Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 513–517

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pharmacophore-Based Discovery of Substituted Pyridines as Novel Dopamine Transporter Inhibitors

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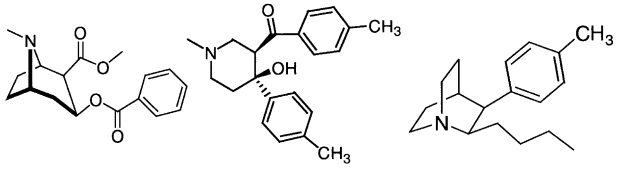
Received 31 July 2002; accepted 6 October 2002

Abstract—Abnormal dopamine signaling in brain has been implicated in several conditions such as cocaine abuse, Parkinson's disease and depression. Potent and selective dopamine transporter inhibitors may be useful as pharmacological tools and therapeutic agents. Simple substituted pyridines were discovered as novel dopamine transporter (DAT) inhibitors through pharmacophore-based 3D-database search. The most potent compound **18** has a K_i value of 79 nM in inhibition of WIN35,248 binding to dopamine transporter and 255 nM in inhibition of dopamine reuptake, respectively, as potent as cocaine. Preliminary structure–activity relationship studies show that the geometry and the nature of the substituents on the pyridine ring determine the inhibitory activity and selectivity toward the three monoamine transporters. The substituted pyridines described herein represent a class of novel DAT inhibitors with simple chemical structures and their discovery provides additional insights into the binding site of DAT.

Dopamine (DA) is a neurotransmitter crucial for normal brain function. The dopamine transporter (DAT) plays a critical role in terminating DA neurotransmission by taking up DA released into the synapse.[1,2] Abnormal DA signaling in brain has been implicated in many pathological conditions such as cocaine abuse, Parkinson's disease and depression.[1–3] The ability of cocaine to bind to the DAT and to inhibit the reuptake of DA has been strongly implicated in the reinforcing properties of cocaine.[2] As such, considerable emphasis has been directed toward DAT as a molecular target for developing a pharmacotherapy for the treatment of cocaine addiction and abuse.[1–4] Novel DAT inhibitors may function as mild and long-lasting stimulants, which may be used as replacement therapy for cocaine addiction.[2,5] These compounds can also function as cocaine antagonists or 'partial agonists' in behavioral models, and may be useful as potential therapeutic agents for the treatment of certain aspects of cocaine abuse and addiction.[2] DAT inhibitors with truly novel chemical scaffolds will also provide new insights into the binding site in DAT.



1 (R-cocaine) **2** **3**

Our group has recently employed a pharmacophore-based 3D-database searching approach for the discovery of novel DAT inhibitors.[4,6–9] Extensive structure–activity relationship studies on cocaine (**1**) and other tropane analogues showed that a tertiary amine at the 8-position, a phenyl group at the 3-position and a carbonyl group at the 2-position may be crucial for their binding to the DAT.[4] Based upon these data we constructed the first pharmacophore model.[4] 3D-database searching using the first pharmacophore model led to the discovery of 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone (**2**)[4,9] and 2-alkyl-3aryl quinuclidines[10] (**3**) among others as novel DAT inhibitors.

Although our very first pharmacophore model was successful in identification of novel DAT inhibitors, several known potent DAT inhibitors like **4**, **5**, **6**, and **7**[11,12] (Fig. 1) did not have a carbonyl group as specified in the

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PII: S0960-894X(02)00943-5

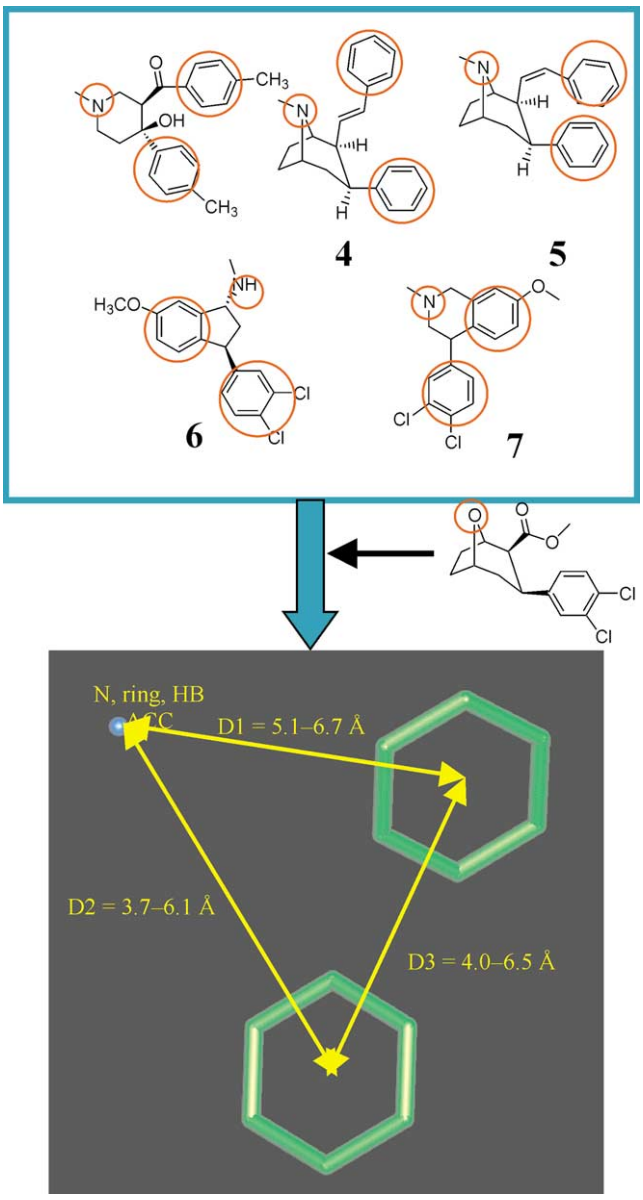
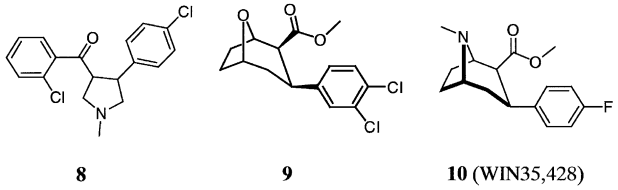


Figure 1. A new pharmacophore model derived from several known DAT inhibitors.

first pharmacophore model. Furthermore, in our design of quinuclidines as a novel class of DAT inhibitors, we also found that the carbonyl group in the original lead compound was not essential.[13] This carbonyl group could be replaced with either an aliphatic or an aromatic hydrophobic group. Taken together, these data suggested that the carbonyl group was not absolutely required for binding to the DAT and inhibition of DA reuptake. Thus, more than one pharmacophore model can be proposed and used for the discovery of novel DAT inhibitors. Based on this idea, we proposed our second pharmacophore model that had the carbonyl group replaced with a phenyl group.[7] 3D-database pharmacophore searching using this second pharmacophore model led to the identification of 3,4-disubstituted pyrrolidines (**8**) as novel DAT inhibitors.[7]



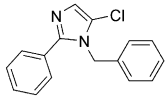
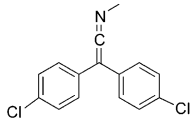
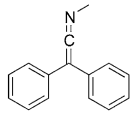
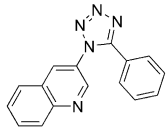
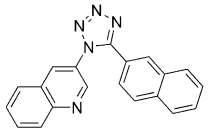
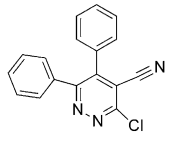
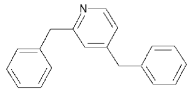
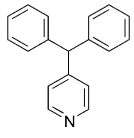
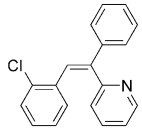
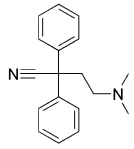
8 **9** **10** (WIN35,428)

Our goal is to identify novel DAT inhibitors with a different binding mode from that of cocaine which would potentially function as cocaine antagonists. Recent site-directed mutagenesis experiments showed that mutation of aspartate 79 (Asp79) to alanine residue strongly affects the binding affinity of both substrate and cocaine analogues containing an amine nitrogen.[14,15] The site-directed mutational experiments thus suggest a direct interaction of Asp79 with the basic amine groups of cocaine analogues. The basic amine group in cocaine analogues and DA becomes protonated (positively charged) under physiological conditions, thus having a strong interaction with the negatively charged Asp79. Thus replacing the basic amine group in cocaine analogues and DA with a functional group that cannot have a strong interaction with Asp79 may lead to DAT inhibitors with novel binding mode to DAT. A recent study has showed that 8-oxa-2-carbomethoxynorbenzotropine (**9**) is a potent DAT inhibitor.[16] In compound **9**, an oxygen atom which can function only as a hydrogen bonding acceptor replaces the basic nitrogen atom in cocaine analogues, indicating that the presence of a basic amino group is not an absolute requirement for high affinity binding to the DAT and a hydrogen bond acceptor in this position of the basic nitrogen can be equally effective for binding to the DAT and for inhibition of DA reuptake. This prompted us to propose a new pharmacophore model (Fig. 1) in which the tertiary nitrogen (sp^3) atom was replaced with a hydrogen-bond acceptor N (sp^2) atom in a ring system. Distance parameters between the nitrogen atom and the center of the two aromatic rings were established based upon low energy structures obtained from the conformational analysis of compounds **2**, **4**, **5**, **6**, and **7**.

Using this new pharmacophore model we searched the National Cancer Institute (NCI)[17] 3D-database that contained 206,876 'open' compounds accessible by the public. The program Chem-X[18] was used for identifying compounds that fit our pharmacophore query. A total of 1104 (0.5%) compounds ('hits') met the pharmacophore requirements as specified in the Figure 1. These 'hits' were only potential DAT inhibitors and they needed to be confirmed for their activity to inhibit the reuptake of DA into striatal nerve endings (synaptosomes) and to displace the binding of [^{3}H]WIN35,428 (**10**) to DAT.[4] Since the binding and uptake assays were quite time-consuming, we used filters for selecting a limited number of 'hits' for testing. The molecular weight of the selected compounds had to be below 500, the number of rotatable bonds had to be below five, the chemical matter had to be novel and attractive for chemical modifications. We selected 10 structurally diverse compounds for preliminary testing.

These 10 compounds were first screened in DA uptake assay. Since we were only interested in fairly potent compounds, we have screened them with a concentration of 1 μM for each compound. Two compounds **17** and **18** were found to have significant inhibition of DA reuptake. The IC_{50} value was estimated by adding the radiolabeled neurotransmitter following equilibration between the test compounds and the transporter.

Table 1. Preliminary results of selected 'hits' from pharmacophore-based virtual screening in dopamine reuptake assay

Compd	Structure	DA K_i (μM)
11		> 1.0
12		> 1.0
13		> 1.0
14		> 1.0
15		> 1.0
16		> 1.0
17		2.3
18		0.2
19		> 1.0
20		> 1.0

Therefore, we were able to use the Cheng-Prusoff equation for classic, competitive inhibition to calculate the K_i values from IC_{50} values in these experiments. Their IC_{50} values were determined using the computer program LIGAND. The K_m values used were 67 nM for [^{3}H]DA, 53 nM for [^{3}H]5-HT, and 54 nM for [^{3}H]NE.[19] While **17** has a K_i value of 2.3 μM, **18** has a K_i value of 0.2 μM, as potent as cocaine (Table 1). Interestingly, both of these compounds belong to substituted pyridines.

Compound **18** represents a novel class of DAT inhibitors with very simple chemical structure and fairly good potency. We have tested six additional new analogues to gain insights into structure–activity relationship for this class of DAT inhibitors (Table 2). Compounds **18**, **21**, **22**, and **23** show quite potent activities in binding and uptake assays, with K_i values 0.079–0.780 μM in binding, and 0.255–1.067 μM in inhibition of DA reuptake, respectively. Compound **18** is the most potent among tested with K_i values of 79 nM in binding and of 255 nM in inhibition of DA reuptake. Despite its very simple chemical structure, **18** is as potent as cocaine in the DA uptake assay. The activities of compounds **18**, **21**, and **22** show that the position of the nitrogen on the pyridine ring (or the position of the diphenylmethyl substituent on the pyridine ring) affects both inhibition of DA uptake and WIN binding (Table 2). Compounds **21** and **22** with the diphenylmethyl substituent at either the *meta*- or the *ortho*-position on the pyridine ring are about 10-fold less potent in WIN binding and 4-fold less potent in inhibition of DA reuptake, respectively, when compared to **18**. Compound **23**, which has a benzyl group in the position of the phenyl group in **18**, is only slightly less potent than **18**. This suggests that a larger

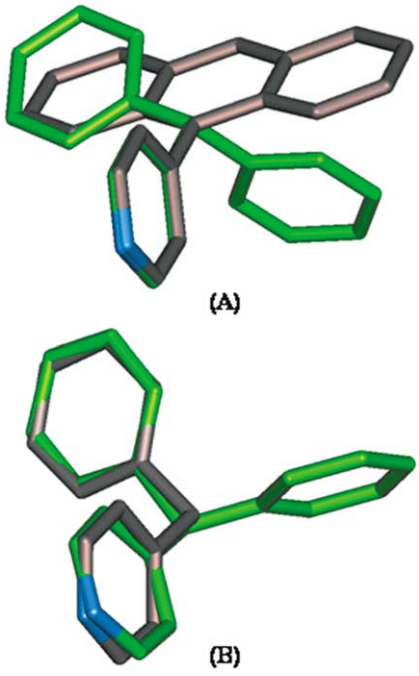
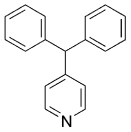
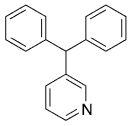
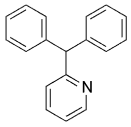
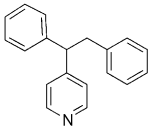
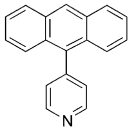
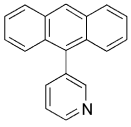
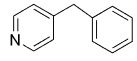


Figure 2. Superposition of the lowest energy conformations: (**A**) compound **11** (green) and compound **15** (grey). (**B**) Compound **11** (green) and compound **17** (grey).

Table 2. Binding affinities of substituted pyridines to DAT and their update activities at the three monoamine transporters

Compd	Structure	K_i (μM)			
		[H^3]WIN binding	[H^3]DA uptake	[H^3]5-HT uptake	[H^3]NE uptake
R-cocaine (**1**)			0.270±0.020	0.155±0.001	0.108±0.004
18		0.079±0.004	0.255±0.008	1.160±0.020	3.46±0.10
21		0.780±0.064	0.860±0.032	12.60±2.700	7.32±0.77
22		0.742±0.026	1.067±0.034	35.00±7.000	5.53±0.45
23		0.099±0.017	0.263±0.003	0.910±0.100	0.393±0.008
24		>10.00	>10.00	>10.00	>10.00
25		>10.00	>10.00	>10.00	>10.00
26		>10.00	>10.00	>10.00	>10.00

Standard deviation was obtained with three experiments.

group can be tolerated at this site for binding to the DAT. Compound **24**, whose structure may be viewed as the diphenyl rings in **18** fused into an anthracene ring, has a minimal activity at 10 μM. Likewise, compound **25**, which also has an fused anthracene ring, is also inactive. Molecular modeling studies showed that the two phenyl rings in **18** have a quite different relative orientation as compared to the corresponding aromatic rings in **24** (Fig. 2A). While the two aromatic rings in **24** have to be in the same plane, the two phenyl rings in **18** cannot be in the same plane in low energy conformations because they are connected to a sp^3 carbon. Compound **26**, which may be viewed as one phenyl ring in **18** being replaced with H, is inactive. Molecular modeling shows that the pyridine and the phenyl rings in **26** can be superimposed on the corresponding rings in **18** (Fig. 2B), suggesting that both phenyl rings in **18** are important for binding to DAT.

To achieve a further insight into the selectivity of these compounds among the three monoamine transporters (DAT, SERT and NET), we also evaluated the activity of the seven monosubstituted pyridines in inhibition of 5-HT and NE reuptake (Table 2). Our data showed that **22** has the highest selectivity, 35-fold, between inhibiting DA uptake versus 5-HT uptake. Compound **18** has the highest selectivity, about 14-fold, between inhibiting DA uptake versus NE uptake. Compound **23**, whose structure is more flexible than **18**, shows no selectivity between DAT and NET, but has the same selectivity as **18** between DAT and SERT. The difference in selectivity for monoamine transporters between **23** and **18** may also be attributed to the size difference between substituents on the pyridine ring. Compounds **24**–**26** show no appreciable activity for up to 10 μM concentration. Our data suggests that the position, size and flexibility of the substituents on the pyridine ring are important for their selectivity among these three monoamine transporters for this class of compounds.

In summary, simple substituted pyridines are discovered as a novel class of DAT inhibitors through 3D database searching using a new pharmacophore model. The discovery of pyridines as fairly potent DAT inhibitors provides a validation to our proposed new pharmacophore model used in our 3D-database searching and further shows that the protonated nitrogen and the ester group in cocaine are not

absolutely required for binding to DAT and other monoamine transporters.

Acknowledgements

The financial support (DA R0111545 to S.W.) from the National Institute on Drug Abuse is greatly appreciated.

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