group instead of a phenyl group at position 3 of the pyrrolidine ring (**11**) resulted in a 2- and 3-fold improvement upon its DAT and NET activities, respectively, but has no effect on its SERT activity, as compared to **5**. A thienyl group instead of a phenyl group at position 3 and a *para*-Cl substituted phenyl ring at position 4 of the pyrrolidine ring (**12**) resulted in a 7-, 3-, and 5-fold improvement in the DAT, SERT, and NET activities, respectively. Thus, analogues **10**, **11**, and **12** are reasonably potent NET inhibitors with K_i values of 0.069, 0.044, and 0.031 μM, respectively. While analogue **12** has a similar activity at DAT and SERT, it is 6 times more potent than cocaine in inhibition of the NET.

The lead compound (**5**) and a number of its more potent analogues (**7**, **10**, **11**, and **12**) all have an *N*-methyl substituent at position 1. To investigate the importance of the *N*-methyl group for their activity, analogues **16**–**20** were evaluated as inhibitors of DA reuptake. Since **16** and **17** are direct derivatives of two most potent inhibitors **11** and **12**, they were also evaluated as inhibitors of SERT and NET. It was found that all these analogues have K_i values greater than 10 μM at DAT, SERT, and NET, indicating that the *N*-phenyl group at position 1 of the pyrrolidine ring is too bulky for achieving potent activity at all these three transporter sites. These results are consistent with our previous SAR studies of 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketones, which showed that an *N*-methyl group is optimal for its activity at DAT and a bulkier group such as *N*-ethyl and *N*-phenyl decreases the activity in inhibition of DA reuptake.[9a,b]

The cocaine antagonism of the two most potent analogues **11** and **12** were evaluated using a functional antagonism assay, as described previously.[15] The IC_{50} values of cocaine in the presence of **11** and **12** 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.[15] If the experimental IC_{50} value of cocaine in inhibition of DA reuptake in the presence of a drug is significantly greater than the theoretical IC_{50} value, the drug is then considered as a potential cocaine antagonist. A DAT inhibitor with a significant functional antagonism suggests that the inhibitor is capable of reducing the binding of cocaine, either by steric hindrance or by an allosteric mechanism, while at the same time having a relatively smaller effect on DA transport. The results are summarized in Table 2.

Table 2. Functional antagonism of analogues **11** and **12**

Compounds	[^{3}H]-Dopamine uptake	
	Experimental IC_{50} (nM) (Mean ± SEM)	Theoretical IC_{50} (nM) (Mean ± SEM)
Cocaine (**1**)	297 ± 22[a]	
11	663 ± 22	
Cocaine + **11** (100 nM)	494 ± 10	333 ± 16
Cocaine + **11** (300 nM)	645 ± 78	420 ± 21
12	308 ± 16	
Cocaine + **12** (30 nM)	460 ± 71	320 ± 16
Cocaine + **12** (100 nM)	641 ± 15	386 ± 19

[a]Standard error was based on three experiments.

As can be seen from Table 2, at each of the two concentrations of **11** used (100 and 300 nM), the IC_{50} value obtained for cocaine in the presence of **11** was significantly greater than that expected from 'same site' antagonism. Therefore, **11** was considered to have weak functional antagonism against cocaine, though it is important to point out that at these concentrations **11** significantly inhibited the DA reuptake alone and that the data are normalized to 100% for the analysis. Similar results were obtained for analogue **12**. Therefore, both **11** and **12** appear to have a weak functional antagonism against cocaine.

In conclusion, 3,4-disubstituted pyrrolidines were discovered as a novel class of monoamine transporter inhibitors through 3-D database pharmacophore searching using a new pharmacophore model. This class of inhibitors has a selectivity profile different from that of cocaine at DAT, SERT, and NET. Two potent analogues **11** and **12** were found to function as weak cocaine antagonists in a functional antagonism assay. Further pharmacological and behavioral studies are under way to investigate the mechanism of functional antagonism of **11** and **12**. Furthermore, extensive SAR studies are being carried out and will be reported in due course.

Acknowledgements

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13. QUANTA, a molecular modeling system, was supplied by Molecular Simulations, Inc., 9685 Scranton Road, San Diego, CA 92121-3752, USA.
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